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Selected Oral Presentations

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OR01

Paired-nickase S.aureus Cas9 system is an efficient and potentially safer *in vivo* treatment for Primary Hyperoxaluria Type 1

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Primary hyperoxaluria type 1 (PH1) is a rare genetic metabolic disorder associated with mutations in AGXT gene, causing hepatic alanine-glyoxylate aminotransferase (AGT) deficiency. Consequently, oxalate is overproduced in the liver and accumulated in kidneys causing life-threatening renal damage. The only curative treatment is liver transplantation, thus, new therapies are required. The inhibition of glycolate oxidase (GO), the enzyme implicated in the synthesis of glyoxylate (precursor of oxalate), has been proven to be an efficient substrate reduction therapy (SRT) to treat PH1. Recently, AAV8-CRISPR/Cas9-mediated in vivo SRT was shown to greatly diminish GO expression, resulting in urine oxalate reduction and prevention of kidney damage. Nevertheless, concerns regarding CRISPR/Cas9 off-target effects should not be underestimated. Our approach to decrease off-target modifications was to use a nickase Cas9 combined with two gRNAs targeting nearby regions on the opposite strand. PH1 mice were treated with D10A-SaCas9 nickase mutants and two gRNAs previously tested efficient for the WT SaCas9. Simultaneous nicks greatly interfered with transcription and translation of Hao1, showing no significant differences with WT Cas9, while individual nicks did not interfere with the target gene expression. As expected, GO inhibition decreased urine oxalate levels and crystal accumulation in kidney parenchyma. Moreover, potential off-target sites for each gRNA were identified by CIRCLE-seq and NGS analysis is being performed in mice treated with WT or nickase SaCas9. In conclusion, dual nickase Cas9 should be preferred over nuclease strategy for more efficient and safer in vivo treatment of this monogenic disease.

OR02

HDR-CRISPR: a novel system to promote Cas9-mediated homology-directed DNA repair

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Precise genome editing requires the harnessing of homologous recombination-based homology-directed repair (HDR) pathway and a proper donor template to precisely seal a designer nuclease (DN)-induced DNA double strand break (DSB). However, competing DNA repair pathways, such as non-homologous end-joining (NHEJ), predominate in mammalian cells, often resulting in HDR frequencies far below the thresholds required for clinical translation. While chemical compounds have been used to synchronize the cells in cell cycle phases when the HDR pathway is most active or, alternatively, to inhibit NHEJ, their potential side effects limit the use of such drugs in clinical settings. To overcome this limitation, we sought to increase at the DSB the local concentration of factors critical for either engaging HDR or inhibiting NHEJ, and generated 13 different Cas9-fusion proteins (referred to as HDR-CRISPRs). We used a traffic light reporter (TLR) system to assess the frequency of DSBs that are either repaired by NHEJ or HDR, respectively, and achieved up to 3-fold increase in HDR-mediated repair events with selected HDR-CRISPRs. Importantly, the simultaneous inhibition of the NHEJ pathway further improved the HDR to NHEJ ratio, leading to an almost even distribution of HDR to NHEJ. These results support our hypothesis that the local recruitment of factors to the DSB to either promote HDR or inhibit NHEJ can modulate the DNA repair choice without altering the physiology of the target cells. We envision that this strategy is readily translatable to clinically relevant applications.

OR03

Targeted gene correction of human hematopoietic stem cells for the treatment of Wiskott-Aldrich Syndrome

ABSTRACT WITHDRAWN

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OR04

Adeno-associated virus in human liver: natural history and consequences in tumor development

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Adeno-associated virus (AAV) is a defective mono-stranded DNA virus, endemic in human population (35–80%). Recurrent clonal AAV2 insertions are associated with the pathogenesis of rare human hepatocellular carcinoma (HCC) developed on normal liver. This study aimed to characterize the natural history of AAV infection in the liver and its consequence in tumor development. Viral DNA was quantified in tumor and non-tumor liver tissues of 1461 patients and in silico analyses using viral capture data explored viral variants and new clonal insertions. AAV DNA was detected in 21% of the patients, more frequently in the non-tumor counterpart (18%) than in tumor (8%). The full-length viral sequences were reconstructed in 57 patients leading to identify two distinct AAV subtypes: one similar to AAV2, the other hybrid between AAV2 and AAV13 sequences. Episomal viral forms were found in 4% of the non-tumor tissues, frequently associated with viral RNA expression and human herpesvirus type 6 (HHV6), the candidate natural AAV helper virus. In 30 HCC, clonal AAV insertions were recurrently identified in CCNA2, CCNE1, TERT, TNFSF10, KMT2B and GLI1/INHBE. AAV insertion triggered oncogenic overexpression through multiple mechanisms that differ according to the localization of the integration site. Clonal AAV insertions were positively selected during HCC development on non-cirrhotic liver challenging the notion of AAV as a non-pathogenic virus. In conclusion, this is the first large scale study that provides an integrated analysis of wild type AAV infection in the liver with the identification of viral genotypes, molecular forms, helper virus relationship and viral integrations.

OR05

Paracrine delivery of therapeutic biologics for cancer

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A fundamental goal of cancer drug delivery is to achieve sufficient levels within the tumour without leading to high systemic concentrations that might cause off-target toxicities. In situ production of protein-based therapeutics by tumour cells provides an attractive alternative to treatment with repeated high bolus injections, as secretion by the tumour itself could provide high local concentrations that act in a paracrine fashion over an extended duration. For this purpose, we have developed a non-oncolytic adenoviral delivery system that allows for targeting of Ad5 to discrete cell types by redirecting viral tropism to cell surface biomarkers through the use of interchangeable adapters. Furthermore, we recently described the engineering of a protein-based 'shield' that is coated on the Ad5 capsid, which, together with the retargeting adapters, allows for improved tumour specificity and prevention of viral clearance. To test this delivery strategy in vivo, SCID-beige mice bearing orthotopic BT474 xenografts were treated with three doses of either a cancer-specific, non-replicative Ad5 that encodes a secreted anti-HER2 antibody, trastuzumab, in its genome, or with the protein therapeutic itself (Herceptin®). We have employed state-of-the-art whole tumour clearing and imaging with confocal microscopy at high spatial resolution in 3D to assess biodistribution, and large volumetric imaging has revealed that the secreted therapeutic diffuses significantly throughout the tumour leading to a therapeutic effect and delayed tumour outgrowth. Moreover, the systemic concentration of antibody is significantly reduced with viral delivery, suggesting that paracrine delivery may be a promising strategy for delivery of biologics with narrow therapeutic indices.

OR06

Base editor-mediated CD33 engineering to improve safety and efficacy of CD33-targeted cancer therapy

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Antigen-specific immunotherapies for myeloid malignancies, including acute myeloid leukemia (AML), have largely focused on CD33, a myeloid differentiation antigen displayed on AML blasts and possibly on leukemia stem cells. Improved survival with the CD33 antibody-drug conjugate gemtuzumab ozogamicin (GO) in AML patients has validated CD33 as immunotherapeutic target. An important limitation of this approach is however the significant on-target/off-leukemia effects attributed to the expression of CD33 on normal myeloid cells, causing severe cytopenia in treated patients. Recent studies, including by our group, have demonstrated that CRISPR/Cas9 nuclease-based editing of CD34+ hematopoietic stem and progenitor cells (HSPCs) conferred protection from CD33-directed drugs. While promising, this CRISPR-based strategy suffers from off-target activity due to cleavage of a nearby CD33 homolog pseudogene and from activation of endogenous TP53-mediated DNA damage responses. To address these limitations, we have explored the use of cytosine base editors (CBE) that introduce precise nucleotide substitutions and circumvent the need for DNA double strand breaks. We investigated 2 different strategies for introducing non-sense and splicing mutations in CD33. CBE-treatment of human CD34+ HSPCs did not impair engraftment and differentiation in a mouse model, while reducing CD33 expression and protecting cells from in vivo GO administration. Next-generation sequencing analysis of blood nucleated cells confirmed the persistence and specificity of CBE-induced mutations in vivo. Together, these results validate the use of CBE for the generation of CD33 engineered hematopoiesis to improve safety and efficacy of CD33-targeted therapies.

OR07

De novo generation of functional human thymus organoids from induced pluripotent stem cells

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A proper functional thymus is required for generation of T cell mediated immunity. This is dramatically illustrated by patients lacking a thymus, such as children with complete di-George Syndrome which is fatal if left untreated. Therapeutic options for such patients are limited and confined to transplantation of small fragments from allogeneic neonatal thymi. Following the concept that an autologous medical product would be advantageous for any condition in which thymic function is impaired, we set out to develop a preclinical strategy to generate functional human thymi from induced pluripotent stem cells (iPSC), as potential autologous stem cell source. Here we describe that human iPSC can be differentiated into induced thymic epithelial cells (iTEPC) following developmental stages that mimic normal development. This protocol is most robust when combined with directed differentiation enforced by lentiviral expression of FoxN1, the master regulator for thymic epithelial cells. When aggregated in organoids and transplanted in nude mice (that lack a thymus), these organoids supported the development of functional T cells with a broad TCR repertoire capable of cytokine production when stimulated via the T cell receptor. Thus, we provide proof-of-principle evidence that a combination of stem cell technology nad gene therapy can restore thymic function.

OR08

Self-assembly of human stem/progenitor cells creates neo-vascularized skin and skin organoids

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Stem/progenitor cells bear the potential to self-organize, creating organoids that resemble the organ functions in vitro. Here we established a humanized skin regeneration mouse model, based on self-assembly of adult as compared to iPSC-derived skin cell lineages forming neo-vascularized human skin. Adult endothelial cells (EC), skin fibroblasts (FB) and epidermal keratinocytes (KC) were propagated in 2D under xeno-free conditions. In addition, umbilical cord blood-derived iPSC were differentiated into iPS-EC,-FB, and–KC. Cell identity and purity were confirmed by flow cytometry and clonogenicity indicating their stem/progenitor potential. Skin organoid formation was performed to investigate cell self-organisation supported by human platelet-derived growth factors. Via life cell tracking sequential organoid assembly starting from stromal-vascular aggregation and followed by superficial anchorage of KC was revealed. Xeno-free human cell grafts, containing a mixture of KC, FB and EC in human platelet lysate (HPL) were transplanted onto full-thickness wounds of NSG mice using a transplant chamber to circumvent murine skin contraction. Two weeks after transplantation, histological analysis demonstrated appropriate cell organization into layered skin and a regular distribution of collagen fibers and ground substance. Immunohistochemistry confirmed the human origin of the grafts and a combination of murine and human neo-vasculature. Quantification showed significantly increased vessel numbers upon co-transplantation of EC compared to limited murine in-sprouting angiogenesis after transplantation of KC+FB only. The data show that self-assembly of human KC+FB combined with co-transplanted EC and HPL can create complex organoids in vitro and human neo-vascularized skin in vivo, building the basis for novel skin regeneration strategies.

OR09

Cell- and vector-engineering approaches for manufacturing high-titer GaLV pseudotyped lentiviral vectors from stable and constitutive producer cell lines

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Lentiviral vectors (LV) are currently the first choice for cell and gene therapy applications when long-term expression is required and have consolidated as a preferential vector in the context of

hematopoietic cells transduction. To cope with current and future demand of LV manufacturing, we have developed LentiPro26, stable and constitutive cell lines to support continuous production of LVs. LentiPro26 cells deliver competitive titers for amphotropic-pseudotyped vectors, but for Gibbon Ape Leukemia Virus (GaLV) envelope, preferable for hematopoietic cells transduction, obtaining higher titers is more challenging. Herein, we present two approaches to enable the manufacturing of high-titer LV GaLV-pseudotypes from stable and continuous producer cells lines. On the vector side, we created a panel of chimeric envelopes based on genetic modification of the cytoplasmic tail. The best envelope from our panel delivers transient titers near 10^7 TU/mL but also induced a strong cytotoxic phenotype. Thus, on the producer cell side, we abolished this phenotype by CRISPR-CAS knock-out of key cellular proteins. Genome-edited cells are capable of stably expressing the highest titer GaLV envelope with no evidence of envelope-induced cytotoxity. This work enables the use of constitutive packaging cell lines of GaLV LV pseudotypes, simultaneously featuring the competitive titres of transient production with the scalability, standardization and versatility of operation modes of stable and continuous production.

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OR10

Generation of an automated GMP-grade protocol in a closed system for the expansion of polyclonal memory γδ-T cells for a "third party" cell bank

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γδ-T cells are cells of the immune system with properties of both innate and adaptive compartment. Their powerful cytotoxic activity against bacteria, virus, tumours, together with their ability to recognize antigens in an HLA-independent manner and their negligible alloreactivity, make them attractive for clinical translation, especially in the perspective of a ''third-party'' T-cell bank. Unfortunately, γδ-T cells represent only a small lymphocyte population and therefore require in vitro expansion for clinical application. In this study, we developed a protocol to manually and automatically expand large numbers of polyclonal γδ-T memory cells, with the possibility of genetic modification to improve their anti-tumour activity. Artificial antigen presenting cells (aAPC) expressing CD86/41BBL/CD40L and the cytomegalovirus-pp65 antigen were used to induce expansion of γδ-T cells. To implement safety, aAPCs have been further modified with the inducible Caspase-9 suicide gene. γδ-T cells expanded 240 ± 109 times (day +21), expressing activation and memory markers maintaining a polyclonal phenotype (predominantly Vδ1). The extensive anti-tumour activity of this population was demonstrated in vitro with different tumour cell lines and leukaemic blasts ($p < 0{,}01$) and in vivo in a xenograft leukemia mouse model when compared to polyclonal αβ-T cells. In none of the cases, alloreactivity was observed. Phosphoproteomic and gene-expression studies reveal features between expanded and activated αβ and γδ-T (ability to operate as APCs, metabolism, pathways activated upon stimulation, phenotype, migration). The automated protocol for the isolation and expansion has been optimized and validated in the Clinimacs Prodigy closed-system, which maintains all the features obtained in the manual process.

OR11

Modelling skeletal muscle laminopathies with human iPS cells and bio-engineered skeletal muscles: Prospects for genetic therapies

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Laminopathies are severe genetic diseases caused by mutations in the LMNA gene, which encodes A-type lamins. Together with B-type lamins, they assemble into a mesh-like structure located beneath the nuclear membrane, providing structural stability and regulating gene expression. Laminopathies affect various cell types in a systemic or tissue specific manner, with the latter including striated muscle laminopathies. Although different mechanisms have been proposed, the precise pathophysiology of laminopathies remains unknown; additionally, therapy development is hindered by their rarity and lack of easily accessible cell types for ex vivo studies. To overcome these hurdles, we used induced pluripotent stem (iPS) cells from patients with skeletal muscle laminopathies such as LMNA-related congenital muscular dystrophy, limb-girdle muscular dystrophy 1B and Emery-Dreifuss muscular dystrophy (type 2 and 3), to model disease-associated phenotypes in vitro. iPS cells from four patients were differentiated into skeletal myogenic cells and myotubes. Characteristic pathological hallmarks, including nuclear shape abnormalities and mislocalisation of nuclear lamina proteins, were observed in LMNA-mutant iPS cell derivatives in proliferation and in differentiation. Notably, modelling in three-dimensional (3D) artificial muscle constructs resulted in higher fidelity recapitulation of nuclear shape abnormalities than in standard monolayer cultures, and identified nuclear length as a reproducible, mutation-specific phenotypic readout. Finally, we will present and discuss current efforts and future applications of this novel iPS cell-based platform to develop genetic therapies for laminopathies and other severe muscle disorders, including viral and non-viral strategies such as LMNA exon-skipping and gene editing.

OR12

Decoy-based gene therapy for Myotonic Dystrophy

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Myotonic dystrophy (DM) is an autosomal neuromuscular disease encompassing two distinct forms, DM1 and DM2, caused by abnormal microsatellite expansions of C(C)TG repeats in the non-coding regions of DMPK and ZNF9 genes, respectively. Mutant RNAs carrying expanded repeats are retained in the nucleus that abnormally sequester MBNL RNA-binding factors hampering their normal function in the regulation of alternative splicing events. Thus, several splicing changes in DM patients have been associated with clinical symptoms such as myotonia, muscle weakness and cognitive defects. Although various therapeutic approaches for DM are under development, to date there is no effective therapy available. Herein, we report a novel gene therapy strategy with the use of an engineered MBNLΔ RNA-binding protein that acts as a CUGexp-decoy to release sequestered endogenous MBNL factors and restore their proper functions. In vitro, the decoy interferes with CUG-expanded transcripts and normalizes splicing abnormalities. An RNAseq analysis confirmed that the vast majority of missplicing events are no more significantly altered in DM1-treated cells, indicating an almost complete recovery of MBNL1 activity. Further in vivo analysis showed that a single intramuscular injection of AAV-MBNLΔ vectors in a mouse model of the disease results in a complete and durable normalization of splicing misregulation up to one year. Moreover, systemic delivery of the decoy using AAV9 vectors improves hallmarks of the disease including abolition of the myotonia and correction of splicing defects. In conclusion, our results support this innovative decoy-based gene therapy approach as an alternate or complementary therapeutic intervention for DM treatment.

OR13

Shortened *ex vivo* transduction for hematopoietic stem cell gene therapy of Hurler disease: impact on hematopoietic reconstitution potential

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San Raffaele Telethon Institute for Gene Therapy (SR-TIGET) has recently started a phase I/II study on transplantation of ex vivo genetically-modified autologous CD34+ hematopoietic stem and progenitor cells (HSPC) in patients with Hurler disease (NCT03488394), implementing for the first time a shortened lentiviral transduction protocol containing prostaglandin E2. Preliminary data from the first five patients treated show rapid hematologic recovery following fludarabin/busulfan-based myeloablative conditioning: the median duration of grade 4 neutropenia and thrombocytopenia was 17 and 0 days, respectively. Median duration of neutropenia (<100 cells/mcl) was 6 days. The drug product (DP) and non-cultured (NC) CD34+ cells from the first 3 patients were xenografted into mice. With the exception of the 4 week time-point where human CD45+ engraftment in peripheral blood was reduced by 50% in DP compared to NC, there were no significant differences between groups during the follow up until 24 weeks, including secondary transplantation. This data-set confirms that the shortened ex vivo transduction protocol preserves HSPC function. Vector integration site analysis on the first patient's DP graft in primary and secondary mice is ongoing and will allow to compare graft clonality with the reference database. To further characterize the impact of ex vivo culture, we performed longitudinal single cell RNA sequencing on CD34+CD90+ cell fractions. Cells were cultured for up to 1 week, showing metabolic changes and stress responses after prolonged culture, which may impact engraftment potential. These results are anticipated to help improve the ex vivo manufacturing process.

OR14

The SUNRISE-PD Study, a clinical trial of AXO-LENTI-PD: a CNS-directed gene therapy for the treatment of Parkinson's Disease

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Parkinson's disease (PD) is caused in part, by the progressive degeneration of dopaminergic neurons in the substantia nigra. The standard of care, L-dopa, is highly efficacious but long-term use is complicated by motor fluctuations from intermittent stimulation of dopamine receptors and off-target effects. Therefore, a therapy that provides a continuous supply of dopamine to the area of greatest loss in PD, namely the putamen, offers the potential for reduced motor fluctuations and off-target effects. AXO-Lenti-PD is a novel gene therapy that delivers three genes critical for de novo dopamine biosynthesis, to the putamen, using a high-capacity lentiviral vector. Transduced neuronal cells produce continuous dopamine from endogenous tyrosine in the striatum. The first-generation construct of this product (ProSavin®) was found to be well-tolerated with all patients displaying some improvement in the UPDRS part III OFF score, which was sustained in some patients up to six years. To further increase the potency of this construct, the second-generation product, AXO-Lenti-PD, was developed, utilizing the same genes but in a different configuration allowing for increased dopamine production per genetically modified cell. AXO-Lenti-PD is being investigated in the two-part SUNRISE-PD study, comprised of a dose-ranging phase to confirm the optimal therapeutic dose, followed by a sham-controlled trial to assess the safety and efficacy of the optimal dose from the first part of the study. Based on data from the lowest dose cohort, AXO-Lenti-PD was observed to be generally well-tolerated, and the data suggests it may have greater efficacy compared to the highest dose of ProSavin®.

OR15

In vivo generated human CAR T cells eradicate B cell leukemia in preclinical mouse models

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Chimeric antigen receptors (CAR) T cells have shown significant clinical benefits to patients with B-cell malignancies. However, production of CAR T cells requires extensive and time-consuming procedures of cell isolation, sorting, transduction and in vitro expansion of T cells. We investigate if CAR T cell

production can be radically simplified by transferring the CAR gene selectively into particular lymphocytes directly in vivo using receptor-targeted lentiviral vectors (LV). We have previously demonstrated that human CD19-CAR T cells can be generated in vivo in huPBMCs engrafted NSG mice as well as CD34+ humanized mice using CD8-LV which specifically targets human CD8+ cells (Pfeiffer et al., 2018). Such in vivo generated CD8+ CAR T cell eliminated CD19+ B lymphocytes in the vector-injected mice. To investigate their anti-tumoral activity, NSG mice were i.v. injected with CD19+ luciferase-encoding Nalm-6 cells. Once the Nalm-6 leukemia was systemically established the mice received human PBMC followed by a single injection of CD8-LV delivering the CD19-CAR. Continuous in vivo imaging revealed substantially reduced luciferase activities in vector-administered mice. Notably, about 7 - 9% of CD8+ cells isolated from the bone marrow of vector-treated mice were CAR+. These had completely eliminated the Nalm-6 cells in bone marrow and in spleen whereas in the control animals high levels of CD19+ cells were detected. This is the first demonstration of a potent functional activity of in vivo generated CAR T cell to control and eliminate tumor cells.

OR16

Trastuzumab derived HER2-specific CAR for trastuzumab-resistant breast cancer: CAR T-cells successfully engage target epitopes that are not accessible to antibodies

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Targeting HER2 by monoclonal antibodies improves the outcome for advanced breast cancer patients, however, therapy resistance is frequent. One important mechanism by which resistance to targeted antibody therapy may develop is epitope masking and steric hindrance through various cell surface and extracellular matrix components such as sialomucins, or the CD44/Hyaluronan complex present in the tumor. In an attempt to overcome this physical barrier, we have created actively moving primary human T-cells with a CD28-zeta chimeric antigen receptor that targets HER2 using a trastuzumab-derived scFv. HER2-CAR T-cell activation was verified by ELISA and cytotoxicity assays using the HER2 positive MDA-HER2 and JIMT-1 cell lines as targets. In co-culture assays, either saturating doses of trastuzumab combined with NK-92 cells or HER2-specific CAR T-cells equally well recognized and killed HER2-positive cell monolayers. Next, we generated JIMT-1 spheroids to compare their effector functions in 3D cultures where cells have established an extracellular matrix. We found that only CART-cells penetrated all the way into the core region of tumor spheroids and exhibited cytotoxic activity there. Coherent with this, combined long-term treatment with trastuzumab plus NK-92 cells only temporarily retarded the growth, but did not induce the regression of clinically trastuzumab-resistant breast cancer xenografts in NSG mice, however, a single dose of HER2-specific CAR T-cells eradicated the tumors and consequentially lead to long-term overall survival. In summary, we show here that actively moving CAR T-lymphocytes successfully combat tumor cells through target epitopes that are otherwise not accessible to passively diffusing antibodies owed to a well-developed ECM.

OR17

Semi-rational engineering of next-generation AAV capsids for muscle gene therapy

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Monogenic muscular diseases such as myotubular myopathy or Pompe disease are life-threatening disorders for which effective treatment options are lacking. Previously, using DNA barcoding and next-generation sequencing, our lab has identified a myotropic peptide (P1: RGDLGLS) whose display retargets systemically delivered AAV9 to skeletal muscle, heart and diaphragm. Concurrent in vivo screening of shuffled AAV capsid libraries in the musculature of mice had resulted in an enrichment of AAV9 sequences in the capsid's C-terminus. Strikingly, insertion of P1 into selected shuffled capsids yielded two variants that surpass all parental AAVs in terms of efficiency and specificity in the muscle. Here, we show that these features are maintained across different mouse strains, except for NMRI mice that exhibit a unique profile in the heart. Moreover, using differential scanning fluorimetry, we found that P1 insertion lowers the thermostability of the capsid scaffold, which may contribute to its superior efficiency. Replacement of P1 with another peptide that also alters physical capsid properties abolished the myotropic phenotype, underscoring the complex function of the P1 peptide. To further dissect its biology, we created an AAV9 library displaying a partially randomized sequence that preserves the RGD motif from P1, based on its integrin binding ability. Interestingly, three rounds of in vivo selection in mice led to the emergence of distinct peptide sequences that share three additional amino acids with P1, next to RGD. Collectively, our data illustrate the power of combining multiple technologies for AAV evolution and characterization as a means to semi-rationally engineer next-generation AAVs.

OR18

Systemic gene transfer with AAVrh74.MHCK7.SGCB increased β-sarcoglycan expression in patients with limb girdle muscular dystrophy type 2E

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Limb girdle muscular dystrophy type 2E (LGMD2E; due to β-sarcoglycan [SGCB] deficiency) manifests with muscle weakness, cardiac involvement, and elevated creatine kinase (CK). We present initial phase 1 findings of AAVrh74.MHCK7.SGCB gene transfer in LGMD2E. This is an ongoing, multiple ascending-dose study in patients with LGMD2E (NCT03652259). Eligible patients were aged 4–15y, with confirmed SGCB mutation (both alleles), negative for anti-AAVrh74, and >40%n on 100-meter walk test. Patients were administered single IV infusion of 5x10^13 vg/kg AAVrh74.MHCK7.SGCB (AAVrh74-mediated therapy; human SGCB gene driven by muscle-specific promoter, MHCK7). Prednisone 1 mg/kg/day was initiated 1 day before study drug, tapering after 30d. Primary endpoints were ≥20% SGCB-positive fibres and safety. Secondary endpoints were CK and functional endpoints. For the first 3 patients (13y, n = 2; 4y; n = 1), robust SGCB expression was observed by immunohistochemistry (IHC),

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with a mean of 51% SGCB positive fibres (range 42–63%) expressing a mean 47% intensity (range 38–57%). Co-localization of α-sarcoglycan was observed by IHC. Western blot showed a mean 36.1% SCGB expression vs normal (range 34–39%). Mean CK levels were reduced by 90% (range 83–97%), suggesting slowed muscle destruction. Two patients had elevated liver enzymes following steroid taper, which returned to baseline. Two patients had transient mild nausea with increased steroid dosing. No other clinically significant lab findings were observed. Gene transfer in patients with LGMD2E following AAVrh74. MHCK7.SGCB infusion appears promising. This is the second gene therapy inducing protein production post-transgene delivery with AAVrh74 vector and MHCK7 promoter, demonstrating potential benefits of a rationally designed delivery system.

OR19

GSE4, a Dyskerin derived peptide enhances pneumocyte growth, reduces apoptosis, DNA damage and lung fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a lethal lung fibrotic disease, with a mean survival of 2–5 years and no curative treatment. Telomere shortening occurs both in sporadic and familiar forms of the disease. The GSE4 peptide is able to rescue cells with telomerase defects from senescence, DNA damage and induce telomerase activity. Here we have investigated the effect of GSE4 expression in vitro in rat alveolar epithelial cells (AECs), and in vivo in a bleomycin model of lung fibrosis. Bleomycin Injured rat AECs expressing GSE4 or treated with GSE4-nanoparticles showed a rescue of telomerase activity, decreased DNA damage, and decreased expression of IL6 and cleaved-caspase 3. In addition, these cells also showed an inhibition of expression of fibrotic markers induced by TGF-β, such as collagen-I and III. Furthermore, treatment with GSE-loaded nanoparticles in a rat model of bleomycin induced fibrosis, increased telomerase activity and decreased DNA damage in proSP-C cells. Both in a GSE4 preventive and therapeutic protocols the peptide prevented and attenuated lung damage monitored by SPECT-CT and inhibited collagen deposition. Lungs of rats treated with bleomycin and GSE4-PLGA/PEI-nanoparticles showed lower expression of α-SMA, increased number of pro-SPC multicellular structures and increased DNA synthesis in proSP-C cells, indicating therapeutic efficacy of GSE4 in experimental lung fibrosis.

OR20

Cardioprotective effect of miRNAs derived from mesenchymal stem cells extracellular vesicles in doxorubicin-induced damage

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Doxorubicin is an anthracycline effective against several types of cancer, but its use is limited due to cardiotoxocity. Nowadays there is no effective treatment to avoid the mentioned cardiac damage. Mesenchymal stromal cells (MSCs) derived extracellular vesicles (EVs) are an excellent candidate to be used as next generation therapy since they showed excellent results in different preclinical models of heart diseases. In this regard, EVs contain cytokines, signalling molecules and different miRNAs, small chains of nucleotides able to regulate the expression of a wide variety of protein in the cells. In this work, we isolated EVs from MSCs and observed that they reduce oxidative stress and senescence trigger by doxorubicin in cardiac cells. In addition, MSC derived EVs partially reverted fibrosis induced by doxorubcin and recovered angiogenesis of coronary microvasculature. As mentioned, EVs deliver miRNAs to target cells. In this regard we identified miRNAs related to cardiatoxicity in MSC derived EVs. Moreover, transfecting these miRNAs individually or in combination in cardiac cells increased their viability and decreased their oxidative stress when treated with doxorubicin. In conclusion, we showed that EVs secreted by MSCs have beneficial effect on doxorubicin treated cardiomyocytes and that the miRNAs carried by these vesicles plays a key role in this effect. This piece of work indicates that EVs enriched in miRNAs could be an effective treatment for doxorubicin damaged heart and opens the door to design synthetic EVs loaded with a combination of therapeutic miRNAs. ACIF/2017/318. FEDER co-financing ''una manera de hacer Europa'' HeCaToS CP-IP 602156-1.

OR21

Intravitreal gene therapy with ADVM-022 for neovascular age-related macular degeneration (phase 1 OPTIC Trial)

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ADVM-022 is a gene therapy product that utilises a proprietary vector capsid (AAV.7m8) carrying an aflibercept coding sequence under control of a proprietary expression cassette. Nonhuman primate studies have demonstrated that ADVM-022 can sustain therapeutic levels of aflibercept expression in the vitreous and aqueous humor 30 months after intravitreal injection. The OPTIC Trial is a phase 1, multicohort, open-label, multicenter study designed to assess the safety, tolerability, functional and anatomic outcomes of a single intravitreal injection of ADVM-022 in patients with neovascular age-related macular degeneration (nAMD) who have previously demonstrated response to anti-VEGF treatment and required frequent injections to control nAMD disease activity. The primary outcome measure for this study is type, severity and incidence of ocular and systemic adverse events. Secondary outcomes include change in best-corrected visual acuity (BCVA), change in central retinal thickness (CST) and number of rescue anti-VEGF treatments received during the study. Primary and secondary outcomes data through 24 weeks from the first

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cohort will be presented (n = 6). Initial findings showed no dose-limiting toxicities and an initial robust anatomic response following administration of ADVM-022 at 6 x 10^11 vg/eye. ADVM-022 is designed to provide sustained therapeutic levels of aflibercept with a single intravitreal injection and has the potential to minimize treatment burden, improve anatomical disease control and real-world vision outcomes for patients with nAMD.

OR22

Phase 2/3 trial to assess the safety and efficacy of Lenti-D autologous haematopoietic stem cell gene therapy for cerebral adrenoleukodystrophy

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Cerebral adrenoleukodystrophy (CALD), a rare, X-linked, metabolic disorder, is characterised by rapidly progressive inflammatory cerebral demyelination leading to irreversible loss of neurologic function and death. Early diagnosis and treatment are key in ensuring optimal long-term outcomes. Lenti-D Drug Product (DP) is an investigational autologous haematopoietic stem cell gene therapy for the treatment of CALD. In an open-label phase 2/3 study (ALD-102), boys (≤17 years) with early CALD were fully myeloablated with busulfan and cyclophosphamide prior to infusion of autologous CD34+ cells transduced with the Lenti-D lentiviral vector. The primary efficacy endpoint is the proportion of patients who are alive and free of major functional disabilities (MFD) at Month 24. As of April 2019, the trial was fully enrolled with 32 patients having received Lenti-D DP (median follow-up 21.2 months, min-max, 0.0–60.2). Fifteen patients have completed 24 months of follow-up; 14 patients remain in ALD-102. Two patients were withdrawn and referred for allo-HSCT before their Month 24 visit; another experienced rapid disease progression resulting in MFDs and death. All other Lenti-D DP-treated patients generally showed evidence of neurologic function stabilisation at their last follow-up. Of patients with evaluable data at Month 24, 15/17 (88%) were alive and MFD-free. To date, there have been no reports of graft failure, GVHD, or transplant-related mortality; recorded adverse events were consistent with myeloablative conditioning. There is no evidence of replication competent lentivirus or insertional oncogenesis. These data suggest that Lenti-D DP stabilises neurologic disease progression and appears to be a promising gene therapy for CALD.

OR23

Targeting pre-existing anti-transgene T cell response for effective gene therapy of Mucopolysaccharidosis type-I

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Mucopolysaccharidosis type-I (MPS-I) is caused by the deficiency of the alpha-L-iduronidase (IDUA) enzyme, that results in glycosaminoglycan accumulation in tissues. The available treatments are enzyme-replacement therapy (ERT) and allogeneic hematopoietic stem cell (HSC) transplantation. An alternative therapeutic option is ex vivo hematopoietic stem cell (HSC) gene therapy and preclinical studies performed in mice demonstrated the efficacy of this approach based on lentiviral vectors in the absence of pre-existing anti-IDUA immunity. However, the clinical efficacy of ex vivo HSC gene therapy can be compromised by pre-existing anti-IDUA immunity, developed by MPS-I patients as a consequence of ERT. To study the impact of pre-existing anti-IDUA immunity on gene corrected HSC engraftment in enzyme pre-treated and immunized mice, we optimize an artificial immunization protocol in MPS-I mice to mimic the effect of ERT in patients. We demonstrate that engraftment of IDUA-corrected HSCs is impaired in pre-immunized MPS-I mice and that the rejection of transplanted cells is mediated by IDUA-specific CD8+ T cells and not by humoral immunity. The selective depletion of IDUA-specific CD8+ T cells allows engraftment of IDUA-corrected HSCs in immunized MPS-I mice. Overall, these data demonstrate, for the first time, the relevance of pre-existing anti-transgene immunity on ex vivo HSC gene therapy for MPS-I and suggest the application of tailored immune-depleting treatments, as well as a deeper immunological characterization of patients, to safeguard the therapeutic effects of ex vivo HSC gene therapy in immune-competent hosts.

OR24

Recombinant AAV-mediated gene transfer to the skeletal muscle is associated to immune modulation of transgene expression in the macaque model

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Recombinant adeno-associated virus (rAAV) provides a clinically relevant platform for efficient and sustained gene therapy. However, preclinical gene transfer studies in large animal models were associated to immune rejection of the transgene product, in particular following intramuscular (IM) vector delivery. In past

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studies, it was generally admitted that the loss of transgene expression is irreversible because of a total cytotoxic elimination of transduced cells. Following IM delivery of a rAAV expressing an immunogenic transgene in the nonhuman primate model, we report here that transgene loss can be only transitory. Despite the detection of humoral and cellular immune responses against the transgene product and the presence of cells infiltrates in the muscle, we were able to recover gene expression after an initial transient loss. Functional viral genomes (vg) were detected until at least five years following gene transfer. Analysis of muscular cell infiltrates revealed the presence of B cells, CD8 T cells and T cells among which FoxP3 expressing T regulatory cells. In conclusion, our data highlight non-conventional host immunity leading to transgene expression immune modulation following IM rAAV-mediated gene transfer. A better characterization of the underlying mechanisms of transient transgene silencing will contribute to more optimal clinical translation of rAAV gene therapy products.

OR25

Lentiviral-mediated Phenotypic Correction of CD34+ Cells from RPS-19-deficient Diamond-Blackfan Anemia Patients

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Allogenic hematopoietic stem cell transplantation (HSCT) currently represents the unique curative treatment for the bone marrow failure (BMF) of DBA patients. Aiming at developing a gene therapy approach for these patients, we have first investigated the HSC content in their BM. Compared to Fanconi anemia (FA) patients, significantly higher numbers of CD34+ cells were observed in the BM of age-matched DBA patients, suggesting that collection of HSCs should not constitute a major limitation in DBA gene therapy. With the aim of correcting the phenotype of RPS-19 deficient HSCs, clinically applicable lentiviral vectors (LV) carrying a codon-optimized version of RPS19 driven by the PGK or the EF1α promoters, were constructed. Studies carried out in K562 cells interfered with anti-RPS19 LVs showed that complementation with either the therapeutic LVs restored the expression of RPS19 and reverted defects in ribosomal biogenesis. Furthermore, transduction of primary CD34+ cells from RPS-19 deficient patients with therapeutic LVs increased the number of hematopoietic colonies as compared to a control group that was transduced with non-therapeutic EGFP-LVs. Moreover, therapeutic LVs reverted the red blood cell differentiation defect characteristic of DBA cells, and preserved the repopulating potential of corrected HSC cells in immunodeficient NSG. Our preclinical studies support that gene therapy should constitute a suitable approach for the treatment of the BMF characteristic of DBA patients.

OR26

Competitive sgRNA screen identifies Mapk14 as a druggable target to improve HSPC engraftment in a proinflammatory environment

ABSTRACT WITHDRAWN

OR27

Interim results from the phase 3 Northstar-2 and Northstar-3 studies of LentiGlobin gene therapy for the treatment of transfusion-dependent β-thalassaemia

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LentiGlobin gene therapy for transfusion-dependent β-thalassaemia (TDT) contains autologous CD34+ cells transduced *ex vivo* with the BB305 lentiviral vector (LVV) encoding β-globin with a T87Q substitution. Phase 1/2 study demonstrated initial safety/efficacy; polyclonal integration in all 18 patients with up to 4.5 years follow-up. Herein are results from the ongoing Phase 3 studies using a refined manufacturing process, Northstar-2 (NCT02906202; non-β^0/β^0 genotypes) and Northstar-3 (NCT03207009; β^0 or β^+ IVS-I-110 mutation at both HBB alleles).

Following G-CSF/plerixafor mobilisation and apheresis, CD34+ cells were transduced with BB305 LVV and infused into patients after busulfan myeloablation. Statistics represent median (min-max).

In Northstar-2 and Northstar-3, 20 and 11 patients were treated as of 13 December 2018 and 12 April 2019 with 8.1(0.5–22.2) and 5.4(1.5–15.9) months follow-up, respectively. Drug product vector copy number in Northstar-2 and Northstar-3 was 3.2(1.9–5.6) and 2.5(1.2–4.3) copies/diploid genome, respectively. All patients with >1 and >5 months follow-up achieved neutrophil and platelet engraftment, respectively.

In Northstar-2, 13/14 patients with ≥3 months follow-up have been transfusion-free for >3 months with haemoglobin (Hb) of 12.2(8.8–13.3) g/dL at last visit. HbA^{T87Q} at Month 6 and 12 was 9.5 (n = 10) and 9.3 g/dL (n = 7), respectively. In Northstar-3, 5/9 patients with ≥3 months follow-up have been transfusion-free for ≥3 months. Total Hb at was 7.8–13.8 g/dL with 59–91% contribution from HbA^{T87Q} (n = 7), at last Hb fraction assessment.

Non-haematologic grade ≥3 adverse events post-infusion (≥3 patients) included stomatitis, febrile neutropenia, epistaxis, pyrexia, and veno-occlusive liver disease. No replication-competent lentivirus or clonal dominance reported.

OR28

Haematopoietic reconstitution dynamics of MPB- and BM-derived Haematopoietic Stem/Progenitor Cells in Wiskott-Aldrich Syndrome patients treated with lentiviral gene therapy

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Haematopoietic Stem/Progenitor Cells (HSPC) gene therapy (GT) is based on infusion of genetically-modified autologous HSPC obtained from bone marrow (BM) or mobilised peripheral blood (MPB). In haematopoietic stem cell transplantation (HSCT), MPB is usually preferred due to higher HSPC yields and faster haematopoietic reconstitution in the recipient. Here, we present an exploratory analysis on the reconstitution kinetics and lineage output of these two sources, up to 3 years after GT, in 14 Wiskott-Aldrich syndrome patients treated with lentiviral vector transduced BM (n = 5) and/or MPB (n = 8) HSPC; 1 patient received genetically-modified BM+MPB HSPC. The groups were similar in the infused product vector copy number (VCN), CD34+ cell dose and transduction level. Deep-phenotyping of CD34+ cell composition before transduction revealed the presence of primitive haematopoietic stem cells with similar phenotypic characteristics in both sources and higher amounts of primitive and myeloid-committed progenitors in MPB compared with BM HSPC. After GT, all patients show stable engraftment of transduced cells. In MPB-GT we observed higher median percentage of transduced colonies, myeloid VCN and integration site numbers compared to BM-GT. The higher content of more committed progenitors in MPB HSPC may explain the rapid neutrophil engraftment and platelet transfusion independence obtained with MPB-GT, in line with the observations in HSCT. Importantly, similar myeloid and lymphoid reconstitution was observed from 1-year post-GT suggesting that both BM-GT and MPB-GT have similar long-term repopulating properties. This study will provide fundamental information on the contribution of distinct sources of HSPC to short-term and long-term engineered haematopoiesis.

OR29

Allele-specific gene disruption through discrimination of a single base change by S. aureus Cas9-KKH prevents progressive hearing loss after AAV-mediated gene delivery

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Since most dominant human mutations are single nucleotide substitutions, we explored gene editing strategies to disrupt dominant mutations efficiently and selectively without affecting wild-type alleles. Single nucleotide discrimination can be difficult to achieve because commonly used endonucleases, such as Streptococcus pyogenes Cas9 (SpCas9), can tolerate up to seven mismatches between guide RNA (gRNA) and target DNA. Furthermore, the protospacer-adjacent motif (PAM) in some Cas9 enzymes can tolerate mismatches with the target DNA. To circumvent these limitations, we screened 14 Cas9/gRNA combinations for specific and efficient disruption of a nucleotide substitution that causes the dominant progressive hearing loss, DFNA36. As a model for DFNA36, we used Beethoven mice, which harbor a point mutation in Tmc1, a gene required for hearing that encodes a pore-forming subunit of mechanosensory transduction channels in inner-ear hair cells. We identified a PAM variant of Staphylococcus aureus Cas9 (SaCas9-KKH) that selectively and efficiently disrupted the mutant allele, but not the wild-type Tmc1/TMC1 allele, in Beethoven mice and in a DFNA36 human cell line. In heterozygous Bth mice, injection of AAV2/Anc80-CMV-SaCas9-KKH-U6-gRNABth led to robust improvement of hearing thresholds, with some animals exhibiting near normal hearing sensitivity (in contrast to uninjected

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animals, which have severe-to-profound hearing loss) up to 1 year post injection. Analysis of current ClinVar entries revealed that ~21% of dominant human mutations could be targeted using a similar approach.

OR30

Intrathecal AAV9-GALC corrects both central and peripheral nervous system disease in canine Krabbe disease in a dose and time-dependent manner

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Globoid cell leukodystrophy (GLD, Krabbe disease) is caused by deficient activity of galactosylceramidase (GALC), which degrades myelin lipids galactosylceramide and galactosylsphingosine (psychosine). Accumulation of cytotoxic psychosine results in widespread central and peripheral nervous system (CNS, PNS) demyelination and death often by two years. In this study of 26 GLD dogs, intrathecal delivery at the cerebellomedullary cistern of high dose (1E14 vg) AAV9 encoding canine GALC into pre-symptomatic GLD dogs completely ameliorates neurologic disease up to 98 weeks of age, >6 times that of untreated GLD dogs, with all 6 treated dogs ongoing beyond 1 year of age. Treated dogs demonstrate normalized nerve conduction velocity, stabilization of MRI, and significant reduction of CSF psychosine and protein. Post-mortem evaluation revealed significant increase in GALC enzyme activity, reduction of psychosine levels, and increase in vector genome copies in the CNS and PNS. Histological analysis demonstrated improved myelination, global expression of GALC, and attenuation of neuroinflammation. Reducing the dose by 20% resulted in doubling of lifespan to ~30 weeks of age; however, an attenuated form of GLD developed including cognitive dysfunction and blindness. Administering high dose at a symptomatic age significantly extended lifespan and delayed disease progression, with one dog still alive beyond 1 year of age. The low dose given at a symptomatic age provided no benefit. Intrathecal delivery of AAV9-cGALC is having unprecedented therapeutic effect in canine GLD; however, narrow dosing and timing windows have been identified and should guide translation of this promising therapy into children with Krabbe disease.

OR31

Widespread, dose-dependent and durable huntingtin lowering in Huntington's disease-relevant brain regions of large mammals with intraparenchymal administration of the AAV gene therapy VY-HTT01

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Huntington's disease (HD) is a fatal, autosomal dominant neurodegenerative disorder characterized by progressive motor, cognitive and neuropsychiatric impairment. There is a high unmet medical need for a treatment to delay the onset or slow the progression of HD. HD is caused by a genetic mutation in the huntingtin (htt) gene in which an expanded cytosine-adenine-guanine (CAG) trinucleotide repeat sequence of more than 36 repeats results in a pathologic protein with a polyglutamine expansion in its N-terminus. The mutant huntingtin protein exhibits toxic gain-of-function which leads to neuronal dysfunction and death, especially in the striatum and cortex. Partial suppression of HTT in the brain has been demonstrated to be both safe and effective in multiple animal models of HD, providing proof-of-concept for an HTT lowering therapeutic strategy. VY-HTT01 is a potent AAV gene therapy encoding a primary miRNA targeting human HTT mRNA selectively for knockdown. Here, we update our previously reported results on VY-HTT01 biodistribution and pharmacology in nonhuman primates (NHPs). MRI-guided convection-enhanced delivery of VY-HTT01 into the putamen and thalamus of NHP resulted in widespread biodistribution of VY-HTT01 vector genomes and mature HTT miRNA, and dose-dependent, robust and durable suppression of HTT mRNA and protein in multiple brain regions that are highly relevant to the HD, including the cortex, caudate and putamen. These results demonstrate the potential of the AAV gene therapy VY-HTT01, with a one-time administration into the putamen and thalamus, for the treatment of Huntington's disease.

OR32

Intrathecal administration of AVXS-301 for amyotrophic lateral sclerosis (ALS): survival extension and SOD1 reduction in mice and nonhuman primates

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ALS is a fatal neurodegenerative disease affecting motor neurons. Twenty percent of genetically-associated ALS cases are linked to superoxide dismutase-1 (SOD1) gene mutations. Previously, adeno-associated virus serotype 9 (AAV9)-mediated delivery of small hairpin RNA (shRNA) targeting human SOD1 produced efficient downregulation in mice and nonhuman primates (NHPs). A single administration of the recombinant vector AAV9-GFP-shRNA-SOD1 extended survival in newborn ALS mice and also at later stages after disease onset. To move toward clinical trials, this vector was modified using an expression cassette for the delivery of shRNA against human SOD1 devoid of foreign transgenes (GFP) but maintaining the same cassette size. The vector was packaged into an AAV9 viral capsid, creating AAV9-SOD1-shRNA (''AVXS-301'' herein). A one-time intracerebroventricular administration of AVXS-301 improved motor function and prolonged survival in the SOD1G93A ALS mouse model overexpressing human mutated SOD1. Brain and spinal cord tissue analysis revealed a significant, dose-dependent knockdown of SOD1 at the RNA transcript and protein levels, indicating the central nervous system (CNS) was successfully targeted. To facilitate dose extrapolation to human patients, we tested this strategy in NHPs including 3–4-year-old cynomolgus macaques and a 10-year-old rhesus macaque. A single lumbar intrathecal administration of AVXS-301 achieved efficient transduction and SOD1 downregulation throughout the CNS, and showed a direct relationship between dose and identified vector genomes in CNS tissue. Furthermore, we observed up to 93% reduction in SOD1 RNA transcript in spinal motor neurons. These results represent an important advance toward clinical trials for ALS patients.

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OR33

Human microRNAs selected by high-throughput screenings enhance CRISPR/Cas9-based homologous recombination in post-mitotic hearts

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The advent of the Crispr/Cas9 technology offers exiting new perspectives for the simple and precise gene correction of hereditary mutations through Homologous Directed Repair (HDR). However, in mammals, this process is largely inefficient, particularly in adult post-mitotic tissues, such as the heart. The main goal of this project was to identify genetic treatments that enhance frequency of CRISPR/Cas9-induced HDR. By robotic high-throughput, high-content microscopy we systematically screened a library of 2024 human microRNAs to search for regulators of HDR-mediated gene correction. We identified 21 miRNAs that significantly increased CRISPR/Cas9-induced HDR events. Interestingly, 10 of top identified miRNAs belonged to only two miRNAs families sharing the same seed sequence. A common and distinctive feature of these miRNAs was to induce expression of key proteins of the HDR pathway, including MRE11, NBS1, RAD50 and RAD51. Another highly effective miRNA, not belonging to either of these two families, regulated expression of p38-beta, suggesting involvement of this MAPK in the HDR process. In mice, the selected miRNAs markedly enhanced CRISPR-induced, AAV-based homologous in frame insertion of a promoterless GFP into the last exon of the myosin regulatory light chain 2 (Myl2) gene both ex vivo and in neonatal and adult hearts in vivo. Together, these results support the therapeutic potential of the transient treatment with miRNAs enhancing HDR together with the use of pro-recombinogenic AAVs for gene-editing of the heart and of other post-mitotic tissues.

OR34

Broad applicability of NHEJ-mediated gene editing to correct mutations in a variety of Fanconi Anemia genes

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Gene editing constitutes a new strategy for the precise gene therapy of hematological disorders. While Homology Directed Repair (HDR) is limited to dividing cells, Non-Homologous End-Joining (NHEJ) constitutes the preferred repair mechanism in quiescent cells, such as hematopoietic stem cells (HSCs). Additionally, since Fanconi Anemia (FA) cells are characterized by defects in HDR, we aimed at exploiting the NHEJ to remove/compensate FANCA mutations by the use of CRISPR/Cas9 system, thus mimicking genetic reversions described in FA mosaic patients. Using this strategy we demonstrated the feasibility to generate therapeutic insertions and deletions (indels) that induced the re-expression of FANCA protein and the phenotypic correction of FA-A cells. Moving towards the clinical application of NHEJ-mediated gene editing in FA, we pursued the correction of mutations described in different FA genes. The generation of specific indels in lymphoblastic cells lines (LCLs) from four different FA complementation groups promoted efficient therapeutic editing events (up to 31%) that conferred a marked proliferative advantage and reversion of the characteristic mitomycin C sensitivity of FA cells. To increase the editing efficiency in LCLs and primary HSCs, chemically modified synthetic sgRNAs (MS-sgRNAs) were used. MS-sgRNAs markedly increased the frequency of editing events in CD34+ cells in comparison to in vitro transcribed sgRNAs (88% vs 13.2%, respectively). Moreover, in vivo studies in transplanted NSG mice confirmed the high editing efficiency in human HSCs suggesting that NHEJ-mediated editing should constitute an efficient strategy for the treatment of the bone marrow failure of FA patients and potentially other blood disorders.

OR35

ImmTOR™ tolerogenic nanoparticles enhance transgene expression after both initial and repeat dosing in a mouse model of methylmalonic acidemia treated with Anc80 AAV-Mut vector

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A major immunological barrier to systemic gene therapy with AAV vectors is the inability to re-dose patients due to formation of vector-induced neutralizing antibodies (Nabs). We have developed tolerogenic nanoparticles encapsulating rapamycin (ImmTOR™), which provide long-term suppression of adaptive immune responses against AAV, allowing for vector re-dosing. Moreover, co-administration of liver-tropic AAV vectors and ImmTOR™ leads to an immediate increase in transgene expression even after the first dose. We tested the safety and therapeutic efficacy of an admixed ImmTOR™ and AAV vector combination in a mouse model of methylmalonic acidemia (MMA) using Anc80, a rationally engineered AAV vector. Repeated co-administration of Anc80 and ImmTOR™ was well-tolerated and led to complete inhibition of IgG antibodies to Anc80. Several expression cassettes were tested with human MUT gene driven by a liver-specific promoter showing the highest therapeutic potential. A more profound decrease of serum methylmalonic acid after initial and repeat injections was observed in mice treated with the combination of ImmTOR™ and Anc80-MUT, which correlated with higher viral genome copy

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number per liver cell (vg/cell) as well as higher hepatic MUT mRNA expression levels. These effects were dose-dependent, with higher doses of ImmTOR™ providing for higher vg/cell levels and lower plasma methylmalonic acid levels while also enabling therapy of juvenile mice with maternally-transferred Nabs to Anc80. Therefore, the admix of ImmTOR™ and Anc80-MUT is a promising approach to mitigate the detrimental impact of Nabs on gene therapy for MMA and may also provide a benefit in enhancing transgene expression at the initial dose.

OR36

Bicistronic AAV gene therapy for Tay-Sachs disease

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GM2 gangliosidosis (Tay-Sachs, Sandhoff disease) are fatal monogenetic diseases that result from a deficiency of hexosaminidase (Hex), which is an enzyme of the ganglioside degradation pathway. These diseases are clinically indistinguishable in humans and are caused by mutations in the alpha and beta subunits, respectively. Symptoms present in children as lack or loss of developmental milestones (sitting, crawling, etc.) and progress to inability to swallow, seizures and eventual semi-vegetative state. Tay-Sachs also occurs in sheep (TSD sheep), and this model is useful in testing of novel therapies. Adeno associated viral (AAV) gene therapy has shown efficacy in animal models, but has the limitation of intracranial delivery using two viral vectors to deliver the therapeutic genes. Here we describe the use of a single, bicistronic AAV9 vector construct to co-express both Hex subunits simultaneously. TSD sheep were injected with 2E13 vg vg/kg intravenously (n=5) or 3E14 vg total via the cerebrospinal fluid (n=2). Studies are ongoing with animals currently surviving as long as untreated TSD sheep, both IV and CSF AAV treated TSD sheep exhibit marked attenuation of neurologic disease as well as normal cognition as measured by maze testing. MRI, EEG, CSF and other fluid biomarker analyses were performed. Two of the IV treated animals were euthanized due to musculoskeletal complications. These data show promise for a minimally invasive treatment for Tay-Sachs and Sandhoff disease using this new vector construct.

OR37

Treatment of methylmalonic acidemia (MMA) by targeted integration of *MMUT* into Albumin with a promoterless AAV vector (GeneRide™) confers a progressive hepatocellular growth advantage in mice

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MMA is a heterogenous inborn error of metabolism most commonly caused by a deficiency of methylmalonyl-CoA mutase *(MMUT)*. Patients suffer from frequent episodes of metabolic instability, severe morbidity, and early mortality. Gene therapy has been explored in MMA mouse models as an alternative therapy to liver transplantation. To minimize the potential of vector-related insertional mutagenesis and preserve MMUT expression after therapeutic gene delivery, we designed a promoterless AAV vector that utilizes homologous recombination to achieve site-specific gene addition of human MMUT into the mouse albumin (Alb) locus. We have previously reported that treatment of different MMA mouse models at birth reduced disease related metabolites and produced durable MMUT expression for more than a year. In older treated mice, RNAscope revealed MMUT positive hepatocytes as distinct and widely dispersed clusters, consistent with a pattern of clonal expansion. Here, we report dose finding studies, biomarker responses, and a time course analysis of MMUT expression after a therapeutic GeneRide™. After a latency period of several months, there is a continuous enhancement of MMUT expression accompanied by weight gain, reduced disease metabolites, increased 1-C-13 propionic oxidative capacity, increased Alb-2A levels, increased Alb-MMUT integration events, and a reduction of the mitochondrial-stress biomarker, Fgf21. The progressive clinical and biochemical improvement in the treated mice is consistent with an expansion of corrected hepatocytes, yielding a greater therapeutic benefit with time, and is accompanied by a predictable pattern of biomarker changes that will facilitate clinical translation.

OR38

Positive cohort 1 results from the phase 1/2, AAV8-mediated liver-directed gene therapy trial in glycogen storage disease type Ia (GSDIa)

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GSDIa results from deficiency in the enzyme glucose 6-phosphatase (G6Pase), essential for glycogenolysis and gluconeogenesis. Decreased endogenous glucose production causes severe fasting hypoglycemia. DTX401 is an AAV8 vector that expresses the human G6PC under the transcriptional control of a liver-specific promoter. The GSDIa phase 1/2 gene therapy study (NCT03517085) is a global, open-label dose escalation trial evaluating the safety, tolerability, and efficacy of a single DTX401 IV infusion in adults with GSDIa. Continual reassessment method is used to evaluate potential doses of 2.0 x10^12 Genome Copies (GC)/kg, 6.0 x10^12 GC/kg and 1.0 x10^13 GC/kg in cohorts of 3 subjects. Three Cohort 1 subjects received DTX401 at 2.0 x10^12 GC/kg. At Week 24, time to hypoglycemia (<3.3 mmol/L) during a controlled-fasting challenge increased from baseline by 79%, 220%, and 20%, respectively. Total daily cornstarch use decreased from baseline to Week 24 by 77%, 44%, and 73% respectively. DTX401 was generally well-tolerated. No infusion-related or treatment-related serious AEs were reported. All AEs were mild or moderate in severity. Subjects 1 and 2 had transient mild elevations in ALT, similar to previous observations with AAV-based gene therapy; both responded well to a low-dose, tapered regimen of prednisone and were off prednisone during the Week 24 fasting challenge. In conclusion, DTX401 had an acceptable safety profile and resulted in clear biological G6Pase activity through Week 24, with an increase in time to hypoglycemia during a fasting challenge and reduction in daily cornstarch. Enrollment for Cohort 2 began at a dose of 6.0 x10^12 GC/kg.

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OR39

Safety, tolerability, biopotency and neurocognitive data of ABO-102 in Transpher A, an open-label, multicenter, single-dose, dose-escalation, Phase 1/2 Clinical Trial in Sanfilippo Syndrome type A (Mucopolysaccharidosis IIIA

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Transpher A is a clinical study assessing safety and efficacy of intravenous ABO-102 (a self-complementary AAV9-based vector expressing the human SGSH gene) for children with MPS IIIA, a disorder caused by loss-of-function mutations in the SGSH gene. General safety, neurodevelopment, biomarkers, liver and brain volumes are measured.

Results: Fourteen patients have been enrolled (Cohort 1, 5x1012 vg/kg, n = 3; Cohort 2, 1x1013 vg/kg, n = 3; Cohort 3, 3x1013 vg/kg, n = 8). ABO-102 was well tolerated, without serious drug-related adverse events. Cohorts 1 and 2 have completed 24 months follow-up with 16.9 months median follow-up in Cohort 3 (8.7–23.9 months). Transient, mild elevation of liver transaminases resolved with protocol-prescribed corticosteroids and 8 out of 14 patients showed mild transient cellular immune responses (ELISpot). A rapid, sustained and dose-dependent reduction in CSF-HS was observed in all patients with a 65% decrease from pre-treatment levels at day 30 (n = 8), 77% at Month 6 (n = 7) and 71% at Month 12 in Cohort 3. Abdominal MRI showed a rapid and sustained decrease in liver volume in all patients, with two patients in Cohort 3 normalizing liver volume by Month 6. In patients treated at younger age (<30 months), neurocognitive function tracked in the normal range at 12–18 months of follow up. Other patients showed signs of cognitive stabilization.

Conclusion: Intravenous administration of ABO-102 in children with MPS-IIIA showed a favorable long-term safety profile and led to sustained and dose-dependent improvement in biomarkers and liver volume, with preservation of neurocognitive development in patients treated at early stages of disease.

OR40

AAV-FGF21 gene therapy mediates healthspan expansion in old mice

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Both body weight gain and insulin resistance increase with aging. Fibroblast growth factor 21 (FGF21) is considered a promising therapeutic agent for these age-related diseases. The aim of this study was to evaluate the potential for extending healthspan and counteracting insulin resistance and body weight gain in old mice by means of the long-lasting secretion of FGF21 into the bloodstream following a single administration of adeno-associated viral (AAV) vectors to the skeletal muscle (Skm). AAV vectors carrying a murine FGF21 coding sequence (AAV-FGF21) or non-coding AAV-Null vectors were administered intramuscularly into each hind limb of 13-month-old C57Bl6 mice. Untreated 2- and 7-month-old C57Bl6 mice were used as younger controls. Old mice treated with AAV-FGF21 maintained the body weight they had at the initiation of the study whereas their age-matched control mice steady increased their weight as animals aged. Furthermore, the weight of the adipose depots and the liver were considerably reduced in 20-month-old FGF21-treated mice and indistinguishable from those of 2-month-old mice. In contrast to null-injected animals, 22-month- old mice treated with AAV-FGF21 showed markedly improved insulin sensitivity, coordination and strength, which were similar to those shown by 2-month-old mice. Assessment of survival and gene expression analysis is ongoing. Altogether, these results demonstrate that AAV-mediated overexpression of FGF21 in skeletal muscle leads to sustained secretion of therapeutically-relevant levels of this factor to the bloodstream and highlight the therapeutic potential of this approach to expand healthspan and counteract age-related insulin resistance and body weight gain in the future.

OR41

Trap and Ambush viroimmunotherapy for tumour recurrence by oncolytic virus driven neoepitope vaccination

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Treatment of established tumours with oncolytic Vesicular Stomatitis Virus (VSV) can lead to regression followed by recurrence. We showed that VSV infection induces IFN-ß-dependent expression of the mutagenic cytidine deaminase APOBEC3B which leads to the selection of VSV-resistant escape tumours (Huff et al., Mol. Ther. Oncolytics, 2018. 11:1–13). Whole genome sequencing of cells which escaped VSV oncolysis identified APOBEC3-induced mutation of the CSDE1 gene as a major driver of escape. One mutation in CSDE1 mutated an MHC binding epitope such that it would bind with a significantly higher affinity (<500nM) than the wild type epitope. We show that a strong T cell response was raised against the mutated CSDE1* epitope in mice vaccinated with CSDE1*-pulsed DC or VSV-CSDE1*. Mice with B16 tumours treated with i.t. VSV-IFN-ß underwent regressions, followed by aggressive recurrence. However, mice treated with i.t. VSV-IFN-ß and vaccinated with CSDE1* survived significantly longer (median survival 75d) and, with anti-PD1, long terms cures were generated. Immune correlative studies confirmed that cured mice had generated potent T cell responses against CSDE1*. Our data show that tumour recurrences express specific mutations which drive escape from frontline therapy, a proportion of which may generate novel, immunogenic neo-epitopes. By combining sequencing data to identify heavily selected mutations in treatment-resistant recurrences, with predictive bioinformatic analysis for possible neo-epitope formation, it is possible to ambush recurrences which have been trapped/forced into a phenotype that facilitates escape from frontline therapy but is itself readily targeted by concomitant vaccination.

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OR42

Local expression of a PD-L1 blocking antibody from a self-replicating RNA vector induces potent antitumor responses

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Immune checkpoint blockade has shown high anti-cancer efficacy, but requires systemic repetitive administration of monoclonal antibodies (mAbs), often leading to adverse effects. To avoid toxicity, mAbs could be expressed locally in tumors. We developed adeno-associated virus (AAV) and Semliki Forest virus (SFV) vectors expressing an anti-PD-L1 mAb (aPDL1) and tested them in a colon adenocarcinoma MC38 tumor model. AAV-aPDL1 and SFV-aPDL1 intratumoral administration led to similar local mAb expression at 24h. Although expression in SFV-aPDL1-treated tumors diminished quickly, it induced >40% complete regressions, being superior to AAV-aPDL1, and to aPDL1 mAb given systemically, or locally in tumors. Furthermore, SFV-aPDL1 showed abscopal effects in untreated MC38 tumors. SFV-aPDL1 antitumor efficacy was also observed in a melanoma model (B16-OVA). The high SFV-aPDL1 antitumor activity could be related to local upregulation of interferon (IFN)-stimulated genes (ISGs), due to RNA replication. Synergy between induction of IFN-I responses and immune checkpoint blockade was confirmed by combining local SFV-LacZ administration and aPDL1 given systemically, a treatment that showed similar therapeutic effects to those of SFV-aPDL1. SFV-aPDL1 promoted tumor-specific CD8 effector T-cells infiltration while AAV-aPDL1 did not induce relevant changes. Furthermore, SFV-aPDL1 upregulated co-stimulatory (CD137/OX40) and co-inhibitory (LAG-3) markers in tumor CD8 T-cells, suggesting possible benefits from combination with mAbs against those receptors. Accordingly, SFV-aPDL1 combined with anti-CD137 mAb showed more pronounced antitumor effects than each single agent. These results indicate that local transient expression of immunomodulatory mAbs using a non-propagative RNA vector inducing IFN-I responses could represent a potent and safe approach for cancer treatment.

OR43

Nuclease-free genome editing by AAVHSC vectors leads to *in vivo* genome correction and amelioration of disease phenotype in a mouse model of phenylketonuria (PKU)

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The correction of pathogenic mutations has great potential for the treatment of genetic disorders. A panel of adeno-associated viruses isolated from normal human hematopoietic stem cells (AAVHSCs) have shown nuclease-free gene editing through the homologous recombination pathway. Here we explore application of AAVHSCs for in vivo nuclease-free correction of phenylketonuria (PKU), a recessive disorder caused by loss of function mutations in the gene phenylalanine hydroxylase (PAH) which results in elevated levels of phenylalanine and decreased tyrosine production. To correct PKU, an AAVHSC vector containing a human PAH cDNA flanked by targeting sequences homologous to the murine Pah gene is administered by a single IV injection into Pahenu2 mice, a disease model. Correction was assessed by monitoring serum Phe levels. Treatment with AAVHSC vectors led to long-term correction of phenylalanine levels while on a normal diet. Molecular characterization of treated mouse livers shows efficient and precise gene editing and expression of human PAH concordant with phenotypic correction. Human-specific editing of human liver was explored using AAVHSC editing vectors constructed with human-specific targeting sequences and administered to mice whose liver is populated with implanted human hepatocytes. Characterization of humanized livers display human-specific gene editing and expression comparable to those sufficient to reverse PKU phenotypes. Further, no ''off-target'' editing of the orthologous mouse loci is detected supporting sequence specificity. Together these results support AAVHSC vectors as a clinically feasible platform to address the genetic liver disorder PKU via targeted genome correction and led to the development candidate, HMI-103, currently in IND-enabling studies.

OR44

A versatile platform for precision exosome engineering

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Exosomes have been shown to be an important intercellular communication system facilitating the transfer of complex macromolecules (RNA, protein, lipid) between neighboring and distant cell types. Selective delivery of exogenous macromolecule cargo tethered to the exosome surface or packaged inside the lumen are key strategies for harnessing the therapeutic potential of exosomes. Using mass spectrometry analysis, we identified two families of proteins that are preferentially sorted into exosomes and can facilitate high density surface display and luminal loading of bioactive molecules through molecular engineering of the exosome producer cell line. Stable expression of prostaglandin F2 receptor negative regulator (PTGFRN), a single-pass transmembrane glycoprotein, or brain acid soluble protein 1 (BASP1), a member of the MARCKS protein family, in a producer cell resulted in multi-log enrichment of these proteins on exosomes. Full length and truncated forms of these ''scaffolds'' were used to display or package a variety of payloads including reporters, cytokines, antibody fragments, vaccine antigens, Cas9, and members of the tumor necrosis factor superfamily. High efficiency exosome surface display and luminal loading of biomolecules mediated by fusion to PTGFRN or BASP1 demonstrated superior activity in vitro compared to exosomes engineered using previously published scaffolds including LAMP2B, pDisplay, and exosome-associated tetraspanins. Furthermore, these engineered exosomes retained potent activity in vitro and in vivo. The engineering platform described is a unique approach for efficient functionalization of the surface and lumen of

exosomes with topologically diverse macromolecules using molecular engineering strategies amenable to scalable biopharmaceutical manufacturing.

OR45

Homology-independent targeted integration to counteract toxic gain-of-functions and loss of transgene expression due to cell proliferation

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Gene therapy with adeno-associated viral vectors (AAV) is safe and effective in both retina and liver, with products that are either on the market or in advanced stages of clinical development. However, in retina canonical gene replacement does not apply to those conditions due to gain-of-function (GOF) mutations, whereas in liver AAV dilution during hepatocyte division represents a major drawback. To address these issues, we designed a flexible homology-independent targeted integration system with 2 AAV vectors: one encoding for CRISPR/Cas9 and one containing a gRNA expression cassette and a donor DNA with a reporter transgene. We measured efficiency of integration in mouse and pig photoreceptors and in mouse hepatocytes after targeting the rhodopsin (Rho) or the albumin (Alb) locus, respectively. Indeed, GOF in RHO are a frequent cause of dominant retinitis pigmentosa (RP), while ALB is transcribed at high levels representing an ideal locus from which to express a secreted protein. We showed 10% and 4% efficiency in photoreceptors and hepatocytes, respectively. An in-depth characterization of on- and off-target integration is in progress. We then used our system to replace Rho, and achieved mild phenotypical rescue in a mouse model of dominant retinitis pigmentosa. Similarly, the integration of a promoterless arylsulfatase B (ARSB), whose deficiency is responsible for the lysosomal storage disease mucopolysaccharidosis VI (MPS VI), in the albumin locus resulted in stable expression of ARSB in newborn MPS VI mice. These data support the therapeutic use of AAV-mediated homology-independent targeted integration in both retina and liver.

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OR46

Engineering potent, small, chimeric, synthetic, RNA-guided nucleases (sRGN) from four uncharacterized Cas9 genes

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Adeno-associated viruses (AAVs) and lipid nanoparticles (LNP) are among the methods of choice for delivering nucleases for in-vivo genome editing. The widely used Streptococcus pyogenes (Spy)Cas9 is specific for a short non-degenerate PAM sequence. However, the large size of SpyCas9, together with its sgRNA and expression elements presents a challenge to the 4.5 kb DNA AAV packaging limit, to the synthesis of long mRNA templates and their stable formulation into LNPs. The best-characterized smaller Cas9s frequently recognize degenerate and longer PAMs, reducing the number of addressable genomic targets. We evaluated four related, previously uncharacterized Cas9 nucleases of ~1050 amino acids. Surprisingly, most were specific for a common NNGG-PAM that enables addressing the same targets as SpyCas9. Using protein-engineering approaches, we altered these genes to generate synthetic RNA-guided nucleases (sRGNs) and demonstrated robust editing in human cells. Analyses using all possible, single-nucleotide mismatched off-targets of a DNA substrate indicated high overall specificity in cell free experiments. Interestingly, different clones displayed a higher specificity for different mismatches at different sites along the target. Comparing the activity of sRGNs with SpyCas9 across 12 targets, we observed overall editing efficiencies on par with the larger SpyCas9. LNP packaging and in-vivo performance vs. SpyCas9 were comparable. Thus, we have generated a set of novel, small sRGNs, from which improved nucleases can be selected for particular targets of interest. These engineered RNA-guided nucleases are expected to be valuable additions to the canon of known genome-editing nucleases that can be employed for human gene therapy applications.

OR47

CAST-Seq, a novel preclinical genotoxicity assay, enables qualitative and quantitative insights in chromosomal aberrations in gene-edited human hematopoietic stem cells

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Genome editing with designer nucleases has shown great promise but also revealed the risk of genotoxicity caused by the insertion of mutations or chromosomal aberrations. Recently developed methods to identify genome-wide off-target activity confirmed those perils but also revealed the restrictions of these assays, including insufficient sensitivity or specificity, or the failure to detect gross chromosomal aberrations. To overcome these limitations, we established CAST-Seq, a method capable of detecting directly in clinically relevant cell types, aberrations derived from on- and off-target activity of CRISPR-Cas nucleases or TALEN, including large deletions, inversions and translocations. Moreover, we detected novel on-target activity mediated aberrations, such as homology-mediated translocations, acentric and dicentric translocations between homologous chromosomes, and large deletions. Validation of the results by deep sequencing and digital PCR confirmed the quantitative nature of CAST-Seq and revealed new insights into the DNA repair kinetics and dynamics in human stem cells.

OR48

In vivo gene knockout followed by targeted gene insertion results in simultaneous reduced mutant protein levels and durable transgene expression

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1: Intellia Therapeutics 2: Regeneron

CRISPR/Cas9-based genome editing offers the potential to cure genetic diseases at their source within the DNA. Using a

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hybrid approach of lipid nanoparticle (LNP) encapsulated CRISPR/Cas9 components in combination with adeno-associated virus (AAV) donor DNA template, we have demonstrated targeted gene insertion in vivo in both murine and non-human primate (NHP) models. Here, we highlight the modularity of this platform to enable complex genome editing events leading to production of therapeutically relevant proteins. One disease of high unmet medical need is alpha-1 antitrypsin (AAT) deficiency. Gene knockout can eliminate the production of the faulty PiZ variant of the protein, while insertion of a wild-type gene copy, or direct repair of the mutant allele, can allow the production of functional circulating protein. Using our novel hybrid LNP-AAV platform in a humanized mouse model, we have demonstrated serial LNP delivery results in distinct genome editing events that facilitate the reduction of circulating PiZ levels by >98% concomitant with sustained expression of wild-type human AAT. The non-viral nature and modularity of the LNP Cas9/gRNA delivery platform enables a series-based dosing approach for targeting multiple genes in the same individual. Furthermore, we have demonstrated feasibility of our hybrid LNP-AAV targeted insertion-based editing in multiple model animal studies. Insertion of human F9 in the albumin locus has achieved normal human levels of circulating FIX protein throughout two months of observation in NHP and remains durable over 12 months of observation in mice.

OR49

A phase 1/2 clinical trial of AAV8 gene therapy in adults with late-onset OTC deficiency: CAPtivate cohort 1+2 results

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Ornithine transcarbamylase (OTC) deficiency is an X-linked disorder resulting in impaired ammonia flux through the urea cycle and subsequent hyperammonemia. Current standard of care includes a protein restricted diet and nitrogen-scavenging agents; however, risk of recurrent hyperammonemic crisis with resulting irreversible neurocognitive damage remains. DTX301, an AAV8 vector containing the OTC transgene, is currently under investigation for OTC deficiency. CAPtivate is a global, multi-center, open-label phase 1/2 dose-escalation trial evaluating the safety and efficacy of DTX301 in adults with late-onset OTC deficiency. Cohorts 1 and 2 each enrolled three subjects; subsequent cohorts will enroll a minimum of three subjects. The primary endpoint is the incidence of adverse events (AEs). Secondary endpoints are changes in ureagenesis rate and 24-hour plasma ammonia AUC. Dosing of three subjects each in cohort 1 (2x10^12 GC/kg) and cohort 2 (6x10^12 GC/kg) is complete. No infusion-related or serious AEs were reported; all AEs were grade 1–2. Mild, asymptomatic ALT increases in three subjects resolved with a tapering course of oral corticosteroids, per protocol. Two subjects (1 per cohort) achieved a normal ureagenesis rate, discontinued all ammonia scavenger medications, and liberalized dietary protein restrictions. All six subjects in cohort 1 and cohort 2 completed the 52-week study period and enrolled into the 4-year extension study. Enrollment in cohort 3 (1x10^13 GC/kg) is ongoing. Early data from CAPtivate indicate that DTX301 is safe and tolerable, and may be a potential new therapeutic option for patients with OTC deficiency.

OR50

Inhibition of proliferation in primary human hepatocytes following *in vivo* AAV-mediated genome editing

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1: Children's Medical Research Institute

Recombinant adeno-associated viral (rAAV) vectors are the current system of choice for *in vivo* delivery of reagents for CRISPR/Cas9-mediated genome editing. However, the effect of viral transduction on the proliferative state of the target cells is rarely considered. Our group has previously shown that transduction with rAAV causes transient inhibition of hepatocellular proliferation in the murine liver. Here we show that inhibition also occurs when transducing primary human hepatocytes *in vivo*. This inhibitory effect was observed when a vector encoding a donor template for homology directed repair (HDR) was delivered alone, and was further amplified when a second vector encoding a user-designed nuclease (SaCas9 and sgRNA) was delivered in combination. These observations were made using FRG mice bearing xenografted primary human hepatocytes, and editing vectors at clinically-relevant doses. A combined dose of 2.5×10^{11} vg/mouse ($\sim10^{13}$ vg/kg) of both donor and nuclease encoding vectors resulted in a 66% reduction in proliferation as determined by BrdU labelling and Ki-67 immunostaining. Transduction with donor vector alone resulted in a 37% reduction in the number of labelled hepatocytes. Moreover, p53-dependent activation of the DNA Damage Response (DDR) was detected in targeted cells. p53 and p21 labelling increased when the editing vectors were delivered to primary human hepatocytes and was accompanied by the formation of nuclear foci of p53-binding protein 1, indicative of DDR activation. These effects of rAAV transduction on hepatocellular proliferation demand further study as they have the potential to affect editing efficiencies, particularly via HDR which is cell cycle dependent.

Poster Presentations

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P001

Development of AAV3B variants with better liver transduction in nonhuman primates by directed evolution

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To generate better capsid for adeno-associated virus (AAV) mediated gene therapy, we have established an AAV directed evolution platform and run AAV3B on it for liver targeting. We used a scorecard approach to generate the initial diversity on AAV3B hyper variable region (HVR) VIII, by first aligning around 180 natural AAV isolates and then picking ten variable, surface-exposed sites within HVR.VIII for mutagenesis. For each site, we selected the amino acids with the highest frequencies according to the alignment and incorporated them into degenerated oligos for the mutagenesis. We then conducted two rounds of selection in human-hepatocytes-xenografted *Fah*$^{-/-}$/*Rag2*$^{-/-}$/*Il2rg*$^{-/-}$ (FRG) mice, by injecting the libraries intravenously and retrieving AAV cDNA from human hepatocytes isolated from those mice to prepare new libraries for the next rounds. The mouse model is an immunodeficient mouse with human hepatocytes re-populated in its liver by selection pressure provided by the fumarylacetoacetate hydrolase (FAH) deficiency. After the two rounds of selection, 16 variants that showed dramatic increase of relative frequencies by next generation sequencing (NGS) were evaluated in nonhuman primates (NHPs) with a validated barcodes system. Most of the 16 variants were clearly better than AAV3B in terms of liver transduction, with some showing high liver specificity. Two variants were further evaluated with a therapeutic transgene for liver gene therapy in NHPs and the preliminary results confirmed the NHP barcode evaluation result. These results indicate that our platform works and provided two AAV3B variants with good liver transduction in NHPs.

P002

Transduction evaluation of novel AAV natural isolates in nonhuman primates using a barcoded transgene system

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Adeno-associated virus (AAV) vectors have been shown to be safe and effective gene transfer vehicles in clinical applications yet they can be hindered by preexisting immunity to the virus and can have restricted tissue tropism. In order to find AAVs that can circumvent these issues we explored the genetic variation of AAV in mammalian tissues by using AAV-single genome amplification: a technique that accurately isolates individual AAV genomes from within a viral population. Five novel AAV capsid sequences from clades A, D, E, and the primate outgroup were recovered and tested as gene transfer vectors using intravenous (IV) and intracerebroventricular (ICV) delivery routes in mice and nonhuman primates (NHP). In NHPs, novel capsids and their respective prototypical clade member controls (AAV6.2, AAV7, AAV8, AAVrh32.33, and AAV9) were made into vectors containing a modified eGFP transgene and unique six base pair barcodes prior to the polyA signal of the transcript. The transgene was modified by deletion of ATG sequence motifs to prevent polypeptide translation and consequent immune response towards a foreign protein. The barcoded vectors were pooled and delivered via IV and intracisterna magna (ICM) routes in cynomologus macaques to assess systemic and central nervous system transduction patterns of the novel capsids. Interestingly the clade E variant, AAVrh90, showed higher levels of transgene RNA expression in NHP liver, heart, skeletal muscle, kidney, and pancreas after IV delivery than all other novel and control capsids tested. The clade A variant, AAVrh91, showed high transduction levels in NHP CNS tissues after ICM delivery.

P003

Restoring the intrinsic AAV2 human hepatotropism attenuated by tissue culture adaptation

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AAVs have risen to scientific prominence owing to their unequivocally successful application as gene therapy vectors. The life-cycle of the prototypical AAV2 is evolutionarily associated with infection of human liver and AAV2-based vectors transduce human hepatocyte-derived cell lines at high efficiency. Paradoxically, clinical trial use of the AAV2 capsid resulted in unexpectedly low therapeutic efficacy, consistent with subsequent observations of inefficient human hepatocyte transduction in a biologically predictive xenograft mouse model of human liver. We have resolved this paradox by determining that the intrinsic hepatotropism of the prototypical AAV2 is attenuated by capsid mutations likely acquired as a consequence of culture adaptation. This was achieved by initial sequence comparisons of the prototypical AAV2 with novel human liver isolates (Poster #010 #YYY), revealing differences in the residues responsible for binding to heparan sulfate proteoglycan (HSPG), hitherto regarded to mediate the interactions between AAV2 and human hepatocytes. In vitro evolution experiments on novel AAV liver isolates were used to recapitulate the acquisition of strong HSPG binding properties in culture, with concomitant improvement of transduction in vitro. In parallel, in vivo re-adaptation of the prototypical AAV2 capsid on primary human hepatocytes in xenograft mouse livers restored its human hepatotropism via mutations that

led to profound HSPG de-targeting. Taken together, these results challenge the paradigm that hepatocyte entry of AAV2 is HSPG-mediated and imply that the inability of AAV2 to effectively target liver is a consequence of tissue culture adaptation.

P004

In vivo screening of an adeno-associated virus capsid library in non-human primate eyes identifies a novel AAV variant with superior retinal penetration and transduction by intravitreal delivery

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1: Adverum Biotechnologies

In recent years, adeno-associated virus (AAV)-mediated gene therapy has shown promise in the clinic for the treatment of ocular diseases. Most gene therapy approaches entail subretinal administration of AAV vectors; while this route of delivery targets retinal cells efficiently, it is limited to the injection site and may result in insufficient expression levels. Moreover, the injection procedure itself introduces the potential for further retinal damage. Intravitreal (IVT) injection is a less invasive procedure, but is far less efficient with current, naturally-occurring AAV serotypes that are unable to cross the inner limiting membrane (ILM), a barrier to efficient retinal transduction of the primate retina. To identify novel AAV capsids that can efficiently cross the ILM and transduce primate retina after IVT administration, we generated a diverse capsid library of 1.2 million variants by insertion or substitution of surface-exposed peptide loops on the VP3 of AAV2.5T, a chimeric variant with low neutralizing antibody prevalence that robustly transduces photoreceptors but cannot penetrate the ILM when injected intravitreally. Three screening rounds of IVT injection identified a novel AAV variant, AAV2.5T.LSV1, that was recovered from parafoveal regions of the retina, where the ILM is the thickest, as well as from the retinal pigment epithelium (RPE). Unlike the parental variant, AAV2.5T.LSV1 carrying the GFP reporter gene exhibited deep tissue penetration via IVT administration as evidenced by robust, widespread cellular expression in the primate retina including Muller glia, photoreceptors and bipolar cells, as well as the RPE. Studies evaluating AAV2.5T.LSV1 expressing a therapeutic transgene are currently underway.

P005

Development of a novel adeno-associated virus capsid for markedly enhanced muscle transduction

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1: The Scripps Research Institute

Adeno-associated virus (AAV) is currently the most promising and clinically utilized vector for gene therapy and the applications for AAV-delivered therapies are numerous. Among them are the delivery of therapeutic antibody genes for expression in muscle tissue to be secreted into blood. However, the current state of technology is limited by the low efficiency with which most AAV vectors transduce human muscle tissue. As a result, high titers are required, which elicit an immune response against both the transgene and the vector. Additionally, practical limits to the size and number of doses that can be administered, and high costs of production, render AAV-mediated therapy inaccessible in resource poor settings. The aim of the current study is to develop a vector that can achieve greater transgene expression with fewer vector particles. Here we show that recombinant AAV9 vectors modified in variable region IV or VIII are capable of targeting a specific cellular receptor highly expressed on muscle cells. These vectors exhibit markedly improved transduction of human and mouse cell lines, and primary human muscle cells in vitro. We also show enhanced transduction of mouse muscle in vivo compared to wild-type AAV9. This approach has the potential to dramatically reduce the technical challenges and cost of AAV-mediated gene therapy. Moreover, the findings could expand opportunities for the development of more efficient vectors in different tissue types as well. We are currently testing the utility of this approach in other AAV serotypes.

P006

Modulating the heparan sulfate proteoglycan binding of AAV2 enhances *in vivo* transduction of human hepatocytes in a xenograft mouse model

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The creation of bioengineered AAV capsids such as AAV-LK03 and AAV-NP59, with enhanced functional transduction of primary human hepatocytes both in xenograft models and in the clinic, was facilitated by directed capsid evolution. Remarkably, AAV-NP59 differs from the prototypical AAV2 (a poorly hepatotropic serotype in humans) by eleven amino acids, providing a unique opportunity to study the structural capsid properties involved in functional transduction of human hepatocytes. To this end, we created a library of AAV2 capsids containing all possible permutations of these eleven variant amino acids and performed in vivo selection screens. This facilitated identification of the key determinants of AAV-NP59's enhanced transduction of primary human hepatocytes. Importantly, the identified residues reduce heparin binding affinity, a finding consistent with our independent observation that attenuation of prototypical AAV2 heparan sulfate proteoglycan (HSPG) binding dramatically enhances human hepatocyte transduction (Poster #003). A separate directed evolution experiment identified two additional AAV2 residues, within the heparin binding region, which further improved in vivo transduction of human hepatocytes in a xenograft liver model. Vector sequestration in HSPG-rich extracellular matrices has been proposed to reduce in vivo transduction. We explored this concept by generating two mutants of the AAV8 (a highly murine-hepatotropic serotype) capsid with increased affinity for heparin, which dramatically reduced AAV8's in vivo performance and thus recapitulated the poor liver transduction seen in AAV2. Heparin binding was subsequently reduced, and the hepatotropic phenotype restored, via introduction of a mutation homologous to one of the critical residues identified for AAV2 and discussed above.

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P007

Development of a novel AAV capsid with improved PNS tropism for treating Pompe disease by intravenous administration

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1: Abeona Therapeutics Inc.

Pompe disease is a lysosomal storage disorder caused by a deficiency in acid alpha-glucosidase (GAA) activity that results in the accumulation of glycogen in the lysosome. Pompe presents as a form of muscular dystrophy that affects both smooth and striated musculature as well as the peripheral nervous system (PNS), with early mortality. Enzyme replacement therapy (ERT) is currently the only FDA-approved therapy to treat Pompe and requires biweekly injections of large quantities of recombinant protein. While ERT reduces the mortality rate of infantile Pompe patients, it fails to completely ameliorate all symptoms of Pompe due to inefficient treatment of the PNS and immune responses to the GAA protein. Previous work has shown that hepatic-specific transgene expression can tolerize animals to the GAA protein and reduce the humoral immune response, but its therapeutic effectiveness is still under debate. We have identified a novel adeno-associated virus (AAV) capsid allowing improved GAA transgene delivery to the PNS and diaphragm, with strong GAA expression also seen in other target tissues such as heart and other muscle tissue after intravenous administration. Testing of both constitutive and tissue-specific promoters with codon-optimized GAA transgene sequences demonstrated a robust reduction in the underlying molecular pathology seen in the gaa-/- knock-out mouse model of Pompe Disease. Proof-of-concept studies in the disease model showed supraphysiological GAA expression levels in target tissues as well as increased secretion for cross-protection to non-transduced cells and are leading to IND-enabling studies.

P008

Nanobody-enhanced targeting of AAV vectors

ABSTRACT WITHDRAWN

P009

Novel AAV capsids for delivery to the retina by intravitreal administration

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The eye represents a unique opportunity for the use of gene therapy due to its immune-privileged status, relatively small size and the availability of non-invasive imaging to monitor safety and efficacy. Historically, AAV administration to the eye has been performed by subretinal injection between the neural retina and underlying retinal pigmented epithelium (RPE). While this method provides the benefit of positioning the vector directly next to its cellular target, it requires a retinal detachment, as well as an operating room and trained retinal surgeon. Conversely, a therapy that is administered directly into the vitreous of the eye would provide a safer and likely more feasible approach. Here, we identify a novel AAV capsid that provides robust expression in both the inner and outer retina after intravitreal administration in mice and non-human primates (NHPs). Administration of this novel AAV to NHP retinal explant cultures demonstrated similar or higher expression compared to AAV8, including expression in the photoreceptors. To evaluate the potential for clinical translation, intravitreal administration to NHPs at 1.5E+12 vg/eye showed strong foveal expression and widespread expression in the peripheral retina within four weeks post-treatment. NHP retinal expression was highest in the photoreceptor, bipolar, ganglion and RPE cells. Importantly, this novel AAV can be administered using a relatively safe method in an out-patient retinal clinic, allowing broader availability to previously under-treated communities.

P010

Nature trumps nurture: Use of naturally liver-tropic adeno-associated viruses as vector platforms for liver-directed human gene therapy

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Our previous discovery and functional characterisation of conserved master hepatic transcription factor binding sites in the 3'-untranslated region of the prototypical human AAV isolate, AAV2, signify its intimate association with the human liver. However, vectors utilising the AAV2 capsid in a liver-directed gene therapy trial resulted in unexpectedly low therapeutic efficacy. This was consistent with subsequent observations of inefficient human hepatocyte transduction in the xenograft FRG mouse model, despite robust targeting of human hepatocyte-derived cell lines. Upon discovering that most primate AAVs evolved to infect the liver, we resolved the paradox of AAV2 human hepatotropism by amplifying AAV capsid sequences directly from human liver samples. Comparison with the prototypical AAV2 capsid revealed functionally validated differences in the heparan sulfate proteoglycan binding domains (Poster #003), thereby attributing inefficient transduction of primary human hepatocytes by the originally

culture-isolated AAV2 (henceforth ''ciAAV2'') to culture adaptation. The development of AAV vectors using capsids isolated directly from liver samples, and naturally evolved to traffic to the liver and target human hepatocytes, harnesses the power of viral evolution and circumvents cell culture attenuation of hepatotropism. In humanised FRG mice, some of the novel and sero-diverse wildtype liver-isolated capsids vectorised to date already outperform the most human liver-tropic bioengineered AAV capsids – LK03 and AAVS3 – currently in gene therapy clinical trials targeting the human liver, but still fall short of the most human hepatotropic capsids our groups have developed. We are further optimising their performance and manufacturability to develop a set of superior, clinically translatable capsids.

P011

Characterization of a novel AAV capsid with enhanced brain transduction following systemic delivery

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1: ReGenX Biosciences

Adeno-associated viral (AAV) vectors are widely used in many ongoing preclinical and clinical gene therapy studies for targeting the central nervous system (CNS). The ability of certain AAV serotypes to cross the blood brain barrier, transduce cells of the brain and spinal cord and successfully deliver therapeutic genes has already been proven clinically. While the current gold standard for CNS gene therapy is AAV9, several attempts are being made by multiple groups to engineer an improved AAV capsid for targeting brain and spinal cord. Here we report the findings from our efforts to develop a better capsid by strategically modifying the capsid for enhanced brain transduction after intravenous delivery. We evaluated the in vivo bio-distribution of a group of modified capsids designed for better CNS transduction in a C57Bl/6 mouse study. One novel capsid, AAV-RGNX-BBB14 showed a statistically significant 87-fold increase in genome copy number in the brain over AAV9 in this study. This vector resulted in global transduction of the CNS as evident in immunohistochemical analysis of different brain regions including striatum, cortex and hippocampus. Further characterization of the transduction profile of this capsid revealed a non-significant reduction in liver genome copy number compared to AAV9, however, the reduction in vector copy numbers in biceps/skeletal muscle (6-fold), heart (2-fold) and lung (8-fold) was significant. Studies are ongoing to evaluate the in vivo biodistribution of this novel AAV capsid in different mouse strains and non-human primates.

P012

Characterization of HPV-related impurities in HeLa producer cell line generated rAAV and in vivo preclinical analysis of HPV-related host cell DNA persistence and gene expression following vector delivery

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1: Ultragenyx Pharmaceutical 2: Sarepta Therapeutics 3: The Janssen Pharmaceutical Companies of Johnson & Johnson

The presence of co-packaged host cell DNA and reverse-packaged plasmid DNA remains a challenge in manufacturing AAV-based clinical products. Although the mechanisms involved in these phenomena are still under investigation, it is known that impurities are packaged at low levels and that certain impurities have been detected in cells following AAV transduction. We utilized a HeLa-based producer cell line to manufacture the clinical grade product DTX201 AAV.hu37 BDD-FVIII (BAY2599023) for the treatment of severe hemophilia A in adults. As the critical roles of the human papillomavirus (HPV) type 18 E6 and E7 proteins in the generation and continued maintenance of HeLa cells are well understood, we undertook an HPV-related impurity risk assessment to investigate the potential risk of oncogenicity following administration of AAV vector. Highly sensitive methods were developed for the detection of HPV18 E6, E7 and the regulatory long control region. Detectable levels of each HPV-related impurity in clinical grade viral preparations were observed utilizing quantitative PCR methods with limits of detection of ≥10 copies for individual HPV targets. A high dose (3 x 1013 GC/kg) of this clinical grade vector preparation was then administered to C57BL/6N mice (effective human dose of 3 x 1014 GC/kg). Following completion of a biodistribution study, these same tissue samples were interrogated for the presence of HPV-related impurities. Importantly, although these impurities were found at detectable levels in the viral preparations, we were unable to detect any HPV-related impurities present as persistent DNA or transcribed RNA in any of the analyzed tissues.

P013

Rapid CMC development and pre-commercial considerations for rAAV gene therapy products for rare diseases

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1: Ultragenyx Gene Therapy

Despite significant progress over the past several years in the process and analytical development of rAAV vectors for numerous clinical gene therapy applications, fundamental challenges of low productivity, poor downstream yield, immature product quality characterization, and limited external manufacturing capacity continue to pose challenges for the biopharmaceutical industry in the CMC development of these products. UGT has established a fast-to-clinic CMC development strategy leveraging two distinct production platforms, implementation of high-throughput centers of excellence, and a state-of-the-art pilot plant to streamline and standardize the development and technology transfer of pre-clinical and clinical candidates to external manufacturing partners. Process Development has resulted in greater than 5-fold volumetric productivity increase and significant improvement in yield across chromatography and filtration steps. Scalability to 250L has been demonstrated within 2–3 months, which significantly decreases time and risk toward GMP manufacturing at up to 2000L scale. Significant development in analytical characterization methods have enabled product quality assessment to maintain comparability and enhance product understanding as we transition toward pre-commercial development. Finally, a risk-based strategy has been implemented for characterization and late-stage development and qualification of the processes and analytics.

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P014

Over-expression of the E. coli single stranded DNA binding protein (ssb) inhibits the loss of Adeno-associated virus (AAV) hairpin loop inverted terminal repeats in transfer vector plasmid preps

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Transient production of recombinant AAV vector in mammalian cells utilises a transfer vector plasmid carrying a transgene flanked by AAV inverted terminal repeats (ITRs). ITRs are required both for packaging of the transgene into the capsid and stability of the recombinant genome in transduced cells. During replication in E. coli, a variable proportion of transfer vector plasmids (~5–100%) suffer a partial deletion of the 5'-ITR in all strains tested, resulting in heterogeneous plasmid preparations, a universal problem in AAV vectorology. Recombinant AAV genomes flanked by a deleted ITR are inefficiently packaged into the capsid in mammalian cells, reducing target vector yields and giving unpredictable in vivo performance. This ITR deletion was postulated to be driven by formation of hairpin structures in the lagging strand of the DNA replication fork. In this model, the hairpin is bridged by an Okazaki fragment, resulting in daughter plasmids lacking the ITR sequence. We hypothesised that over-expression of the E. coli single stranded binding protein (ssb), that binds all single stranded DNA in the bacterium, including single stranded DNA at the replication fork, would prevent secondary structure formation. The ssb gene was cloned into an AAV transfer vector plasmid, dramatically increasing levels of ssb protein in bacteria and reducing ITR loss 10-fold in the condition tested. Other factors may contribute, but addition of ssb to AAV transfer vector plasmids has largely solved the ITR instability issue, eased cloning steps for new AAV gene therapies and will potentially improve quality and yield of vector.

P015

AAV p40 promoter expression in the absence of Rep proteins

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Vectors based on adeno-associated virus type 2 (AAV2) are promising tools for gene therapy, as evident by recent approvals for therapeutic use. AAV2 p40 promoter controls the expression of the cap gene, which encodes the structural proteins VP1–3, the assembly-activating protein (AAP) and the protein X. It is generally assumed that p40 is inactive in the absence of AAV Rep proteins. However, p40 activation in cells transduced with rep-deficient vectors was previously reported (Tratschin et al., 1986 and Ye et al., 2006). Similarly, we detected strong cap expression in HEK293T cells transfected with rep-deficient AAV plasmids and in HuH-7 and HepG2 cells transduced with rep-deficient vectors. Mapping of the transcription start site to the 3' end of p40 promoter confirmed that the observed AAV capsid expression was p40-mediated. We hypothesized that the constitutively active spleen focus forming virus (SFFV) promoter located upstream of the p40 promoter, contributed to the activation of the AAV endogenous promoter. Interestingly, however, strong p40 activity was also detected in constructs lacking the SFFV exogenous promoter, which prompted us to investigate the mechanisms involved in p40 activation. To do so, we have evaluated the contribution of the AAV inverted terminal repeats (ITRs) and cap 5' untranslated region (5'UTR) to p40 activity in numerous immortalised cell lines using plasmids and AAV vectors, as well as in vivo in mice using AAV vectors. This study has the potential to improve our understanding of AAV2 biology and to influence the design of p40-containing, rep-deficient AAV vectors and selection platforms.

P016

Casein kinase 2 phosphorylates Rep78 of adeno-associated virus (AAV) type 2 Rep78 with effects upon its biochemistry and boosts AAV replication

ABSTRACT WITHDRAWN

P017

Scalable AAV production using stable helper virus-free AAV producer cell lines based on CEVEC's CAP-GT cells

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Over the past decade, unprecedented insights into the molecular mechanisms of inherited and acquired human diseases shifted gene therapy into the focus of clinical medicine. Results from clinical trials evidencing low immunogenicity, broad targeting spectrum and long-lasting transgene expression appointed recombinant adeno-associated virus (rAAV) as the most promising gene transfer vehicle. Consequently, the expanding clinical demand for rAAV production underscored a critical need for scalable manufacturing processes capable of generating large yields of pure rAAV particles. Using an innovative approach based on our proprietary serum-free suspension cell line, we have developed a novel AAV production platform based on stable AAV producer cells. This inducible system overcomes scalability issues by enabling the manufacturing of viral vectors in suspension culture. To generate the stable rAAV producer cell lines, suspension CAP-GT cells were genetically modified to stably express Rep proteins, as well as the adenoviral helper functions E2A, E4orf6 and VA RNA. The resulting AAV pre-packaging cell lines were further genetically modified by stable integration of the capsid gene. Subsequent addition of the transgene flanked by the ITRs resulted in inducible rAAV producer cell lines. For a robust and scalable AAV production process, upstream process development using the ambr15 system as scale-down model was performed which was followed by a scale-up into disposable stirred tank bioreactors. In conclusion our stable helper virus-free AAV production platform tackles the challenges posed by the high demand for AAV production at industry scale by offering a reproducible, scalable and cost-efficient delivery of high-titer and high-quality viral vectors.

P018

AAV9.hCLN2 (RGX-181) improves survival and neuropathology in TPP1m1j mice, a model for CLN2 Batten disease

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CLN2 (late infantile neuronal ceroid lipofuscinosis type 2) disease is a rare, genetic disorder caused by a mutation in the tripeptidyl peptidase 1 (TPP1)/CLN2 gene. A deficiency in TPP1 leads to lysosomal dysfunction and profound neurodegeneration. CLN2 disease is diagnosed from 2–4 years of age and progresses to a loss of cognitive abilities, motor function, vision and early death. RGX-181, a recombinant adeno-associated virus of serotype 9 (AAV9) containing a human CLN2 (hCLN2) expression cassette, was evaluated in TPP1m1j mice, a model of CLN2 disease. RGX-181 was administered by intracerebroventricular (ICV) injection to groups of male and female TPP1m1j mice (4–5 weeks old) at escalating doses. At 13 weeks of age, a subset of animals (5/sex/group) were euthanised for analysis of TPP1 activity and neuropathology. Additional animals (5/sex/group) remained on study to evaluate survival. As expected, mortality in untreated TPP1m1j mice occurred between 11 to 18 weeks of age. No improvement in survival was observed at the lowest dose, whereas, at 36 weeks (study ongoing) there is 100% survival in animals at the highest dose tested and 70% survival in animals at a mid dose level. At 13 weeks of age, increases in TPP1 activity were observed in the serum, brain, spinal cord and liver at all doses. Neuropathological analysis showed a pronounced reduction in the neuropathological outcome measures that correlated with the ongoing survival. These preclinical results demonstrate the potential therapeutic efficacy of RGX-181 to prevent clinical manifestations of CLN2 disease.

P019

Finding the perfect match: Directed AAV vector development guided by Targeted High Expression

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The power of AAV Directed Evolution for identifying novel vector variants with improved properties is well established, as evident by numerous publications reporting bioengineered AAV variants over the last decade. However, most capsid variants have been identified using either replication competent selection platforms or PCR based recovery method. Those strategies can bias the selection towards efficiently replicating viruses or vector variants that are unable to complete the intracellular journey, therefore not leading to productive expression, respectively. The aim of this study was to develop and validate a novel AAV selection platform, which allows for rapid identification of novel highly functional variants based on transgene expression in target cells. We engineered an AAV selection platform, named Targeted High Expression (THE) platform, that encodes a reporter transgene and AAV capsid libraries. In contrast to other platforms, our design allows for selection of capsids based on transgene expression in the target cells. Initial data indicate that our platform works with a range of promoters and cell types and that the design allows for efficient packaging of encoded AAV variants. An initial test-selection experiment on the human hepatoma-derived cell line HuH-7 confirmed that our novel design robustly supports the Directed Evolution process and helps identifying highly functional novel AAV variants. Furthermore, results of cross-packaging studies suggest that the THE platform is not prone to uncontrolled cross-packaging, which further validates this novel design as an attractive alternative method for AAV engineering.

P020

Optimization of elution conditions for immunoaffinity chromatography of AAV5 and AAV9 vectors

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Recombinant adeno-associated virus (rAAV) vectors are the most convenient instruments for gene therapy of severe inherited diseases due to their safety, lack of pathogenicity and low immunogenicity in humans. To date a number of AAV-based systems for therapeutic transgene delivery have been described and evaluated in vivo. Effective upstream and downstream processes will facilitate drug launching for mostly incurable diseases. Moreover development of a universal method based on affinity chromatography is an attractive strategy that enables scalable and versatile one-step purification process for rAAV vectors

screening. The most commonly used protocols applied for rAAV immunoaffinity chromatography using commercially available resins utilize relatively harsh conditions at low pH for AAV elution procedure. But relative instability of several rAAV serotypes at low pH conditions previously was considered to be a limitation for purification by affinity chromatography. In the present work we described an approach for an optimization of elution conditions for affinity chromatography of rAAV5 and rAAV9 with improved yield and vector stability. Implementation of modified conditions for rAAV elution in affinity chromatography resulted in a yield increase of more than 40%-60% by vector genomes (qPCR) depending on serotype. We also showed that the results of a transduction test of the eluted vectors was comparable to those obtained for the vectors purified by iodixanol gradient ultracentrifugation. Thus, the new approach allows us to purify fully functional rAAV vector while avoiding harsh conditions of standard elution protocol for commercially available resins for AAV affinity chromatography.

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P021

Cryo and negative staining EM – a comparison study with AAV capsids

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Academic and industrial labs frequently use AAV vectors for the development of gene therapies. A robust and reliable quantification of rAAV titers is indispensable, to ensure safety and efficacy of these therapies. There has been an ongoing debate about the reliability of determination of the full and empty capsid ratio using negative staining electron microscopy (EM). As an alternative, cryo-EM has been suggested to be less prone to artifacts and thus its results more reproducible for determining empty and full capsids. PROGEN now conducted a study to compare negative staining EM and cryo-EM with regard to discrimination between full and empty capsids and accuracy of the determined ratios. AAV preparations of the AAV serotypes 3, 5, 6 and 9 as well as AAV8 W217L, which is equivalent to the ATCC RSM, were used for this study. The analysis of the two determination methods resulted in almost identical ratios using the same AAV preparations for both, the negative staining and the cryo-EM analyses. This result supports the validity of the negative staining EM method, if a well-established protocol is used. The outcome of this study confirms the quality and reliability of PROGEN's calibration process and the strict quality control stated for the AAV Titration ELISAs. For the calibration of PROGEN's AAV2 and AAV8 Titration ELISAs, the available ATCC RSM standard material is used. For all other serotypes, PROGEN developed an in-house standard characterized by several methods, including negative staining EM for the determination of full to empty capsid ratios.

P022

Capsid engineering overcomes barriers toward Adeno-associated viral (AAV) vector-mediated transduction of endothelial cells

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Due to their key functions in vascular homeostasis, Endothelial cells (EC) are important targets in gene therapy and regenerative medicine. However, Adeno-associated viral (AAV) vectors of various serotypes transduce EC inefficiently. In order to identify barriers limiting the EC transduction, we performed an in vitro AAV peptide display selection on primary human macrovascular EC. This screen led to the identification AAV-VEC, a capsid variant, which showed a 3-fold improved transduction efficiency compared to the parental AAV-2 serotype. AAV-VEC also outperformed first-generation targeting vectors in EC transduction. When analyzing AAV-VEC infection path, the most significant change in EC-AAV interaction for AAV-VEC compared to AAV-2 was observed for vector uptake. Notably, AAV-VEC transduced not only proliferating EC, but also quiescent EC and iPSC-derived endothelial progenitor cells (iPSC-EPC). The high susceptibility of these iPSC-EPC to AAV-VEC is of particular importance as it paves the way for new applications in regenerative medicine and gene therapy.

P023

Advantages of homologous recombination- over transposition-based systems to generate recombinant baculovirus for AAV vector production

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Viral vectors derived from the AAV have a great potential for gene delivery in a wide range of gene therapy indications, but manufacturing at pharmaceutical scale is a big challenge for the industry. The baculovirus-insect cells system is one of the most scalable platforms to produce clinical grade AAV, but the standard procedure to generate recombinant baculovirus based on Tn7 transposition is time consuming. Moreover, we recently shown that baculoviral sequences adjacent to the AAV ITRs are preferentially encapsidated into the AAV particles. This observation implies a safety concern for clinical applications due to the presence of bacterial or antibiotic resistance genes. Here, we investigated a fast and safe system to generate baculovirus based on homologous recombination (HR) for its use in AAV manufacturing. Indeed, we found that HR-based system shown an increased stability of the cassette of interest over passages assessed by qPCR (rAAV genome) or Western blot (Rep-Cap sequences). Moreover, we confirmed the absence of undesirable genes into the final product by PCR. Importantly, we tested these materials to produce AAV vectors and we obtained similar yields compared to the baculovirus generated by transposition procedure. Finally, we also demonstrated that it is possible to generate baculovirus deleted in chitinase

and/or cathepsin genes using the HR system. Deletion of those genes may avoid capsid protein degradation during AAV production. Overall, this study highlights the importance of the upstream process and starting biologic materials to generate more efficient and safer AAV biotherapeutics. Keywords: baculovirus, homologous recombination, Tn7 transposition, AAV

P024

Chemically modified AAV vectors for gene therapy

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Recombinant AAV vectors are now becoming therapeutic products. Despite clinical trials and commercialization of the first treatments, AAV have shown some critical limitations: (i) high doses are usually required to achieve therapeutic effect; (ii) broad biodistribution to non-target tissues; (iii) loss of efficacy in the presence of pre-existing neutralizing antibodies. Our team has developed a chemical technology to modify amino groups of AAV capsid (lysine and arginine) with the goal of overcoming those limitations. Chemical modifications resulted in reduced interactions with neutralizing antibodies. Moreover, the addition of a ligand on the capsid surface redirected the vector tropism and increased selectivity towards specific cells. Here, we described a new chemical modification of the tyrosine residues. Indeed, previous publications showed that AAV's tyrosine mutations improved significantly its transduction efficiency. We hypothesize that chemically modified tyrosine could induce the same effect. As a proof of concept, N-acetylgalactosamine ligand, with a diazonium salt specific reactive function for tyrosine, was synthetized and bioconjugated to AAV2 in order to selectively target hepatocytes. By using Dot and Western blot we demonstrated that this ligand was covalently linked to AAV capsid. Further characterization of this vector is ongoing and results will be presented. In summary, chemically modified vectors represents a promising alternative to genetically modified capsids for gene therapy applications.

P025

AAV development program: towards next generation of livertropic AAV variants

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The therapeutic use of recombinant vectors based on Adeno-Associated Virus (rAAV) has come of age. It is thus far the only in vivo viral gene delivery tool to achieve regulatory approval by the US Food and Drug Administration or the European Medicines Agency. rAAV vectors are the technological foundation underpinning numerous recent successes in human gene therapy trials, with high-profile examples targeting diseases of the central nervous system, eye and liver. These successes hinge on continuing progress in the development of AAV-based gene delivery systems, driven primarily by advances in capsid development. To this end, the Children's Medical Research Institute (CMRI) and LogicBio Therapeutics have established a joint program, with the goal to develop the next generation of AAV vectors for liver-targeted gene delivery. It builds on novel insights into the relationship between AAV capsid structure and function, access to bespoke parental AAV libraries, and proprietary AAV selection platforms. For the liver, this includes access to normal and patient primary hepatocytes for library screening and vector candidates' evaluation in vitro and in vivo in animal models. This unique combination of academic and industrial expertise allows the team to develop a highly sophisticated AAV pipeline, which combines selection of the most functional AAV variants with an early focus on improving manufacturability and immunological profiles. Here we present the vector selection and validation pipeline as well as a set of novel AAV variants optimised for highly efficient functional transduction of primary human hepatocytes with high-yield manufacturability.

P026

Development of purification step for several AAV serotypes using POROS™ CaptureSelect™ AAVX affinity chromatography

ABSTRACT WITHDRAWN

P027

Development of an improved novel AAV capsids for intramuscular delivery

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Muscle-directed gene therapy is a promising approach for treatment of severe genetic disorders. Systemic, intravenous (IV) delivery of recombinant adeno-associated virus (AAV) vectors can transduce multiple skeletal and cardiac muscles in whole body. However, muscle transduction efficiency may be reduced due to viral vector dilution, and wide vector distribution may cause off-target effects. These limitations may be addressed by localized, intramuscular (IM) AAV dosing. To increase localized transduction efficiency in muscle we screened a library of AAV

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capsids and their derivatives for enhanced muscle delivery and two novel capsids, AAV110 and AAV214, were identified. AAV110 was tested by IM injection of AAV110/EGFP vector (1E+11 vg, 100 μL) into each leg of C57BL/6 mice and compared with the same dosage of AAV9/EGFP vector. Ten days after dosing, biodistribution analysis revealed 16-fold higher muscle transduction by AAV110/EGFP vector compared to AAV9/EGFP vector. Surprisingly, sciatic nerve transduction was also significantly increased (29-fold) for AAV110 compared to AAV9, while several other tissues tested (lung, liver, testis, kidney, eye and brain) showed more than 25-fold decreased transduction with AAV110/EGFP compared to AAV9/EGFP dosed by IM. Direct fluorescence imaging and IHC analysis confirmed that transgene expression correlates with biodistribution data. AAV214/EGFP was similarly tested in rats and demonstrated a similar increase in tropism compared to AAV9. AAV214 demonstrated high muscle transduction efficiency not only by IM delivery, but also by IV dosing. Both AAV110 and AAV214 are promising candidates for the treatment of muscular disorders.

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P028

Critical aspects in implementing a precise and reliable droplet digital PCR method as dose measurement assay for determining vector genomes in rAAV- based Gene Therapy products

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Gene therapy is a promising technology that could potentially be used to therapeutically address symptoms of rare diseases caused by genetic disorders. Recombinant adeno associated virus (rAAV) emerged during the decade as the vector of choice for gene therapy. Assessment of rAAV vector genomes as a measure of active ingredient has become industry standard. However, standard technologies used for determining vector genomes such as quantitative PCR (qPCR) often lack accuracy and precision. This may cause variability in the production process when adjusting target concentration as well as variability in the actual dose administered to patients. Droplet digital PCR (ddPCR) has been identified as potential technology to overcome this problem. In our studies qPCR technology was compared to ddPCR technology in terms of accuracy, precision and dilutional linearity. Furthermore, the impact of changing the amplification target region on vector genome quantification has been assessed for each technique. Finally, the influence of high vector genome titers on ddPCR reliability when applying standard sample preparation procedures was evaluated and compared to optimized sample pre-processing.

P029

SuRE: an unbiased, genome-wide assay to identify regulatory elements for custom-made gene expression

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Effective gene therapy requires robust expression of the therapeutic gene. Expression needs to be stable over prolonged periods and is often preferred to be cell-type specific. Moreover, in some cases inducible expression is required. Therefore, identifying the right regulatory element to drive expression of the therapeutic gene is an important determinant for gene therapy efficacy. Typically gene expression is studied by profiling RNA levels. Such work however does not tell us which DNA elements regulate gene expression. Chromatin profiling can help us to identify such regulatory elements, but is bad at predicting how they perform in isolation in an expression vector. Ideally one would exhaustively screen the entire human genome for elements that can appropriately regulate gene expression by themselves in an expression vector. Gen-X is a spin-off from the Netherlands Cancer Institute that employs the novel SuRE assay to screen >300 million DNA elements for their capacity to drive gene expression. We demonstrate how the SuRE assay provides an unbiased, genome-wide profile of regulatory elements. Also we illustrate how such regulatory profiles in different cell types or conditions can be used to identify tissue-specific regulatory elements and elements that are induced by a stimulus. Gen-X is open to explore projects with industry and academic partners.

P030

The new AAV3 Titration ELISA – continued tradition of reliable AAV titer determination

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AAV vectors are frequently used by academic and industrial labs for the development of gene therapies. A growing number of serotypes are being used for various diseases. To ensure safety and efficacy of these therapies, a robust and reliable quantification of rAAV titers is indispensable. Meeting this need of the gene therapy community, PROGEN developed a new AAV3 Titration ELISA for reliable determination of total capsid titer. The AAV3 ELISA was developed following the well-established workflow for other PROGEN AAV serotype ELISAs1,2. A purified AAV3 gold standard material was generated and quantified by multiple qPCR and electron microscopy experiments in several labs to determine the ratio filled/empty capsid and quantification of viral DNA. Each lot of AAV3 Titration ELISAs is calibrated using this internal gold standard and compared to previous lots in order to ensure minimal lot-to-lot variation. Following the tradition of offering consistent, robust and reliable titer determination for most relevant AAV serotypes, the AAV3 ELISA complements PROGEN's portfolio. 1 Lock, M. et al. Characterization of a recombinant adeno-associated virus type 2 Reference Standard Material. Hum Gene Ther. 10, 1273-85 (2010) 2 Ayuso, E. et al. Manufacturing and characterization of a recombinant adeno-associated virus type 8 reference standard material. Hum Gene Ther. 11, 977–87 (2014)

P031

Evaluation of rAAV vectors engineered for muscle gene delivery

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Recombinant adeno-associated viral (rAAV) vectors demonstrate great promise as the leading platform for in vivo gene delivery. A variety of rAAV vectors enable delivery to multiple tissues, including the muscular system, for the treatment of many genetic and other complex diseases. Some natural serotypes as well as engineered rAAV capsids exhibit enhanced widespread biodistribution to muscle, which could reduce the total dose required. Previously, muscular gene delivery was performed by direct injection of rAAV vectors into muscle. More recently, intravenous (IV) administration of rAAV has been increasingly employed to facilitate distribution to many types of muscle and is now the preferred administration route for several clinical trials. As rAAV drug products advance clinically, analytical tools to characterize these complex products are needed. Examples include characterization of rAAV vector stock titer, purity and its genome integrity. Development and validation of these and additional assays will enable full characterization of rAAV vectors. Another critical tool is development of an in vitro potency assay, which is discussed here. rAAV vectors with capsids of various natural serotypes and engineered capsids were compared to estimate their efficacy for systemic administration and their potential for the development of treatment for Duchenne muscular dystrophy. In applications that require systemic administration of high doses of vectors, such as Duchenne muscular dystrophy, these analytical tools and assays have the potential to further characterize and improve rAAV products, and thereby improve safety and efficacy of gene transfer therapy.

P032

Adenoassociated virus (AAV) serotype 3-based vector as an alternative vehicle for liver-directed gene therapy

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AAV-based vectors are vehicles of choice for liver-targeted gene therapy, as shown in the recent clinical trials for hemophilia. In most of these studies, AAV5, AAV8, and AAV9-based vectors are used, wherein the patients with neutralizing antibodies (NAb) are excluded. In addition, re-administration of an AAV vector with the same serotype is not feasible with NAb. In this study, we searched for an alternative AAV serotype for liver-directed gene therapy to overcome existing NAb. Several AAV serotypes were screened with mouse and human liver cell lines. With murine hepatocyte line TLR3, AAV8 was the most efficient, followed by AAV2 and AAV9. With human cell lines HepG2 and Huh7, however, AAV3 showed the strongest or near-highest expression, while AAV8 and AAV9 allowed limited expressiom. Subsequently, AAV vectors encoding firefly luciferase were intravenously given to mice to monitor transgene expression in vivo. In consistent with the in vitro data and previous studies by others, intravenous injection of AAV8 and AAV9 vectors resulted in robust and sustained transgene expression in the mouse liver, while AAV3 exhibited limited expression. This result was recapitulated with AAV vectors encoding coagulation factor VIII and IX; significant expression was observed with AAV8 and AAV9, while AAV3 produced negligible clotting factors. Our cell line-based analysis implicated that AAV3 represents an alternative serotype for liver-directed gene therapy and the necessity of further preclinical studies. Translation of murine in vivo data to clinical indication should be considered with caution, and studies with other species than mouse may be required.

P033

Improving the high-throughput sequencing of adeno associated viral vector genome using an optimized PCR-free protocol

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The increasing number of successful gene therapy clinical trials using AAV augurs a bright future for these small viral vectors. However, the development of new drugs goes hand in hand with higher quantity and quality standards. In particular, new methods to assess the level of residual DNA in AAV vector stocks are needed, considering the potential risk of co-transferring oncogenic or immunogenic sequences with the therapeutic vectors. Our laboratory has developed an assay based on high-throughput sequencing (HTS) to exhaustively identify and quantify DNA species in recombinant AAV batches. In combination with a computational analysis of the single nucleotide variants, the single-stranded virus sequencing (SSV-Seq) also provides information regarding AAV genome identity. Here, we have optimized this protocol to improve the sequencing coverage over GC-rich regions in the AAV genome. Three PCR-free kits have been tested to prepare sequencing libraries and reduce the coverage bias due to the PCR amplification step. Among those, the NxSeq AmpFREE Low DNA kit allows to reach the best concentration of sequencing libraries with a negligible amount of unligated adapters. The PCR-free protocol requires four times more biological material than the original SSV-Seq, i.e. 4x1011 versus 1x1011 vg, and consequently is proposed as an alternative method for the analysis of rAAV genomes that are composed of regions with high GC-content as the CAG promoter. HTS-based assays represent today the most accurate way to fulfill regulatory requirements concerning AAV vector quality and purity to improve its safety and efficiency. Keywords: AAV, residual DNA, sequencing, vector genome

P034

Effective stabilization of viral vectors in liquid using an algorithm-based development approach

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1: LEUKOCARE AG

Replication-deficient recombinant viral vectors such as adenovirus serotype 5 (Ad5) and adeno-associated virus (AAV) represent a rapidly growing field of vaccine development and gene therapy. Viral vectors are prone to degradation induced by stress related to manufacturing, storage and distribution. This poses a significant hurdle for the development and administration of vector-based pharmaceuticals such as vaccines or gene therapeutics. To develop effectively stabilizing formulations for Ad5, we applied an algorithm-based development strategy including Design of Experiment. Ad5 was stored for short term at

37 °C and 25 °C as accelerated aging temperature to identify the most effective stabilizing excipients and combinations thereof. These accelerated aging conditions were shown to be predictive for real-time storage at 5 °C. Several of the excipients indicated a neutral influence, whereby a well-balanced combination of the components was important regarding concentration and stabilizing interactions. The predictive power of this approach was confirmed by long-term storage of two iteratively improved formulations tailored for Ad5. The highly efficient pre-selection strategy of stabilizing excipients by an algorithm-based development approach and accelerated aging model enables the generation of best-in-class stable liquid formulations for viral vectors in short development time. Stable titres can avoid lyophilization or high overage and thus reduce manufacturing costs. This approach is transferred to AAV and could have beneficial impact when applied early in downstream processing. A transfer to the frequently applied gene therapy vector system AAV is currently being implemented and an update will be presented at the conference.

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P035

Scalable chromatographic enrichment of full AAV capsids

ABSTRACT WITHDRAWN

P036

Enhanced Adeno-associated virus transduction in endothelial cells

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1: A.I. Virtanen Institute, University of Eastern Finland 2: Heart Center, Kuopio University Hospital 3: Gene Therapy Unit, Kuopio University Hospital

Gene therapy can be a powerful therapeutic option for a multitude of disorders, including those requiring angiogenesis. Viral vectors can be improved by specific targeting to cell types, such as endothelial cells (EC). These cells are critical for developmental and physiological vessel formation and are an important cell target for therapeutic angiogenesis. The aim of this study was to develop Adeno-associated virus (AAV) vectors that specifically express in EC and to optimise AAV transduction of EC. Endothelial primary cells and cell lines were transduced with different serotypes of AAV, time course analysis was performed, and media conditions were tested. As expected AAV2 and 6 transduced human EC the best, while AAV8 and 9 did so poorly. Reporter gene expression demonstrated rapid transduction of AAV6 reaching a maximum already within 24h, in contrast AAV2 was slower and peaked between days 4–6. Surprisingly, media had a significant effect on AAV2 transduction efficiency such that Dulbecco's Modified Eagle's Medium (DMEM) significantly increased AAV2 transduction efficiency in EC compared to their own media. We could attribute some, but not all, of the media difference in transduction efficiency to the presence of fetal bovine serum (FBS) and heparan sulphate in EC media. Further studies are ongoing to elucidate the media-effect on AAV transduction, in addition to validate endothelial-specific vectors that have been in development.

P037

Molecular design optimization towards the development of a high scale rAAV8 production process using BEVS

ABSTRACT WITHDRAWN

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P039

Development of new AAV extraction protocol for titration AAV using quantitative PCR and ELISA

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Precise and robust analytical methods of titration of recombinant adeno-associated viral vectors (rAAV) are crucial for correct monitoring of rAAV titer during manufacturing process and for precise dozing of rAAV drug product for clinical trials and for approved rAAV products. The precision of AAV tittering during upstream process depends on the efficiency of rAAV particles extraction from AAV particle-producing cells. Conventional methods such as the freeze-thaw or sonication methods are time-consuming, require special equipment and usually have low extraction efficiency. Although there are commercially available kits for high efficiency AAV extraction, these kits are usually applicable for qPCR titration and cannot be directly used for ELISA measurements due to the effect of unknown components. Thus, new universal methods for high efficiency AAV extraction are needed to be applied both for qPCR and ELISA measurements and for reducing the costs of extraction. Here we describe a new optimized protocol for high efficiency AAV extraction from AAV particle-producing cells. We show that our protocol allows us to extract AAV from cells with the efficacy as high as it was reported previously for commercially available kits. Moreover, AAV material obtained from AAV particle-producing cells with our new protocol can be applicable directly for qPCR and ELISA measurements to detect both DNA-containing AAV and total assembled capsid particles. Besides that, the costs of our new optimized protocol for high efficiency AAV extraction is much low than existing commercially available kits which allows us to reduce the overall costs of AAV titering.

P040

Self-repressing ‘helper’ adenovirus enabled efficient manufacture of adeno-associated viral vectors without contamination by adenovirus or small drugs

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1: University of Oxford 2: Oxford Genetics Ltd

Adeno-associated virus (AAV) shows great promise as a gene therapy vector, however current manufacturing regimes cannot scale to meet increasing demands. Here we describe a new synthetic biology approach to regulate the ‘helper’ adenovirus replicative life cycle to allow the clean and efficient manufacture of AAV vectors. We achieved this by genetic manipulation of the adenovirus Major Late Promoter (MLP) in situ to enable temporal regulation of its late proteins using a tetracycline-controlled system. Modification of the adenovirus MLP in situ to provide inducible repression has not previously been demonstrated, primarily because the virus DNA polymerase coding sequence is in the opposing DNA strand. We show that strategic insertion of tetracycline repressor binding sites into the MLP and encoding the tetracycline repressor under its transcriptional control, allows for normal adenovirus replication in the presence of doxycycline but only enhanced genome amplification and early gene expression (important for the ‘helper’ functions) in its absence. Using this negative feedback self-repression system, we demonstrated delivery of adenoviral ‘helper’ functions and AAV DNA to yield >4-fold increases in AAV vector production, with particles exhibited improve encapsulation and viral transduction, and a reduction in contaminating adenoviruses by up to $\sim$2.5 million-fold. Moreover, by infecting AAV packaging cells this novel approach allows for a scalable, single agent, AAV vector manufacture system that is free of plasmid DNA and contaminants, with considerable advantages over standard production approaches.

P041

A new gene therapy method to assess transduction efficiency at the single-cell level

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Many preclinical and clinical studies of gene therapy (GT) are based on the use of lentiviruses as the vector of choice. Assessment of the vector titer and transduction efficiency of the cell product are critical for these studies. Efficacy and safety of the corrected cell product are commonly determined assessing the vector copy number (VCN) by qPCR. This optimized and well-established method in the GT field is however based upon bulk population averages which can lead to misinterpretation of the actual VCN of the transduced cells. Therefore we introduce here a single-cell based method that allows to unmask cellular heterogeneity in the GT product, even when antibodies are not available. We use the Invitrogen PrimeFlow RNA Assay®, a flow cytometry based assay, with customized probes to detect our transgenes of interest to determine transduction efficiency, promoter strength and cellular heterogeneity on murine and human stem cells. Indeed, customized PrimeFlow RNA Assay® shows good specificity and sensitivity to detect the transgenes. We observed high correlation of the standard VCN obtained by qPCR with the percentage of transduced cells expressing the transgene and/or their MFI. Differences in promoter strengths can readily be detected. Hence, we show a customizable method that allows to determine the “real” VCN of the actual transduced cells from a GT product, being adaptable to other therapeutic genes for which antibodies are not available or cumbersome for flow cytometry. The method also allows co-staining for surface markers to analyse differential transduction efficiencies in subpopulations of target cells.

P042

Synergistic effect of combined administration of an oncolytic adenovirus and Bifidobacterium spp. supplements in a mouse model of melanoma

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In the last decade, cancer immunotherapy has delivered impressive results in clinical settings. However, efficacy has not been consistent probably because of a number of environmental and genetic factors influencing the outcome. Recent studies have shown that intestinal microbiota can affect checkpoint inhibitors-based immunotherapy outcome both in animal models and patients. In particular, Bifidobacterium seems to have a role as a positive regulator of antitumor immunity in vivo by promoting pro-inflammatory signals in innate immune cells. We hypothesized that modulation of host microbiota could also synergize with active immune therapy, such as oncolytic viruses. Oncolytic viruses can infect and lyse tumor cells causing the release of tumor-associated antigen therefore stimulating an antitumoral immune response. We decided to investigate whether administration of Bifidobacterium could positively affect response to oncolytic vaccines. At this aim, we administered a mix of Bifidobacterium to C57BL/6J mice inoculated with a syngeneic B16-OVA melanoma cells and then treated them with either oncolytic vectors or a mock treatment and compared melanoma growth rate with groups that did not receive bacterial supplementation. Mice treated with Bifidobacterium supplements showed an increased response to the oncolytic adenoviral therapy compared to control groups. In order to characterize the effect of Bifidobacterium administration on gut microbiota, we analyzed fecal microbiome at different time-points during the experiment with 16S rRNA sequencing. We also characterized possible correlations between perturbation of gut microbiome and systemic immune responses to the oncolytic adenovirus, determining the phenotype of antigen-specific and memory T-cell after the different treatments.

P043

Development of secreted nucleases to eliminate residual DNA during viral vector manufacture

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1: Oxford BioMedica

Viral-based therapies have become promising for the treatment of a variety of genetic diseases. Batch-consistency and the reduction of contaminants such as DNA from the final vector product are highly desirable, especially when direct in vivo delivery of high dose viral vector is required. A standard approach to minimize DNA contamination is the use of GMP grade recombinant nucleases (e.g. Benzonase®, SAN) to treat vector harvest prior to downstream purification. However, lower nuclease activity of some of these nucleases at physiological salt typically requires a second nuclease treatment during downstream processing of lentiviral vectors, which exposes the vector to elevated temperatures and potential loss in activity. We have developed a highly efficient alternative approach that uses secreted nucleases co-produced with viral vectors. This can be achieved by either: [1] co-expression of nuclease and vector components within the same cells or [2] co-culturing vector producing cells with nuclease-expressing Helper cells. We show data on the use of endonucleases from serratia marcescens, vibrio salmonicida, and vibrio cholerae engineered to be secreted from adherent and suspension viral vector manufacturing cells and compare their performance with standard nucleases. These secreted nucleases efficiently clear residual DNA from the final product without an impact on vector titres. We also describe modifications to nucleases that optimise activity in a wide range of pH and physiological salt levels as well as across different vector platforms. Further, we address our efforts on the optimisation of the use of Helper cells transiently or stably expressing nucleases in vector production bioreactors.

P044

Impact of production impurities on potency of Adeno-associated virus vectors: systematic comparison of density gradient and affinity purification

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Adeno-associated virus (AAV)-derived vectors are the vehicles commonly used for development of gene therapies. For research, their purification is typically based on density gradients (DG), while purification for clinical application requires chromatography for improved purity. Although the existence of differences in purity are well known in the field, to date no study has systematically investigated their impact on vector potency. We compared a standard DG purification method to an affinity chromatography (AC) method we developed. SDS-PAGE revealed multiple protein impurities in DG preparations and mass spectrometry identified that only 5.49 ± 2.9 % (mean±SD) of proteins in DG preparations were AAV vector proteins, in contrast to 93.24 ± 4.9 % for AC. While 541 different contaminating proteins were detected in DG preparations, only 14 were identified in AC. Systemic injections of $1 \cdot 1010$ or $1 \cdot 1011$ viral genome copies (VGC) of AAV9-EGFP from both manufacturing methods were performed to compare cardiac vector potency in mice. AC purified vectors resulted in significantly increased vector potency on DNA, RNA and protein levels in the heart, with more pronounced effects at the high dose. At $1 \cdot 1011$ VGC/animal, vector DNA copy numbers were increased more than 10-fold, while transgene mRNA copies were increased more than 500-fold. EGFP protein expression was increased 30-fold and a similar pattern was obtained from the liver, suggesting that vector potency but not the bio-distribution profile is altered by contaminating proteins in vector preparations. Our data demonstrates the effect of contaminations in vector preparations and suggests the use of ultra-pure vectors already at basic research level.

P045

Accelerating lentivirus manufacturing to the clinical

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Lentiviral vectors (LVVs) have been increasingly used as gene and cell therapy vectors for the treatment of acquired and inherited diseases. However, the bioprocess of LVVs poses several challenges since the viral particles to be effective must maintain their

infectivity. LVVs face the downstream challenges common to retroviridae family of vectors namely short half-lives at room temperature, sensitivity to pH variations and salt concentrations, and shear stress that compromises the translation to the clinic. To address the downstream bottlenecks we report an improved lentivirus purification process for phase I and II clinical trials and present a case on the use of new materials as a replacement for traditional centrifugation. 3D printing customized matrices and membrane chromatography will contribute to the available toolbox of chromatography-based downstream processing. We implemented a scalable protocol for lentiviral vector that is easy to transfer to GMP environment, combining microfiltration, anion-exchange, and ultrafiltration membrane technologies towards maximization of infectious virus recovery, allowing generation of clinical-grade viral vectors without the need for cleaning validation in a cost-effective manner. The process developed allowed a volume reduction of up to 100 and a maximum concentration of infectious particles up to 2x108 IP/ml. Assessment of LVVs quality was carried out and infectivities from 8 x104 and 5 x104 IP/ng p24 indicate that purified clinical vectors show high infectivity. The work under developing can be further extended in the future to redesign biomanufacturing strategies for other viral particles or other fragile macromolecules.

P046

Automated HEK293 suspension cell adaptation to new media and use of new DoE approach to optimize AAV production in suspension utilizing the ambr®15 platform

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A major bottleneck to translate gene therapy approaches based on adeno-associated viral vectors (AAV) into the clinic is the manufacturing at large scale. The most common platform for producing AAV at laboratory scale is the transient transfection of adherent HEK293 cells. Nonetheless, this technology is difficult to scale up, while suspension cultures are easier to move into large bioreactors. Several parameters are key to develop this technology; the selection of the cell line, the culture media and transfection reagents/conditions. Here, we have used Sartorius ambr®15 system to adapt two commercially available HEK293 suspension cells (Expi293f, ATCC CRL-1573.3) into 7 culture media. Ambr®15 is a high throughput, automated 15mL microbioreactor platform running up to 48 independent cultivations at the same time. Using a direct or a fully automated sequential protocol we have been able to adapt both cell lines into 6 media. Next, we selected the 3 best candidates to screen transfection conditions using a DoE protocol with the Sartorius MODDE® software. The protocol tested several variables: transfection agents (PEImax, Expifectamine and PEIpro), ratio PEI/DNA, total DNA, ratio helper: vector, complex time and cell density. Preliminary data showed a productivity in the range of 4E10 vg/mL (harvest) and identified those parameters with bigger impact on the AAV titer. New DoE protocols will be tested to further optimize this productivity and results will be presented. Notably, this work has been performed in <6 months, highlighting the advantages of using ambr®15 for process development activities in the context of AAV manufacturing in suspension cultures.

P047

Comparison of lentiviral vector production in two fixed-bed bioreactors

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For a long time commercial scale viral vector manufacturing was a bottleneck in the field. Biggest challenges were related to those vectors, such as lentiviral vectors, requiring plasmid transfection of adherent cells in large volumes. We have previously developed a commercial scale third generation lentiviral vector manufacturing process utilizing PEIpro® mediated transfection of adherent cells using iCELLis fixed-bed bioreactors that currently have cell growth surface up to 500m2. Using process parameters optimized in iCELLis Nano, lentiviral vector production was tested and compared in another fixed-bed bioreactor, Scale-X (Univercells), that in the future, Scale-X technology will have cell growth surface up to 2400 m2. Process parameters used in iCELLis Nano were easily transferred to Scale-X and productivity was comparable to iCELLis Nano. Likely, due to composition of the fixed-bed cell distribution was found more equal compared to iCELLis Nano, and moreover, media consumption was slightly smaller in Scale-X. Thus, there is a new promising option for commercial scale virus production on market that can be used for adherent cells.

P048

Scalable and high-titre lentiviral vector production from cGMP-compliant clonal suspension HEK293 cell line

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1: Oxford Genetics Ltd

Lentiviral vectors (LV) present huge potential for cell and gene therapy, allowing long-term genetic modification of a wide range of cell types. The positive clinical data around new therapeutic approaches for a range of disorders, such as CAR-T, is resulting in increased demand for viral vectors and manufacturing capacity. Currently, the industry standard still involves use of adherent cells in processes that lack scalability, robustness and have high associated cost of goods. Oxford Genetics (OG) has developed a proprietary 3rd generation LV packaging plasmid system with codon optimised sequences, reduced vector backbones and minimised inter-cassette homology for improved LV production efficiency and safety profile. OG's LV plasmid system has outperformed major competing LV packaging systems in terms of viral productivity and related quality attributes (e.g. I/P ratio) in both adherent and suspension HEK293T/HEK293 cell lines. Upstream process development has been performed for improved LV production with the above plasmid system in OG's cGMP-compliant clonal suspension HEK293 cell line HEK293OX_BCD_SP7D5. Cell seeding density, plasmid ratio, transfection reagents/ protocols, post-transfection supplementation regime and harvest time-course have been optimised utilising DoE design with AMBR15 bioreactor system. An infectious titre of 1E8 TU/mL has been consistently achieved using the optimised production method across various scales of shake flasks and 0.5–1L stirred tank bioreactors,

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demonstrating a scalable and efficient LV vector production platform which enables rapid transition to large scale vector manufacture.

P049

Development, optimization and scalability of the Pro10 rAAV manufacturing process

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1: Asklepios Biopharmaceutial, Inc

Recombinant adeno-associated virus (rAAV) has emerged as the viral vector of choice for in vivo gene delivery. The use of rAAV along with its proven safety and efficacy in human clinical trials has underscored the need for manufacturing technologies capable of generating commercial grade quantities of highly pure rAAV vectors. To date, generating vector quantities sufficient to meet the expanding clinical and commercial demand is a hurdle for the current manufacturing platforms. The Pro10™ manufacturing system is based on the triple plasmid transfection of a suspension-adapted, HEK293 cell line that is capable of universal manufacturing of all serotypes and chimeric capsids of AAV achieving purified titers of 1x1017 vector genomes from 250–500L SUB cultures. Replacement of plasmid DNA with structurally distinct closed linear double stranded DNAs (DoggyboneTM DNA; dbDNA) manufactured synthetically via rolling circle amplification has led to improvements in safety, 3–4 fold increases in rAAV productivity with less transfected DNA and overall scalability of rAAV vector manufacturing. dbDNA is one of several recent improvements to the Pro10™ manufacturing system that have been optimized and established to achieve commercial grade rAAV vector quantities for supply to patients.

P050

Impact of full and empty particle concentration on product quality and *in vivo* efficacy of HMI-102 in a mouse model of phenylketonuria

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1: Homology Medicines Inc

Phenylketonuria (PKU) is an autosomal recessive disorder where a mutation occurs in the PAH gene on chromosome 12 that results in a deficiency of the enzyme, phenylalanine hydroxylase (PAH). Currently, the course of treatment is restrictive diets, nutritional supplements, or daily subcutaneous injections of an enzyme substitution therapy or a small molecule therapy. Here, a gene therapy approach is described using novel AAVHSC15 vector packaging a human PAH cDNA, HMI-102, as a prospective one-time administration treatment for PKU targeting the genetic cause of the disease. During AAV manufacturing, the vector product contains both full and empty capsids. Historically, empty capsids have been considered a process related impurity and the impact of empty capsids in vivo has been debated depending on delivery method, target tissue, and clinical outcome. In this study, we examined whether varying amounts of empty capsids can influence HMI-102 biological activity and potency, in vivo using the PAHenu2 murine model of PKU. The data demonstrated that a wide range of empty capsid ratios selected resulted in comparable levels of vector quality, transduction efficiency, and biological activity. Furthermore, phenylalanine (Phe) levels of dosed mice were significantly reduced and remained within the therapeutic range throughout the study duration. While a general awareness of empty capsid ratios can be used by manufacturers to find the proper balance to scale and purify the vector product long term, the varying amounts examined did not impact the biologic activity of HMI-102 in normalizing Phe levels in a murine model of PKU.

P051

Lentiviral/Retroviral Vector large scale manufacturing

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1: MolMed S.p.A.

MolMed is a medical biotechnology company focused on research, development and clinical validation of innovative therapies to treat cancer and genetic rare diseases. Lentiviral vectors, produced with transient quadri-transfection in 293T cell line and retroviral vectors, produced with a stable packaging cell line, are used in gene therapy studies. In the context of advanced clinical studies or commercial phase, an important manufacturing challenge is the optimization of vector production in large-scale platforms. To address this issue and to obtain a scalable and robust process, we are developing processes with cells in adhesion using Pall iCELLis® fixed-bed disposable bioreactors and with suspension cells in a culture system without animal derived reagents in stirred tank bioreactor. Transfection agent of choice is PEI-PRO HQ by Polyplus that allow efficient transfection of packaging cells without toxicity issues. The optimized parameters included seeding cell density, DNA concentration, timing and volumes of harvest. Final vectors were characterized for infectious viral titre, particle content, process related impurities and then compared to vectors produced by current GMP processes based on Cell Factories. Downstream process was appropriately scaled up and designed in order to accommodate larger volumes of vectors from bioreactors. Single use Tangential flow Chromatography using AKTA Ready Flux and DEAE chromatography step using AKTA Pilot and Axichrom columns were successfully applied both in scaled down model and in final full scale with good removal of process related contaminants and no detrimental impact on vector infectivity and stability.

P052

Chromatographic approach for separation of AAV capsid variants

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During rAAV vector production in mammalian and insect cell line-based platform, a spectrum of structural variants are co-synthesised wherein empty capsids are present in dominant proportion. Presence of these empty capsids in a clinical grade material reduces the potency and poses a significant immunogenic burden on the patient. Current ultracentrifugation-based methods for removal

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of empty capsids though effective often exert operational complexity and necessitate the availability of specialized centrifugation equipment. Here, we report an alternative chromatographic process for the separation of AAV capsid variants. The proposed cation-exchange chromatography method explores the potential of a pH gradient based elution protocol for high-resolution separation of AAV charged variants by loading an affinity-chromatography purified AAV material. A systemic developmental approach incorporating continuous and discontinuous pH gradient was applied to find an optimal pH conditions for selective elution of empty and genome containing capsids of AAV. These findings were further extended to modulate the binding conditions to achieve selective, exclusive binding of genomic capsids over empty capsids on the ion-exchange medium with a resultant eluate fraction rich in genome containing capsids. Further characterization of the purified material via analytical ultracentrifugation, total capsid quantification, negative staining electron microscopy, and transduction unit will be discussed. Moreover, the results of chromatographic process characterization, including process scalability, will also be presented.

P053

Development of a Scalable platform for AAV manufacturing

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1: Cell Therapy Catapult

AAV vectors are an appealing tool for both ex-vivo and in-vivo gene therapy. The number of AAV gene products entering early and late phase clinical trials is significantly on the increase. High cost of goods, low process yield, and poor product characterisation are all metrics that require substantial development and improvement within this emerging field. Currently most AAV vectors are manufactured using an adherent process and the demand currently outstrips capacity. Whilst switching from a classical 2D approach to a suspension process using single use bioreactors might be appealing to meet the requirement in term of doses and patient number for late stage of development (e.g Phase III study), this can come at the expense of laborious and costly comparability study. Hence, a reliable, low risk manufacturing platform delivering at the desired scale should be identified early on during development. Therefore, it becomes clear that there is a need to develop the next generation of upstream platform processes using a suspension cell line in STRs. Following Quality by design principles, we sought to develop this platform for AAV manufacturing. Using a scale down model, we investigated the impact of a broad range of process parameters using a design of experiment approach on AAV productivity. Scalability of the newly designed process as well as the impact of our USP on full capsid enrichment during our purification process has been investigated. Overall, our latest efforts in developing an end to end scalable suspension platform for AAV manufacturing will be presented.

P054

Analytical development and automation of lentiviral vectors

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1: Oxford BioMedica

Oxford BioMedica (OXB)'s LentiVector® platform is at the forefront of lentiviral gene therapy and our analytics platform is a key part of this. The commercial scale manufacture of lentiviral vectors (LV) presents unique problems in QC release testing with many of the methods requiring days or weeks to complete with multistep cell culture combined with quantitative analytical readouts. Lengthy protocols, operator-to-operator variability, and a heavy dependence on manual setup are common shortfalls. To successfully meet the challenges for commercial supply of vectors, further gains in throughput and productivity by enhancing processes and automating analytics are needed. Many of the analytics performed on each batch can be improved through the use of automation. Liquid handling robotics are being implemented in OXBs qPCR platform to increase throughout while improving precision and reducing operator error. Residual protein analysis is moving away from traditional ELISA kits and towards nanofludic systems. Key safety assays such as the replication competent lentivirus (RCL) also provide an oppurtunity for the use of automated work flows, freeing up operators and ultimately speeding up the process of batch release while retaining sensitivity, accuracy and precision. Beyond QC, automation can have a significant impact on process development activities and reduce the time taken to generate the data required to support process improvements before GMP manufacturing is even initiated. The platform approach taken by OXB in their analytics allows seamless progression for R&D through to QC utilising the new technologies described here.

P055

Single cell analysis of lentiviral integration to support *ex-vivo* gene modified cell therapy development

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1: Cell Therapy Catapult

Ex-vivo gene-modified cell therapies are increasingly being developed for the treatment of monogenic diseases and various cancers. The generation of genetically modified cells often entails the use of lentiviral or retroviral vectors, which randomly integrate into the host genome posing some risks of insertional mutagenesis and leading to therapy-induced secondary malignancies. Currently, safety tests are limited to the analysis of viral integrations on populations of cells. However, population studies do not account for cell-to-cell variations in the number of viral copies integrating into the genome and the risk that rapidly proliferating clonal populations could compromise the efficacy and the safety of the product. To ensure that the quality and safety of cell therapy products are monitored during development and manufacturing, we designed a novel droplet digital PCR (ddPCR)-based strategy to analyse transduced cells at a single cell level. With this approach we unravel the population heterogeneity in the number of viral integrations per single cell and provide a rapid label-free measure of transduction efficiency. While measuring two critical quality attributes (vector copy number and transduction efficiency), this novel single cell assay may be used to benchmark the safety of cell therapy products by evaluating the proportion of cells with an undesired vector copy number. This innovative approach constitutes a paradigm shift to safety and efficacy of cell therapy products and may support the development of consistently high-quality products for the treatment of a wide range of genetic diseases.

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P056

A BAC-cloning platform for development of stable producer cell lines for commercial scale lentiviral vector manufacture

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Ex-vivo lentiviral vector-mediated autologous haematopoietic stem cell (HSC) gene therapy has shown great potential in the treatment of monogenic disorders of the immune, metabolic and erythroid systems. One of the main barriers limiting patient accessibility to such advanced therapies includes the substantial time and costs incurred by manufacture of gene modified cell products. This is attributed to the complexity and lack of capacity to generate sufficient clinical grade lentiviral vector for transduction of patient HSCs. To address the challenge of producing commercial scale vector, a new platform has been developed which incorporates packaging and therapeutic transgene components into a single bacterial artificial chromosome (BAC) DNA construct, which allows for a single step transfection and thus stable integration into a packaging cell line. This process is a highly efficient approach for transfer of several genes, compared to sequential gene transfer and includes inducible expression cassettes within the BAC-construct to permit control of vector production, eliminating issues associated with long-term expression of viral proteins. Selection of a single, clonal line allows consistent and reproducible high titre viral production from each batch. BAC-cloning technology therefore delivers a rapid and powerful system for the generation of high titre producer cell lines for lentiviral production. Here we describe an evaluation, including the stability and titres achieved, of lentiviral producer cell lines generated for therapeutic programs targeting X-linked Chronic Granulomatous Disease and Transfusion-Dependent Beta-thalassemia. Application of this approach may deliver a long-term solution to the vector manufacturing hurdles gene therapy biopharmaceutical organisations are currently facing.

P057

Generation of cGMP-compliant stable packaging cell lines for inducible recombinant adeno-associated viral (rAAV) vector production

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1: Oxford Genetics Ltd

Since the first rAAV therapy product was approved by European Medicine Agency (EMA) in 2012, rAAV based gene therapy has raised significant interest in treating a wide range of diseases. Several drugs have been released in the market and many more candidates are being evaluated in clinical trials. However, the desire to address wider-patient subsets and to facilitate systemic administration drives the requirement for improved rAAV manufacturing technologies. To facilitate a more scalable, robust and cost-effective rAAV production process, Oxford Genetics has developed stable rAAV packaging cell lines based on a clonal derivative of a cGMP-banked suspension HEK293 cell line. This cell line was isolated based on growth profile and transient rAAV production, aiming at providing optimal starting material for cell line development. An all-in-one plasmid, combining Oxford Genetics' proprietary reconfigured AAV packaging elements and a non-antibiotic induction system, has been integrated into the clonal HEK293 cell line in a single step, enabling controlled expression of cytotoxic viral genes and establishment of the stable cell lines. The clonal packaging cell lines have been screened for transgene presence and rAAV production, and will be subsequently tested for long-term stability. The process of clonal cell line isolation and stable cell line development is fully documented and animal component free, ensuring full traceability and cGMP compliance. In parallel, the all-in-one plasmid has been integrated into a site-specific integration landing-pad cell line by Recombinase-Mediated Cassette Exchange and demonstrated inducible rAAV production, indicating an alternative approach for predictable and rapid viral stable cell line development.

P058

Interposition of genes in anti-HIV lentiviral vector affects its titer and antiviral activity

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A promising approach to HIV therapy is to introduce protective genes into HIV-sensitive cells to make them resistant to the infection. Genes which suppress HIV before integration are the most efficient. It is necessary to combine several genetic agents to prevent the appearance of resistant viruses. We used lentiviral vector to deliver microRNA against CCR5, fusion inhibitor V2O, and restriction factor hu-rhTRIM5a to cells. However, lentivector titer decreases with the size of the insert. Hu-rhTRIM5a is also known to significantly reduce the titer of the vector. To optimize the structure of vector we varied interposition of genes and the location of regulatory sequences. We have designed 5 variants of lentiviral vectors, each containing 2 expression cassettes with different promoters. We inserted 3 antiviral genes (Trim and V2O coding proteins and miRNA against CCR5) and EGFP in different order in these vectors. Genes encoding proteins regulated by the same promoter were separated by 2a peptide. The titers of lentiviral vectors differed drastically from 2x10E4 to 2x10E6 pfu/ml. SupT1 cells were transduced with the generated vectors using the same MOI. The expression levels of the TRIM5a gene were similar in all cell lines. However, the expression levels of V2O varied up to 4 times. Transduced SupT1 cells were infected with NL4-3 HIV strain and the resistance of the cells to the virus was investigated. The maximum anti-HIV activity correlated with the vector titer and expression of V2O gene.

P059

LentiVIP delivery suppresses systemic inflammation which results in beneficial outcome in Type 1 Diabetes

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Pancreatic beta cells are destroyed as a result of autoimmune attack in Type 1 diabetes (T1DM) which leads to insulin deficiency. Vasoactive intestinal peptide (VIP) possesses insulinotropic and anti-inflammatory properties, and is a novel therapeutic agent for the treatment of diabetes. However, VIP is extremely sensitive to peptidases (DPP-4) and thus requires constant infusions or multiple injections to observe any therapeutic benefit. To solve this, a 3rd generation of HIV-based lentiviral vector carrying VIP gene (LentiVIP) was generated to provide a stable gene expression in vivo. Although, LentiVIP injection protected against STZ induced DIO animal model of Type 2 diabetes as shown recently, 1 it is not known whether or not LentiVIP administration would reverse STZ-induced hyperglycemia in diabetic animals. To check this, a multiple-low dose STZ-induced T1DM experimental animal model was also developed in rats for testing purposes. LentiVIP delivery into animal model of T1DM reduced hyperglycemia, improved glucose tolerance, and prevented weight loss. Decrease in serum CRP levels, and serum oxidant capacity, in addition to improved antioxidant capacity were also observed in treated animals. All these beneficial results suggested that therapeutic effect of LentiVIP might be due to the repression of diabetes induced systemic inflammation as well as its insulinotropic properties. TUBITAK-215S820 1- HIV-based lentivirus-mediated vasoactive intestinal peptide gene delivery protects against DIO animal model of Type 2 diabetes. Tasyurek HM et al. Gene Ther. 2018 Jul;25(4):269–283.

P060

Improved delivery of tumor specific oncolytic adenovirus type 5 with NK-92 carrier cells in cancer treatment

ABSTRACT WITHDRAWN

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P061

The establishment of highly sensitive real time-PCR-based detection methods of GMP-manufactured biological products for 14 human viral pathogens

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In order to control any possible viral contamination that may occur during the GMP manufacture of biological products when using master and/or working cell banks derived from human-sourced raw materials, it is necessary to develop fast, reliable, and highly sensitive in vitro testing methods to detect potential adventitious contaminants. Among these, 14 human viral pathogens, namely HIV-1, HIV-2, HTLV-1, HTLV-2, HAV, HBV, HCV, HHV-4, HHV-5, HHV-6, HHV-7, HHV-8, B19, and SV40, should specifically be tested for to ensure that biological products derived from human cell substrates are free from contamination. Here, highly sensitive, modular detection methods utilising RT-PCR were developed that include the use of fluorogenic probes and feature detection limits of 50 to 100 copies. These methods were then validated to establish each assay as a standard operating procedure conforming to ICHQ2(R1) and characterised by comparing them to in vitro adventitious assays and next generation sequencing (NGS)-based detection assays with regards to sensitivity, specificity, and precision in detecting viral contaminants. Our results showed that the RT-PCR-based detection of the 14 human viral pathogens is highly sensitive, reliable, and may be used in the quantification of the viral pathogens. The comparison data shows that each detection method has unique features, advantages, and disadvantages, and suggests the use of NGS-based methods as a complementary tool to be deployed alongside our assays for the enhanced identification of potential adventitious agents at high sensitivities during screens of various biological products.

P062

Industrial Lentiviral vector manufacturing using advanced process analytical technologies

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Oxford Biomedica is a pioneer in gene and cell therapy with a prominent global position in lentiviral vector research, development and production. Utilising its proprietary LentiVector® delivery platform, Oxford Biomedica has produced gene and cell therapy candidates for therapeutic applications in the fields of oncology, neurology and ophthalmology. Faced with the challenge of consistently manufacturing lentiviral vectors for gene therapy products at commercial scale within rigorous tolerances for titre, purity and potency, Oxford Biomedica has developed a scalable, serum-free suspension manufacturing process utilising

stirred tank bioreactors and state-of-the-art bioprocessing facilities. In conjunction with Innovate UK, Oxford Biomedica has been working with collaborators to develop novel Process Analytical Technologies (PATs) including Raman spectroscopy and refractive index profiling to enhance process control during vector manufacture and to support future process intensification. In situ Raman spectroscopy performed within 5L stirred tank bioreactors and offline chemometric modelling enabled the generation of predictive models to allow real-time evaluation of process performance and lentiviral vector production during the manufacturing process. In addition, refractive index profiling was used to detect real-time changes in culture composition related to cellular metabolism. The probing of various parameters in real-time enabled the application of automated adaptive process control, potentially leading to the rapid optimisation and fine-tuning of bioreactor operating parameters for individual cell lines and enhancing overall process performance. The use of PATs has the potential to facilitate the development of a next generation bioprocess capable of consistently delivering large quantities of high-quality vector for clinical and commercial purposes.

P063

PTG1plus, a novel yransfection agent for a better AAV productivity in large-scale bioreactors

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Polyethylenimine (PEI) is the most common transfection agent used to produce AAV vectors in suspension HEK293 cells. Using PEI, we developed an AAV manufacturing process at 200L scale, which robustly delivered around 1E13 vector genome copies (vg)/L. However the very high quantities requested for AAV-mediated gene therapy for neuromuscular disorders prompted us in exploring improvements in the suspension process for AAV manufacturing, and in particular novel transfecting agents. Here we report the study of a new superior transfecting agent PTG1+ (Polytheragene, Evry, France) in the framework of the production of AAV vectors. This histidinylated polyethylenimine was optimized by Polytheragene to enhance two of the major limitations encountered with currently available transfection reagents: low efficiency in suspension cells and high cytotoxicity. We have thus evaluated and optimized transfection parameters with PTG1+ in disposable shake flasks. The process was then evaluated in 2L and 10L glass bioreactors and finally scaled up from 50L to 200L in single-use bioreactors. While the transfection efficiency appeared comparable to that observed with PEI, the vector titers and the ratio of full/empty capsids were significantly improved. Batches of 200L consistently provided around 2E16 vg with more than 50% of full capsids. PTG1+ transfection agent thus displayed significant benefit in terms of AAV productivity and quality. These observations were confirmed with several serotypes and transgenes. This process was successfully transferred for large scale GMP manufacturing (at Yposkesi, Corbeil Essonne, France).

P064

Enabling stable lentiviral vector producer cell line generation: identifying barriers to high productivities

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Gene therapies require efficient delivery technologies being lentiviral vectors one of the preferred choices when permanent modification of stem and T cells is required. To develop a gene therapy product using lentiviral vectors an efficient bioprocess, granting high yields and generating high quality batches to ensure high target cell transduction is required. The ideal manufacturing processes would rely on stable producer cell lines and perfusion systems, enabling high cell density and long term productions, as established for gammaretroviral vectors. The aim of this work is to generate stable lentiviral vector cell lines and identify and tackle the main causes hindering high cell productivities. One of the barriers to establish constitutive lentiviral vector producer cell lines is the cytotoxicity it induces. To address this obstacle we either eliminated or reduced the cytotoxicity of lentiviral vector components through the use of an engineered gag-pro-pol and non-toxic envelopes. Using a stringent selection and a novel approach to cell line generation we were able to introduce all lentiviral expression cassettes by chemical transfection. Lentiviral producer cell lines constitutively and stably producing infective titers above 106 TU.mL-1.day-1 were successfully established. Bioreaction studies show sustained vector production over 10 days. The producer cell clones are being characterised for stability in culture, cassette integration copy number and cassette gene expression which are revealing further insights on the key parameters related to infectious particles productivity yields and cell line stability. This work discusses lentiviral vectors production main barriers, as well as, strategies and technologies enabling its effective manufacturing.

P065

3D printing in bioprocessing: Chromatographic purification of viral vectors

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Viral vector purification still poses some challenges due to the high dosages required as well as the vector's quality, purity and safety that needs to be achieved in the final product. The increase interest in the use of viral based biopharmaceuticals contributed for the development of new materials and new ways of operation in its manufacturing process. The potential of 3D printing has been already explored in different areas such as bioprocessing, creating new opportunities in the design of new porous beds with control over the size, shape, position and orientation. Here, we report the production and use of 3D printed cellulose chromatographic columns for the purification of Adenovirus and

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Lentiviral vectors. The columns were functionalized with two different ligands: diethylaminoethyl (DEAE) and hydroxyapatite, that have already been reported for virus purification. We demonstrate that using these 3D printed chromatographic supports, adenoviruses were purified with a recovery yield of 69 ± 6 % of viral genome particles, while maintained their size and shape after electron microscopy analysis. Lentivirus were purified with a recovery yield of 57 % of transducing units (TU). These findings suggest the powerful of 3D printing technologies to be applied in the viral vector manufacturing field to improve downstream process efficiency.

P066

Application of the design of experiment method for AAV vector production in suspension cells

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Adeno-associated virus (AAV) is generally considered as an optimal gene delivery vector for in vivo gene therapy because it is not associated with any human or animal diseases and does not appear to alter the biological properties of host cells upon infection. Also, high titer production of recombinant AAV vectors in small scale culture systems entail benefits for the construction of scale-up culture systems which is important for commercializing AAV based gene therapy. In most experiments for optimizing recombinant AAV vector manufacturing process, one-factor-at-a-time (OFAT) experiments were commonly adopted. But the OFAT method is inefficient compared to changing factor levels simultaneously because it leads to an unnecessarily large number of experimental runs. To overcome the limitation of the OFAT method, we examined the design of experiment (DoE) method using AAV2 three plasmid systems in Expi293F suspension cells. Because the transfection step is critical for achieving high-titer vector yield, we first tested seven effective parameters associated with transfection by screening method. Two factors, cell density and PEI: DNA ratio, were identified that highly influence AAV production. Therefore these two parameters were further tested with response surface methodology for precise optimization. Additionally, the ratio of three plasmids was optimized by using mixture design. Taken together, AAV2 was produced successfully with yields of 1.36e+12 Vg in 20ml scale cell culture under optimal conditions. The optimization of AAV production using DoE in small scales of Expi293F cells demonstrated the potential for scale-up and the industrialization of AAV manufacturing.

P067

High-throughput data capture and analysis of scale down lenti viral vector production systems during process development

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Large-scale production of lentiviral vectors (LV) for therapeutic applications in gene therapy is necessary to achieve the full potential of this technology and meet clinical and commercial demand. A greater understanding of how cells function during the different phases of viral vector production is necessary to fully optimise production conditions. An obstacle to gaining this knowledge is the complex nature of viral vector manufacture and fundamental research and process development at a commercial scale is resource intensive owing to the multistep and multicomponent design space. Resulting DoE (design of experiments) are multifactorial and difficult to perform manually with the added problem that data sets are extremely large, making conventional approaches to computational analysis challenging. OXB is developing capabilities to digitally transform its approach to developing its LentiVector® platform by utilising automation and cloud-based computing to maximise analytical throughput and interrogation of data. Here we present an automated approach to design, execution and analysis of bioprocessing data which has revealed patterns, trends, and associations previously undetected and is paving the way for the development of a system of intelligence to aid in the rapid development and optimisation of lentiviral production. Such a model can then be used to gain further insights with a view to maximise volumetric productivity and vector titres, reduce the cost of bringing life-changing gene therapies to patients and to accelerate adoption of these transformational medicines.

P068

Innovative cell-based therapy to treat Huntington's Disease

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Huntington's disease (HD) is a neurodegenerative disease characterized by a mutation in the huntingtin gene (htt) that leads to the formation of an aberrant protein (mHTT). During HD, a devastating loss of striatal medium spiny neurons (MSNs) occurs that is associated to a decrease of brain-derived neurotrophic factor (BDNF), a potent pro-survival factor for MSNs. Bone-marrow mesenchymal stem cells (hBM-MSCs) are a promising tool for cell therapy due to their beneficial paracrine actions for neurodegeneration. We hypothesise that the transplantation of BDNF-expressing hBM-MSCs and committed neuronal precursors can protect and replace the damaged MSNs in HD. We have optimized hBM-MSCs

expansion, in parallel to the design and large-scale production of BDNF-expressing lentivirus (LVs) in good manufacturing practices (GMP) environment. We have transduced hBM-MSCs with BDNF-LVs and quantified BDNF release by ELISA. Furthermore, we have differentiated human pluripotent stem cells to generate functional MSNs. We anticipate that the application of this combined therapy in mouse models of HD will slow down the progression of the disease. Such an approach could be used to treat other neurodegenerative diseases that also display loss of neurons in specific brain areas. For that reason, we are developing a novel and scalable cell therapy, produced under GMP, that fulfils the manufacturing needs for new advanced therapy medicinal products (ATMPs). This work has been supported by Ministerio de Ciencia, Innovación y Universidades; the ISCIII; and the Generalitat of Catalunya, Spain; CHDI foundation, USA and Marie Skłodowska-Curie Innovative Training Networks (TRAINING4CRM) No.722779.

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P069

Retrospective analysis of factors influencing AAV titers based on 1300 preparations

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As Adeno-associated virus (AAV) continues to become the vector of choice for in vivo gene delivery, it is important to understand the factors contributing to its efficient production. Here we report a detailed retrospective analysis of over 1300 AAV preparations to improve our understanding of factors contributing to viral yields. AAV preps were produced using a helper-free HEK293 cell system followed by iodixanol gradient purification and tittered using qPCR. While the core principals of this production system remained unchanged for the 1300 productions, we noted significant variability in viral titers over time. Histogram representations of the distribution of viral titers across all preps versus preps of a single serotype revealed a narrower range of titers for certain serotypes. Other variables such as the length of the transgene, its configuration, and its promoter were integrated in a linear regression analysis to weigh their potential impact on viral titers. A significant correlation coefficient was seen for the contribution of capsid serotype but generally low r2 values pointed towards implication of other factors. Indeed we noted a variability of several log units even for the same AAV production repeated over time pointing towards qPCR as a major source of noise masking other variable's contribution. We identified the viral capsid serotype and qPCR-based tittering as major sources of variability in AAV titers. Analysis of AAV titers obtained by absolute quantification using digital droplet PCR may help obtain more stable titers and bring a better understanding of parameters contributing to AAV packaging efficiency.

P070

Scalable single-use technology to meet gene therapy & vaccines production demands

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1: Univercells

The demand for viral vaccines and viral vectors for gene therapy is growing exponentially, pushing for new production capacities. However, producing these therapies using traditional technology face several limitations including scalability, flexibility to accommodate a range of therapies and vectors with ease, and cost-effective production. To address these challenges, Univercells has developed the scale-X™ portfolio of single-use, fixed-bed bioreactors, accommodating viral production from process development to medium and large-scale commercial production. The scale-X bioreactor systems are operated in fed-batch or perfusion modes, with in-line clarification and capture processes operated in simulated continuous & automated mode reducing the number and complexity of operations compared with traditional equipment. Direct linear scalability is ensured by the novel structure design of the fixed-bed, ensuring cell homogeneity and even media distribution throughout the scales. In addition, the physical and chemical conditions are kept the same across scales, ensuring a seamless scale-up. This innovative solution significantly reduces the manufacturing costs of viral gene therapy and cell-based vaccines, offering an excellent opportunity for emerging countries & producers to enable local manufacturing of affordable costs. Based on case studies using VERO and HEK293 cells for sIPV and adenovirus production, this presentation will demonstrate how the scale-X system can improve reproducibility, reduce both capital and operating costs and remove the scalability bottleneck between clinical trials and commercial production. Results such as cell culture dynamics, productivity, and key process quality indicators will be demonstrated as well.

P071

Integrating design of experiments to improve Lentiviral purification

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1: iBET 2: ITQB 3: Pall Life Sciences

Lentiviral vectors (LVVs) have rapidly became a powerful tool for gene and cell therapy since they can stably modify the genome in dividing and nondividing cells. In fact, they are being use in several clinical trials as in vivo or ex vivo delivery vectors. Currently, the low stability of the virus, mostly due to the fragility of the membrane envelope, as well as the low production titters are hampering the clinical-to-market transition of gene and cell-based therapies. To overcome this issue, there is the need to produce high volumes and consequently use operation units that allow an efficient virus recovery and concentration. A suitable purification process comprises a clarification and capture steps, followed by virus formulation and sterile filtration. Anionic exchange chromatography (AEX) is one of the most critical operation that could lead to the virus self-inactivation. The major focus of this work is the improvement of the capture step in a high throughput manner. Design of experiments (DoE), a powerful statistical method, is used to unravel the relevant factors by tuning the more suitable operation conditions. Our strategy included different membranes evaluation and study several parameters such as ionic strength in the elution step, pH and the amount of virus loaded onto the membrane. A significant improvement was observed in the Mustang Q membrane resulting in 50% increasing in the chromatography yield. These results were validated by running purifications processes at larger scales.

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P072

Comparative analysis of lentiviral vector final formulation conditions for the *ex vivo* transduction of primary human T-cells

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Lentiviral vectors (LVs) are widely utilized vehicles for delivering transgenes into T cells in clinical applications. However, maintaining high viral titers during the production, purification, and storage of LVs remains challenging due to the fragile structure of the lipid-enveloped virus. We assessed the effects of twelve final formulation buffers on the stability of LVs during storage before the ex vivo transduction of T cells isolated from healthy donor-derived leukapheresis products. The buffers included trehalose or sucrose and MgCl2 in PBS and 50 mM HEPES, and human AB serum in PBS and X-VIVO 15-medium. First, T cells were transduced with a Green Fluorescent Protein-LV (LV-GFP) stored for over seven days in all the twelve buffers at -80°C. The stability of thawed LV-GFP in seven selected buffers during six-hour and 24-hour incubation periods at RT and +4°C was also assessed. Next, the assays were repeated with a CD19-targeted chimeric antigen receptor-LV (LV-CD19 CAR) stored in three selected buffers. LV-particle titers were measured by p24 ELISA, and LV-morphology was assessed with transmission electron microscopy. LV-functional titers were determined using flow cytometry and qPCR. Finally, CD19-CAR-LV in the three buffers were used to produce CD19-CAR T cells, which were characterised by flow cytometry and in vitro functionality assays. Our preliminary results show that HEPES-based buffers maintain LV-stability at -80°C more effectively than PBS-based buffers or X-VIVO 15-medium. This study will provide valuable data for the process development of genetically modified T-cell therapies.

P073

Development of automated platforms for high-throughput screening of clonal viral packaging and producer cell lines

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Development of viral packaging and producer cell lines represents a more efficient, robust, cost-effective and scalable approach to address the increasing demands for viral vector manufacture. Currently, the main cell line development (CLD) strategy for viral packaging/producer cell lines is based on random integration, which introduces multiple copies of viral packaging elements and/or transgenes into the host cell genome. However, the unpredictable integration process requires generation and screening of large numbers of clones to obtain the final cell lines with desired productivity, making the CLD process time-consuming and labour-intensive. Here, Oxford Genetics has developed automated platforms to facilitate high-throughput (1000 clones per batch) screening of clonal viral stable cell lines with reduced manual operation and improved precision. Oxford Genetics' main cell culture automation platform consists of a Solentim Cell Metric imager and Cytomat incubator integrated with a Hamilton liquid handling system which automatically scans the post-sorting 96- or 384- well plates, enabling monitoring of clone growth and generation of clonality reports. With the input of hit-picking lists generated by the Cell Metric imager, the Hamilton system consolidates the selected clones into 96-well master plate format, and subsequently extracts genomic DNA and sets up PCR for screening of transgene presence. The positive clones are subcultured on the platform in 96- and 24-well deep well plates, providing materials for evaluating viral production. Titres of produced viral vectors are determined by automated qPCR, ELISA or infectious titre assay, informing the shortlisting of clones with the best performance for further characterisation in larger scales.

P074

Development and scale-up of a bench-scale bioreactor process for transient lentivirus production using a suspension-Adapted HEK293T clone

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1: Merck Millipore

In recent years, the use of lentiviral vectors for gene therapy applications has become increasingly popular. As a result, there has been an increased demand for manufacturing of these viral vectors in large volumes for clinical trials. Current production processes are labor intensive and make use of adherent, flat stock cultures. Bioreactors enable production of large volumes of viral vectors with improved environmental control (pH, dissolved oxygen, mixing and temperature) and reduced production costs. Here, we describe the development and scale up of a suspension-based, transient lentivirus production process. The effects of pH, dissolved oxygen and mixing on growth of the cells and viral vector production were tested in 3 L Mobius® Single Use Bioreactors. Optimal process parameters were defined for the cell growth and lentivirus production phases, and the bench-scale bioreactor lentivirus production process has been scaled up in 50 L Mobius® Single-Use Bioreactors.

P075

Adherent HEK293t cells cultured in the Pall iCELLis® bioreactor with OptiPEAK HEK293t blood-free chemically defined media exhibit robust and rapid population doubling times

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1: Pall Life Sciences 2: Invitria

In the past five years, the Pall iCELLis bioreactor has emerged as the leading workhorse for clinical manufacturing of viral vectors and vaccines. However, despite the recent success of the

iCELLis bioreactor, some challenges remain. To date, the vast majority of products manufactured in the iCELLis bioreactor have utilized serum-containing media formulations that were developed primarily for traditional flatware, creating a performance hurdle when transitioning to a three-dimensional culture. InVitria has developed OptiPEAK HEK293t, a Blood Free and Chemically Defined media optimized for use in the iCELLis bioreactor's unique dynamic environment. Chemically defined and blood-free medias afford manufacturers less CMC and Regulatory hurdles to overcome by mitigating the supply chain risk and variability that comes from serum. Adoption of InVitria's media formulation in the iCELLis bioreactor can result in efficient cell attachment, cell population doubling times comparable to traditional serum-containing medias, and acceptable post seeding growth lags. In this poster, we demonstrate the successful implementation of OptiPEAK HEK293t blood-free media formulation in the iCELLis bioreactor for adherent HEK293t. We begin by demonstrating rapid and uniform cell attachment to the iCELLis fixed bed followed by robust expansion kinetics found to be equivalent to those observed in serum-containing media. These results enhance the CMC regulatory pathway for product approval by removing a critical supply chain risk and reducing risk associated with highly variable serum composition in large scale clinical manufacturing.

P076

SerLess: an adherent HEK293-derived producer cell line for manufacturing lentiviral vectors in the absence of animal-derived serum

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1: VIVEbiotech

Up-scaling lentiviral vectors (LVV) manufacturing poses a number of challenges for process developers in order to generate a gene therapy product in large quantities to commercial phases. VIVEbiotech is CDMO specialized in LVV manufacturing currently suppling cGMP LVV-batches to clients based in UE, US and Australia to attend their PhI/II clinical trials. VIVEbiotech is committed to follow-up projects to PhIII and commercial phases by increasing its manufacturing capabilities by 1Q2021. Commercialization requirements limit the use of products derived from animals during manufacturing due to safety reasons. Elimination of the serum from culture media of producer cells makes a standardized platform affordable, minimizing inter-batch variability and contributing to a cost-effective process. VIVEbiotech's manufacturing is based on adherent HEK293Tgrowth in fixed bed iCELLis bioreactors from PALL. VIVEbiotech has developed a producer cell line derived from HEK293T adapted to grow in adherence without serum needs. The cell line obtained (SerLess) has been characterized as to its growth profile, transfectability and LVV productivity in both 2D and 3D cultures. PEI transfection has been optimized in the special culture media to levels identical to the parental cell line. Next steps are clonal isolation and characterization to ensure FDA requirements for ATMPs. The talk aims to present how to accomplish a process of adaptation of LVV-producer cell line to grow without serum keeping the vector production according to the state-of-the-art yields and summarizing the ongoing projects to improve productivity of LVV in a cell-basis focusing on critical steps of the morphogenesis of the vector.

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P077

An *in vivo* method for detecting bovine herpes virus 1 as a possible adventitious contaminant utilising real-time PCR analysis

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1: Korea Institute of Toxicology 2: Chungbuk Health & Science University

Manufactured biological products may potentially be exposed to adventitious virus contamination from essential components utilised in the process, including fetal bovine serum or porcine trypsin. As such, detection methods for adventitious viruses are necessary, yet many of these processes have not been systematically characterised in vivo in preclinical settings. Here, we describe an in vivo method for detecting adventitious viruses under the context of artificial viral contamination using embryonated chicken eggs (9 – 11 embryo days), as well as suckling (< 1 day postpartum) and adult mice (6 weeks old). Bovine herpes virus 1 (BoHV-1) was selected as the model agent due to the use of bovine-derived raw materials in production processes. BoHV-1 was inoculated into the allantoic cavity or yolk sac of embryonated chicken eggs, from which fluid was collected after 3 and 8 days of observation and inoculated into other eggs. Two weeks after inoculating suckling and adult mice intraperitoneally and intracerebrally, all surviving mice were necropsied and their tissue emulsified (minus skin and viscera) to inoculate additional mice. A 42% mortality rate was observed in the egg group that had been inoculated with fluid collected from yolk sacs of eggs injected with BoHV-1, with the virus detected in all dead eggs by real-time PCR. 29% of suckling mice inoculated with the emulsified tissue of BoHV-1-inoculated mice died as well. Therefore, we propose adopting this real-time PCR-based in vivo method using embryonated chicken eggs and suckling mice to detect potential contamination by adventitious viruses in manufactured biological products.

P078

Cost modeling comparison of static, suspension and fixed-bed bioreactors to manufacture commercial gene therapy products

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1: PALL Biotech

Adeno-Associated Viral vectors (AAV) are powerful tools used for in vivo gene delivery to cure congenital diseases. AAV can be efficiently produced by transient transfection of HEK293 cultured in static 2D multitray system (MT) and this method is widely used at small scale for preclinical and clinical studies. However, use of this system at commercial scale (>200 L) comes with significant risk and cost. To overcome this scaling challenge, bioreactor based systems can be used but a choice must be made between suspension or adherent based processes. Cost modeling is a useful tool that can be used to determine the impact of particular process choices at large scale. In this study, we compare AAV manufacturing costs for three different upstream processes: static MT, suspension bioreactor and fixed-bed bioreactor; using transient transfection in HEK293 cells in serum-free media as expression system at the 200 and 1000 L scale. Utilizing the BioSolve◆ software for modeling

entire processes in cGMP conditions, cost structure is determined and sensitivity analysis is used to identify parameters that can leverage the cost of goods (CoGs).
◆Biosolve is a trademark of Biopharm Services Ltd.

P079

High quality transfection reagents for therapeutic virus production

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1: Polyplus Transfection

Gene- and cell therapy-based medicines are experiencing resurgence due to the introduction of ''next generation'' transfer viral vectors, which have demonstrated improved safety and efficacy. Adeno-Associated Virus (AAV) and Lentivirus are very commonly used in therapeutics and often produced using PEI-mediated transient transfection in HEK-293 or HEK-293T cells. The critical raw materials needed for cGMP vector production must be sourced from approved suppliers and should have gone through a rigorous testing program to reduce the risk of introducing adventitious agents into the production process. Polyplus-transfection® now provides PEIpro®, the unique PEI-based transfection reagents available in different quality grades, allowing a seamless transition from process development with PEIpro®-HQ to cGMP biomanufacturing with PEIpro®-GMP. Transfection performance of PEIpro® and its high-quality counterparts PEIpro®-HQ and PEIpro®-GMP make them particularly well suited for therapeutic virus production. In this poster, we will show that they are defined as best-in-class transfection reagents since their optimized PEI polymer chemistry contribute to reach high transfection and virus production efficiencies while using low DNA amount. In addition, we will present data demonstrating that this range of PEIpro® quality grade reagents is compatible with different cell culture adherent and suspension systems and ensure reproducible virus production yields at any scale to efficiently manufacture viral vectors for advanced clinical trials.

P080

Advantages of standardization and large-scale manufacturing of helper plasmids for viral vector production

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1: Aldevron

Success in gene and cell therapy has increased demand for plasmid DNA used to produce viral vectors, both in quantities for commercial products as well as the breadth of different vectors for the expanding number of programs in development. Our data and analysis indicate that standardization and large-scale production of helper plasmids, those that are the same regardless of the specific viral vector produced, represents an opportunity to significantly reduce timeline, cost, and risk. We have developed processes to quickly produce a set of helper plasmids that consistently generate functional viral vectors, are immediately available for research and clinical production and are free of any royalties or future payments. To meet production scale requirements, our technical operations team has developed and deployed a platform based around a single-use, 300 L fermentation device and process train capable of purifying up to 100 grams of a plasmid in a single processing event. The manufacture of the output from an individual fermentation process can take as little as seven days. Standardization of lentiviral plasmids supports consistency and efficiency at large scale and across multiple programs. Our data show the scalability of fermentation for a typical plasmid at 30 L and 300 L, with specific yield maintained as scale is increased. Our data show the performance of lentiviral vectors produced with optimized plasmids, indicating improved performance over other plasmid systems. The availability, cost, freedom to operate, and consistency of these plasmids will help address the growing demands of cell and gene therapy.

P081

Accelerating advancement in gene therapy by improving downstream purification of viral vectors

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1: Thermo Fisher Scientific

The area of gene therapy has been of growing interest due to its potential of correcting the genetic cause of the disease rather than chronically treating symptoms. Viral vectors have shown to be most successful in delivering therapeutic genetic material into target cells and the adeno associated virus (AAV) sub-classes have emerged as the vector of choice for many therapies. The lack of scalable manufacturing platform technologies for viral vector purification is one of the major challenges the field faces. In order to increase productivity and meet market needs, efficient commercial manufacturing capabilities need to get established. By reducing the number of purification steps and maximizing productivity, affinity chromatography already offers a significant improvement to the downstream process of biomolecules. This technology offers scalability and process consistency, thereby providing a platform solution to the industry. Herein we outline the benefits of implementing affinity chromatography as platform in the downstream purification of viral vectors, using the CaptureSelect™ technology as the basis of generating high-binding affinity ligands. The methods described will reveal the benefits of affinity chromatography related to specificity, capacity, process yields as well as process scalability for the purification of AAV viral vectors. Specifically, the properties of POROS™ CaptureSelect AAV8, AAV9, and AAVX affinity resins will be discussed as tools to capture and purify a broad range of AAV serotypes. The utilization of these affinity resins will be a significant improvement to the downstream processing, by reducing the purification steps and maximizing productivity, offering scalability and processing consistency.

P082

Improvement of lentivirus manufacturing in large-scale bioreactors using PTG1plus, a novel cationic transfection agent, in suspension HEK 293T cells

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Lentiviral vectors (LV) are increasingly used to treat genetic diseases and cancer via ex vivo transduction of hematopoietic stem cells and T-lymphocytes respectively. For these purposes, large amounts of LVs are needed. Nowadays, the most common manufacturing process consists in transfecting adherent HEK 293T cells, usually grown in cell factories, with 4 different plasmids and calcium phosphate. This method is robust and efficient but cumbersome, and shows limited scalability. To address these drawbacks, a manufacturing process based on transient transfection of suspension cells with a new transfection agent, PTG1plus, an histidinylated polyethylenimine (from Polythéragène, Evry France), has been developed. The optimal transfection parameters were first determined at shake flask scale. When compared to classic PEI, PTG1plus increased significantly the lentiviral productivity and allowed a reduction of plasmid consumption, and thus important cost savings at large scale. The process was then evaluated in 2L and 10L glass bioreactors, and finally scaled up to 50L and 200L single use bioreactors. At 200L scale, the process leads to robust LV productions. A 200L batch delivers 2 to 3E12 IG (Infectious Genomes) of a given therapeutic LV. This process was successfully transferred for large scale GMP manufacturing (at Yposkesi, Corbeil Essonne, France).

P083

Continuous perfusion for an order of magnitude increase in lentiviral vector production

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1: Artemis Biosystems 2: Human Health Therapeutics, National Research Council of Canada

Lentiviral vectors (LVV) represent a key tool for gene and cell therapy applications. The production of these vectors in sufficient quantities for clinical applications remains a barrier, driving the field towards the development of cell suspension processes which are more amenable to large scale production. The HEK293 cell line grows well in suspension at cell densities between 10–20 million cells/mL thus offering direct scalability, and produces a green fluorescent protein (GFP)-expressing Lentiviral vector which can be continuously harvested to increase LVV yields. In all perfusion runs harvests were collected and the LVV-containing supernatant was kept on ice at -4 degrees centigrade until clarification (once daily). This study demonstrated that LVV production using a novel and scalable perfusion process increased viral titers 30-fold compared to batch processing and reached a cumulative total yield of 6.25 x 10^11 TU/L bioreactor culture. The perfusion approach at 10–20 million cells per mL is easily amenable to large scale production of Lentiviral vectors.

P084

UPV: production of customized high quality viral vectors at the joint UAB-VHIR technological platform

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Viral vectors are widely used tools for gene transfer, because of their high transduction efficiency, and the ease and flexibility to genetically express or inhibit one gene or a combination of genes in specific areas and periods of time. They can be used safely in vitro and in vivo, applying standard procedures in a controlled setting. Vector production however, requires the application of specialized techniques, access to expensive equipment and biological safety laboratories. The Vector Production Unit (UPV) is a UAB-VHIR technological platform (Universitat Autònoma de Barcelona - Vall d'Hebron Research Institut) staffed by experienced and highly qualified personnel. Since its opening in 2003, the UPV has been dedicated to the design, production and purification of viral vectors for basic research and gene therapy pre-clinical studies for both public and private laboratories, being nowadays the main Vector Core in Spain for AAV and Ad vectors. The UPV has been authorized by the Spanish ''Ministerio de Agricultura, Alimentación y Medio Ambiente'' to work with Biosafety-level-2 GMOs. ID: A/ES/14/I-29. Also, the UPV has recently implemented guidelines, policies and processes in compliance with the ISO 9001:2015 quality standards. Key products and services: - Assessment in the selection and design of vectors - Construction of the therapeutic cassette - Production, purification and characterization of: - AAV vectors: Concentrations starting at 2e12vg/mL. Serotypes: AAV1, AAV2, AAV5, AAV6, AAV8, AAV9, AAVrh10, AAV.PHPeB. Contact us to request information of other pseudotypes. - Adenovirus vectors: Concentrations starting at 1e12pp/mL. Serotypes: Ad5, Ad5/40, Ad5/52, CAV2. -Quality control assays

P085

Addressing viral vector manufacturing challenges – innovative solutions in the AAV production workflow

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1: Thermo Fisher Scientific

Over the last decade, the field of gene therapy has made significant advancements resulting in a major paradigm shift in medicine. Currently, a handful of therapies in Europe and the USA are approved and commercially available for patients. The pipeline of therapies in phase II and III clinical trials is rapidly growing year over year. Delivery of the therapeutic gene is most often done through viral vectors and Adeno-Associated Virus (AAV) has become the vector of choice in many gene therapy applications. Although a great deal of progress has been made in AAV manufacturing, many challenges in the workflow still exist. Each production process for viral vectors has its own unique impediments and AAV is no exception. A successful production workflow must be consistent, reproducible, and scalable and deliver pure and high-titer product free from impurities so it can pass regulatory requirements. Each step in the manufacturing process poses unique challenges. Working together with gene therapy developers, Thermo Fisher Scientific has realized innovative solutions that span the entire viral vector production workflow, from cell line development to final fill and finish. Here we present our current technologies developed for improving viral vector manufacturing and outline new developments in the field of purification of AAV and other viral vectors.

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P086

RNase-PF: an animal-free recombinant solution for the removal of host-cell RNA

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For the high-quality production of gene therapy constructs, bacterial host-cell RNA is one of the major contaminants that need to be removed. The level of undesired host-cell RNA molecules should be undetectable in the final product. The application of animal-derived (for e.g. bovine) RNA degradation enzymes, as known from kit-based DNA preparations, is discouraged by the regulatory authorities. In this work, a systematic approach is described, that started with the cloning of the gene for the RNase from Bacillus amyloliquefaciens along with its complementary inhibitor. Furthermore, the expressed enzyme was secreted into the periplasm, followed by a controlled release into the extracellular medium. We developed an optimized purification procedure wherein, the target protein was concentrated down using a tangential flow filtration unit. The retentate from this step was filtered through a 0.2 μm membrane and applied to a cation-exchange chromatography column after adjusting binding conditions. Following a stepwise elution through a salt gradient, the relevant fraction was isolated and quantified for total protein and RNase activity. Hydrophobic interaction was tested as a downstream chromatography step, which resulted in a significant reduction of non-target host proteins. The active enzyme fraction was further polished by an anion-exchange membrane, which effectively removed all traces of the recombinant production plasmid. The effectiveness of this step was ascertained through qPCR. Currently, the production of the active enzyme was still only at low levels (4 Kunitz Units/mL). Nevertheless, the purified enzyme fraction was tested in-house for the purification of plasmids from optimal production strains.

P087

The Coding RNA and LncRNA expression profile analysis of stem cells from the apical papilla after depletion of SIRT7

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Objective: To explore SIRT7 effects on the gene expression file of stem cells from the apical papilla (SCAPs), and to reveal the possible mechanism of SIRT7 on the senescence of SCAPs.

Materials: SCAPs were isolated and cultured. SIRT7 shRNA was used to knock down the expression of SIRT7 in SCAPs. After library construction and RNA sequencing, differentially expressed genes were identified using Cuffdiff with the false discovery rate (FDR) ≤0.05 and fold-change ≥2. Pathway and Gene ontology (GO) analyses were conducted to elucidate the changes of important functions and pathways after SIRT7 gene knockout. Gene set enrichment analysis (GSEA) was performed and the FDR score smaller than 0.25 was considered significant enrichment of a gene set.

Results: The most striking GOs between SIRT7 depletion and control SCAPs were mainly response to nucleus, nucleolus, cytoplasm, protein binding, and intrinsic apoptotic signaling pathway. Signaling pathway analysis revealed the top five pahways—Metabolic pathways, Pyrimidine metabolism, Protein processing in endoplasmic reticulum, PI3K-Akt signaling pathway, p53 signaling pathway. According to the result of GSEA, genes were mainly enriched in cell cycle process and cell proliferation. Moreover, TGF-β signaling pathway and cyokine-cytokine receptor interaction pathway were also significantly enriched.

Conclusion: SIRT7 may affect the functions of SCAPs through cell cycle, cell proliferation, and apoptosis pathway.

P090

Efficient and robust NK-cell transduction with baboon envelope pseudotyped lentivector: a new tool for CAR NK cell immunotherapy

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NK-cell resistance to transduction is a major technical hurdle for developing NK-cell immunotherapy. By using baboon envelope pseudotyped lentiviral vectors (BaEV-LVs) encoding eGFP, we obtained a transduction rate of 30 ± 2,2% in freshly isolated NK-cells and 87 ± 6.7% in NK-cells obtained from the NK cell Activation and Expansion System (NKAES), even at low MOI, with a sustained transgene expression for at least 21 days. BaEV-LVs outperformed Vesicular Stomatitis Virus type-G (VSV-G)-, RD114- and Measles Virus (MV)- pseudotyped LVs ($p < 0.001$). mRNA expression of BaEV receptors, ASCT1 and ASCT2, was detected in freshly isolated NK cells and NKAES, with a higher ASCT1 expression in NKAES. Transduction with BaEV-LVs encoding for CAR-CD22 induced a robust CAR-expression on 43% of NKAES cells, which allowed the specific killing of NK-resistant pre-B-ALL-RS4;11 cell line. Remarkably, by using a larger vector encoding a dual CD19/CD22-CAR, separated by T2A, we could, despite a low viral titer, transduce and re-expand dual-CAR-expressing NKAES, which killed efficiently and specifically both CD19KO or CD22KO, RS4;11 cells. These dual CAR expressing NKs might thus overcome tumor evasion to single CAR-therapy. Our results suggested that BaEV-LVs could be the long-awaited tool that will help bringing NK-cell-based immunotherapy to the clinic and study NK-cell biology much more efficiently.

P091

Generation of Chimeric Antigen Receptor (CAR) macrophages from Human Haematopoietic Stem/Progenitor Cells (HSPCs)

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Immunotherapy using chimeric antigen receptor (CAR) technology has demonstrated profound efficacy to enhance T cell cytotoxicity in hematologic malignancies, whereas translation to solid tumors has been challenging. A major hurdle constitutes the immunosuppressive tumor microenvironment (TME), in which macrophages represent a highly-abundant cell population. We hypothesize that transfer of the CAR concept to macrophages will modulate and enhance their functionality and will help to overcome the immunosuppressive TME. Proof of principle studies were performed with a CAR designed to target the carcinoembryonic antigen (CEA). CARs containing a CEA-specific single chain antibody ectodomain, an IgG1 spacer, a 2B4 transmembrane/endodomain and DAP12 as signaling domain were generated in the context of a SIN lentiviral vector using the CAG promoter. Mono-Mac-6 cells were transduced with the CAR vector and stable long term CAR expression was documented by flow cytometric analysis. Primary CD34+ HSPCs were transduced with the CAR vector (efficiency up to 35%), sorted and further differentiated into macrophages. CAR expression was confirmed by flow cytometric and Western blot analysis. Differentiation and expansion capacity of CD34+ cells (factor 10.0 ± 1.6 CAR vs. 10.2 ± 1.7 ctrl) was maintained upon introduction of the CAR construct. CAR-expressing macrophages exhibited similar morphology, surface marker expression (CD45+, CD11b+, CD14+ and CD163+) and basic functionality (phagocytic activity) as unmodified macrophages. Thus, our data demonstrates the feasibility to generate CAR macrophages from human HSPCs maintaining desired macrophage properties. Macrophage signal transduction upon CAR binding using CEA+ cell lines or a CEA single chain-idiotypic antibody is currently being analyzed.

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P092

GMP grade CD44v6 CAR-engineered T cells control tumor growth in lung and ovary adenocarcinoma bearing mice

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1: MolMed S.p.A

The challenge of CAR adoptive T-cell therapy is its application to the field of solid tumors, where the identification of a proper antigen has emerged as the major drawback to success. The variant domain 6 of the hyaluronate receptor CD44 (CD44v6) is a glycoprotein implicated in tumorigenesis, tumor cell invasion and metastasis and represents an attractive target for CAR T cells therapies. T cells, transduced with a gamma retroviral vector expressing CAR-CD44v6DN and a mutated form of the Herpes Simplex Virus Thymidine Kinase (HSV-TKmut2), display antitumor activity in CD44v6+ acute myeloid leukemia and multiple myeloma mouse models. A Phase I/IIa, open-label, multicenter clinical trial in patients with relapse/refractory MM or AML is under active recruitment. Moreover, in several independent experiments we demonstrated that CD44v6 CAR T cells mediate significant antitumor effects in adenocarcinoma bearing mice. Given the promising results obtained in pre-clinical models with research grade produced CAR T cell, we tested the antitumor effect of GMP grade CD44v6 CAR engineered T cells. CD44v6 CAR T cells, produced according to the two manufacturing processes, display the same memory phenotype and the same expression level of activation markers. Percentage of transduction before selection and expression level of CAR at the end of the process were comparable, no differences were observed in expansion and persistence in peripheral blood. Importantly, GMP CD44v6 CAR T cells control tumor growth and extend overall survival. Thereby, we concluded that changes introduced in the manufacturing protocol had no impact on the efficacy of the product.

P093

Single adenoviral vectors armed with HPV oncogene specific CRISPR/Cas9 as efficient tumor gene therapy tools for HPV related cancers

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Human Papillomaviruses (HPV) cause malignant epithelial cancers such as cervical carcinoma, anogenital cancer, head and neck cancer as well as non-melanoma skin cancer by expressing viral oncoproteins. Oncogene disruption by CRISPR/Cas9 has been shown to reverse oncogenic pathways and drive cancer cells into apoptosis. To translate these findings towards in vivo applications we constructed, amplified and purified two single gene deleted, high capacity adenoviral vectors (HCAdVs) armed with all components of the CRISPR/Cas9 machinery including specific guide RNAs for the E6-oncogenes of the major high-risk HPV types, HPV16 or HPV18. Cervical cancer cell lines Siha, Hela and Caski containing integrated HPV16 or HPV18 genomes and HPV negative A549 lung cancer cells were transduced with HPV type-specific CRISPR-HCAdVs respectively. Upon vector transduction, we applied CCK-8 viability assays to assess cell viability and cell proliferation, Caspase 3/7 glow assay to measure apoptosis induction and in cell western analysis for p53 quantification. Adenoviral delivery of HPV type-specific CRISPR/Cas9 resulted in increased cell death of HPV positive cervical cancer cell lines, whereas HPV negative A549 cells were unaffected. Compared to untreated or HPV negative control cells transduced cervical cancer cells showed decreased proliferation and viability, increased apoptosis induction and p53 re-accumulation. This suggests that HCAdVs armed with an HPV-E6 specific CRISPR/Cas9 system can serve as potent HPV specific cancer gene therapeutic agents.

P094

Human Vδ1+ T cells; an allogenic 'off-the shelf' T cell therapy platform

ABSTRACT WITHDRAWN

P095

CRISPR-mediated targeting of fusion oncogenes for cancer-directed therapy

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Fusion oncogenes (FOs) are common in many cancer types and are powerful drivers of several human cancers, including leukemia, lymphoma, and sarcoma. Over 350 recurrent FOs have been identified/characterized to date. Because their expression is exclusive to cancer cells, FOs are attractive therapeutic targets; however, specifically targeting the resulting chimeric products is challenging. We have developed a simple, efficient and clinically relevant genome editing strategy for targeting fusion oncogenes using CRISPR/Cas9 technology. It allows for robust disruption of the FO specifically in cancer cells without affecting exonic sequences or protein expression of germline non-rearranged alleles in non-tumor cells. This strategy is applicable to target different fusion oncogene isoforms or different patient-specific breakpoints making this a universal approach for any given cancer-associated fusion oncogene. Functional assays show that lentiviral or adenoviral delivery of CRISPR-Cas9 components targeting FOs leads to efficient and specific cancer cell death in vitro, and reduced tumor burden/mortality in vivo in preclinical cancer xenograft and PDX models. We provide a proof-of-concept for a universal CRISPR-mediated genome editing strategy targeting cancer-associated fusion oncogenes as an innovative cancer-directed advanced therapy.

P096

Transmembrane topogenesis and folding of extramembrane domains of NaPi2b transporter under conditions of malignant transformation can lead to the appearance of tumor-specific epitopes

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Searching for specific targets for cancer diagnosis and treatment is one of the most important problems in clinical oncology. In our work, we put forward a new concept of the emergence of tumor-specific epitopes of integral membrane proteins by changing their topology under mutations, glycosylation and tumor conditions including low pH and hypoxia on the model of Na + -dependent phosphate-cotransporter NaPi2b. NaPi2b (SLC34A2, NaPi-IIb, NPT2b) belongs to the SLC34A2 transporters family and is involved in maintaining phosphate homeostasis in the human body. NaPi2b express in a number of normal and malignant tissues. It is an integral membrane protein with a large extramembrane domain 4 (EMD4, 234–362 aa), 8 transmembrane domains, N - and C - ends located in the cytoplasm. We have cloned the epitope for antibodies MX35 and L2 (20/3) within 311–340 aa region of NaPi2b, which recognition depends on disulfide bonds, glycosylation and is canceled by the mutation at the T330V position. Topology of the protein undergoing significant changes in hypoxia and low pH conditions, when its N-terminal fragment can be localized outside the cells. This reorientation makes it as a potential tumor-specific domain. The obtained data that was supported according to the Russian Government Program of Competitive Growth of KFU are of both fundamental and applied importance in the development of new targeted antitumor drugs of high specificity.

P097

Charge balance rule in the assembly of integral membrane proteins in tumor cells

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According recently formulated and experimentally tested ''dynamic'' Charge Balance Rule the charge character of extramembrane domains (EMD) and collective charge of membrane surface may act in concern to determine transmembrane domain orientation and phosphatidylethanolamine (PE) fulfills its topogenic function. Na-dependent phosphate-cotransporter NaPi2b appears to be an ideal model protein to test Charge Balance Rule due to net negative charge of its N-termini (NT) (-7) which however predicted to stay inside. Fluorescent

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confocal microscopy with site-directed antibodies directed against the NaPi2b N-, C-termini, L epitope (EMD4) and phosphatidylserine (PS) specific FITC-lactadherin, PE specific antibiotic Ro09-0198 and propidium iodide were used respectively to monitor changes in topology of this membrane protein, topography of both aminophospholipids and integrity of ''resting'' and stressed OVCAR (ovarian cancer) cells reversibly subjected to normoxic/hypoxic conditions and normal/low pH. We found that that NaPi2b adopts a transmembrane topology with its N-terminal domain facing the cytoplasm of cells that is consistent with predicted orientation of NT and violation of Positive Inside Rule in these cells. However, NaPi2b adopts an inverted topology with its N terminal domain facing the outside of OVCAR cells challenged to low pH and hypoxia. This reorientation coincides with surface expose of anionic PS and net neutral PE. Thus, results, that was supported by Russian Government Program of Competitive Growth of KFU, show that flipping of NT is fully reversible and may be driven electrophoretically by change of external pH and facilitated by surface exposure of PE and/or PS in the accordance with Charge Balance Rule.

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P098

Pigs versus rodent models for assessing performance of serotype chimeric Ad5/3 oncolytic adenoviruses

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Engineering Oncolytic serotype-chimeric vectors utilizing Adenovirus type 3 receptors (OAd5/3) has greatly improved oncolytic potential of adenovirus-based vectors. Clinical translation of these vectors has been challenging due to a lack of replication-permissive animal models. In this study, we explored pigs as a model to study performance of oncolytic OAd5/3. As a control, the fiber-unmodified OAd5 vector was used. We analyzed binding, gene transfer, replication, and cytolytic ability of OAd5 and OAd5/3 in various non-human cell lines (murine, hamster, canine, porcine). Among all cell lines only porcine cells supported binding and replication of OAd5/3. Syrian hamsters supported OAd5 replication but showed no evidence of productive viral replication after infection with OAd5/3. Next, primary organ biodistribution and safety profile of systemically-injected OAd5 and OAd5/3 were determined in immunocompetent pigs (1, 6 hours, day 1–7 post-infection). Viral DNA and replication-dependent gene-expression were observed in lungs, spleen, lymph nodes. The levels of OAd5 and OAd5/3 DNA in liver were almost negligible throughout the experiment, indicating a very different pattern of adenoviral biodistribution in pigs compared to rodents. No liver toxicity was observed in pigs despite very high doses (5x10^11 and 3x10^12 viral particles) of systemically-administrated adenovirus. These studies validated the pig as a valuable model for preclinical testing of OAds utilizing Ad3 receptors. These studies have also demonstrated the flaws of using rodent models for bio-distribution and toxicity analyses of Oncolytic Adenoviruses. Importantly, the FDA has agreed with our approach to use pigs for the IND-enabling toxicology studies to determine safety of OAd5/3-based vectors.

P099

Clinical translation of a novel NIS-expressing oncolytic adenovirus for pancreatic cancer therapy and imaging

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The oncolytic viruses encoding sodium-iodide symporter (NIS) are attractive tools to achieve radionuclide imaging and therapy of cancer. Previously we designed an NIS-expressing oncolytic adenovirus utilizing the deltaE3 system with enhanced oncolysis mediated by overexpression of adenoviral death protein (ADP). Although this vector, OAdNIS-ADP (-), was effective in detection regimens, we were concerned that ADP cytolytic effect may diminish iodine uptake. Here we assessed performance of OAdNIS-ADP(+) and OAdNIS-ADP(-) vectors in pancreatic adenocarcinoma (PDAC) and lung cancer models. SPECT/CT studies confirmed that OAdNIS-ADP(-) produced efficient 99mTcO4–uptake in PDAC patient derived xenografts that was maintained until day 36, and significantly outlasted ADP(+) and control AdCMV-NIS (currently in clinical trials for prostate cancer). In lung cancer models, the therapeutic effect of ADP(-) combined with 131I outperformed that with OAdNIS-ADP(+) or AdCMV-NIS combination therapies. Quantitative analyses of 131I uptake in tumor tissues showed that OAdNIS-ADP(-) retained higher 131I uptake than OAdNIS-ADP(+). The safety profile of OAdNIS vestors was demonstrated in immunocompetent pigs which underwent systemic injection of high titers and showed no systemic toxicity. Our short-term goal is to conduct a Phase I Trial to investigate the safety of intratumorally-injected OAdNIS followed by 123I imaging and 131I therapy in patients with locally advanced PDAC. Our overarching goal is to treat metastatic disease by intravenously-administered OAdNIS. To test the therapeutic potential of systemically injected OAdNIS we are developing a PDAC model in KRASG12D/+/TP53R167H/+ Miniature Transgenic Pigs expressing one mutant allele of the TP53 gene and Cre-inducible mutant KRAS.

P100

Induction of immunogenic tumor cell death by a novel immune-oncolytic Vaccinia virus strain leads to robust antitumor immune responses directed against neo-epitopes

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Immune-oncolytic Vaccinia viruses (VACV) are vectors designed to selectively replicate in and destroy cancer cells, at the same time that they can redirect the immune response towards the tumor: tumor-selective viral replication leads to localized inflammation, transiently overcomes tumor-mediated immunosuppression, and releases relevant tumor antigens. In the last years, it was described that inducing an Immunogenic Cell Death (ICD) in cancer cells is key for activating effective

antitumor immune responses. However, VACV strains used to date as oncolytics demonstrated not effective in such induction due to codify for proteins that effectively block the release of Damage-Associated Molecular Patterns (DAMPs). Here, we present a novel VACV strain named Immune-Oncolytic Vaccinia virus Ankara (IOVA). Such strain includes several deletions in key genes involved in the interaction with the host immune system, and demonstrated very efficient in ICD induction: contrary to Western Reserve (WR) and Copenhagen strains, IOVA induces high levels of calreticulin translocation in all cancer cell lines tested. In addition, IOVA also increases the release of other molecules associated with ICD, such as ATP and HMGB1. Importantly, deletions included in IOVA strain do not hinder viral replication in tumor cells, and increase the capacity of the virus to kill cancer cells in 25 times in certain cell lines. In mice tumor models, ICD activated by IOVA translates into a rapid induction of T cell responses directed against tumor neo-antigens, and dramatically improves antitumor activity and survival of mice. IOVA is thus a promising candidate for clinical evaluation in cancer patients.

P101

A new promising CAR.CD30 T cell therapy associated with long persistence and high activity for treatment of CD30 positive lymphomas

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The prognosis of many patients with relapsed CD30+ lymphomas remains poor and novel therapies are desirable. We designed and pre-clinically validated a CAR construct carrying a novel anti-CD30 scFv linked to CD3-ζ through two costimulatory domains, namely either CD28.4-1BB or CD28.OX40. We also added in the construct the ΔCD34 as selectable marker and the inducible Caspase-9 as safety switch. Anti-tumor efficacy and persistence of both CAR.CD30 were evaluated in vitro and in vivo. Independently from the costimulatory domain used, both constructs showed similar transduction efficiency of T cells. Nevertheless, CAR.CD30 incorporating CD28.OX40 was associated with more stable expression of the CAR over time, during extensive in vitro culture ($84.72 \pm 5.30\%$ vs $63.98 \pm 11.51\%$ CAR+T cells at 30 days after transduction; $p = 0.002$). This finding was also associated with a superior anti-lymphoma activity in vitro by CD28.OX40-CAR when challenged at high tumor/effector ratio (8:1) ($37.45\% \pm 27.65\%$ vs $62.85\% \pm 10.90\%$ of residual tumor respectively; $p = 0.03$) and after multiple tumor stimulations. Moreover, antigen-specific stimulation was associated with higher levels of IFNγ (8306.03 ± 3745.85pg/ml), IL2 (13492.68 ± 5837.77 pg/ml) and TNFα (17661.00 ± 11113.27 pg/ml) production by CD28.OX40-CAR compared to CD28.41BB-CAR (6617.81 ± 3025.67 pg/ml, $p = 0.040$; 7616.67 ± 4464.06 pg/ml, $p = 0.008$; 5824.63 ± 1823.73 pg/ml, $p = 0.02$; respectively). In NSG mice, we proved that CD28.OX40-CAR had superior anti-tumor control and persistence than 28.41BB-Ts, leading to a significant reduction of bioluminescence and an increased overall-survival in treated mice (60% vs 10%, at 180 days, $p = 0.0014$). Overall, these data suggest that the costimulatory combination of CD28.OX40 is crucial for improving both persistence and antitumor efficacy of this approach in lymphoma model.

P102

Transgene codon usage adaptation, a novel strategy for the design of armed oncolytic adenoviruses

ABSTRACT WITHDRAWN

P103

Analysis of tumour reactivity and Vβ repertoire of melanoma TIL patients

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Tumour-infiltrating lymphocyte (TIL) therapy is an exciting prospective immunotherapy, which has shown good response rates in trials for immunogenic cancers such as melanoma. However, the T-cell composition and tumour-reactivity of TIL is still relatively undefined, which affects our ability to interpret clinical response data. To address this, we have validated a number of markers which show activation of the T-cells against tumour, in a flow-cytometric based assay. Using these markers, we have set up matched patient TIL and tumour co-cultures for several patients treated with TIL therapy. Through these assays, we have highlighted that the variability of tumour reactivity

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between patients is surprisingly high, with some patient TIL having little-to-no tumour-reactivity against matched tumour in vitro. We have also compared the phenotype and Vβ repertoire of a number of patient TIL samples, to give an indication to the potential skewing and clonal expansion within different patients. By combining the tumour-reactivity and Vβ analysis, we can identify potential tumour-reactive clones within TIL, which can be expanded, sequenced and validated for TCR transfer studies. Through the identification and validation of tumour-reactive clones, new TCRs can be isolated and interrogated for new target selection, opening up new avenues for cancer immunotherapies.

P104

New gene-editing strategy for allogeneic NKG2D-CAR T cell production

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Chimeric antigen receptor (CAR) T cell therapy has arose as one of the most promising therapeutic strategies in cancer treatment. Target antigen selection is crucial to the success of the cell therapy since it defines the off-target toxicity, specificity, and efficacy. NKG2D is an important activating receptor which recognizes multiples ligands absent on normal cells, but induced as a result of cellular stresses on cancer cells. Along with receptor selection, the method for CAR delivery into the cell is a major issue. Typically, CARs are transduced by using randomly integrating vectors. This approach may result in oncogenic transformation and transcriptional silencing. On the other hand, targeting a CAR to a specific locus with CRISPR/Cas9 has previously shown promising results for obtaining efficient CD19-CAR T cells. The purpose of this study was the development of allogeneic NKG2D-CAR T cells by performing a novel genome targeting approach. To that end, T cells were collected from peripheral blood mononuclear cells from healthy donors. Then, a novel CRISPR/Cas9 strategy was used in combination with AAV6 transduction to induce NKG2D-CAR expression and inhibit TCR expression in T cells. Targeted CAR integration was proved by PCR whereas NKG2D-CAR and TCR expressions were determined by flow cytometry. Our new gene-editing method that involves the combination of AAV transduction and CRISPR/Cas9 technology resulted in an average of 30 % TCR-deficient T cells expressing NKG2D-CAR. Additional studies are on the way to get universal CAR T cells for human use.

P105

Analysis of the interaction of mesenchymal stem cells overexpressing interferon alpha-17 and human neuroblastoma in co-culture *in vitro*

ABSTRACT WITHDRAWN

P106

Pre-clinical proof-of-concept for CAR-T Cells directed against the tumour antigen 5T4 in the treatment of haematological malignancies

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Chimeric antigen receptor (CAR) T cell therapy is an emerging field in which T cells are re-programmed to recognise tumour specific antigens expressed on the surface of cancer cells. CAR-T cells specific for CD19 are currently used to treat patients with B cell leukaemia/lymphoma with impressive outcomes. At Oxford Biomedica, we have developed a Lentivector product (OXB-302) to generate CAR-T cells directed against the tumour antigen, 5T4. An excellent target for cancer immunotherapy, 5T4 shows minimal expression on normal tissues, is expressed on the majority of solid tumours and is also present on cancer stem cells. However, little was known about the expression of 5T4 on haematological malignancies. We have now screened over 100 samples collected from patients with haematological cancers for expression of 5T4 by flow cytometry. Our data shows that cells isolated from the majority of patients with Chronic Lymphocytic Leukaemia, Multiple Myeloma, and Acute Myeloid Leukaemia were positive for 5T4. Moreover, in the majority of instances, we also showed that 5T4 was expressed most on cells that also expressed markers of cancer stem cells. Subsequently, we have demonstrated that CAR-T cells specific for 5T4 are functional and active against 5T4 expressing cell lines. In summary, we believe that 5T4 represents a very attractive antigen for targeting with CAR-T cells.

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P107

Validation of tumor suppressor microrna In liver tumorigenesis using hepatocyte-specific hyperactive piggybac transposons

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Dysregulation of microRNAs (miRs) is associated with cancer development and progression. There are only limited in vivo studies establishing a possible causal relationship between miR expression and the initiation and progression of hepatocellular carcinoma (HCC). To identify and validate miRs that inhibit HCC development in vivo, we established a semi-high throughput in vivo screen using hyperactive piggyBac (hyPB) transposons specifically designed to overexpress miRs in hepatocytes (designated as PB-miR). PB-miRs encoding 15 different miRs were therefore transfected into the liver of mice that were chemically induced to develop HCC. In this slow-onset HCC model, miR-20a significantly inhibited HCC. Next, we developed a more aggressive HCC model using PB transposon-mediated hepatocyte-specific overexpression of HRASG12V and c-MYC oncogenes that accelerated HCC development in mice after only 6 weeks. The tumor suppressor effect of miR-20a could be demonstrated even in this rapid-onset HRASG12V/c-MYC HCC model consistent with significantly prolonged survival and decreased HCC tumor burden. To our knowledge, this is the first study establishing a direct causal relationship between miR-20a over-expression and liver cancer inhibition in vivo, supporting its role as a putative tumor suppressor gene. Moreover, these results demonstrate that hepatocyte-specific hyPB transposons are an efficient platform to screen and identify miRs that favorably affect overall survival and HCC tumor regression, underscoring their therapeutic and diagnostic relevance.

P108

miRNA-193a function as a tumor suppressor gene in human head and neck cancer; Development of a novel therapeutic strategy

ABSTRACT WITHDRAWN

P109

Development of ''Artificial neoepitope''-presenting exosomes for novel cancer immunotherapy

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Tumour-derived exosomes carry some of the same proteins as the originating cells, and can be regarded as miniatures of the parent tumour cells. Although many attempts have been made to utilize them as cancer vaccines, it failed to induce antitumour immune responses. One of the most probable reason would be absence of the efficient neoantigens in the exosomes. Tumour specific mutant neoantigens would be most likely to elicit effective immune responses. However, tumour cells generally express only non-mutated self-antigens, which display weak immunogenicity. To overcome these problems, we introduced an immunogenic signaling molecule, ESAT-6 antigen of Mycobacterium tuberculosis, into the exosome-producing tumour cells as an ''artifical neoantigen'' by gene modification. B16 melanoma cells were transfected with a ternary complex consisting of ESAT-6-pDNA, polyethyleneimine, and acidic polysaccharide. Thanks to the low cytotoxicity and durable high expression efficiency of the ternary complex system, ESAT-6 presenting exosomes (ESAT-Ex) having higher amount of ESAT-6 epitopes could be obtained than by the transfection with a conventional plasmid/polyethyleneimine binary complex. Intratumoural injection of ESAT-Ex induced evident tumor growth suppression in mice. The possibility of immune response induction by the exosomes was then examined. ESAT-Ex were injected twice into mouse foot pads. When the leukocytes collected from the mice were incubated with B16 cells, they intensively accumulated around the tumour cells, and secreted high amount of IFN-gamma. DCs were generated from mouse bone marrow, and treated with the exosomes. The DCs stimulated by ESAT-Ex exhibited significantly improved antitumour activity in tumour-bearing mice.

P110

Targeting the tumour microenvironment with anaerobic bacteria

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Necrosis is a common histological feature to solid tumours that offers a unique opportunity for targeted cancer therapy as it is strictly absent from normal healthy tissues. Tumour necrosis provides a suitable environment for germination of the non-pathogenic anaerobic bacterium Clostridium sporogenes from endospores, resulting in tumour-specific colonisation. Modest anti-tumour activity of C. sporogenes alone encouraged the development of 'armed' vectors (Clostridia Directed Enzyme Prodrug Therapy, CDEPT), whereby germinated bacteria express a prodrug activating enzyme, resulting in the generation of active drug selectively in the tumour microenvironment. The current study identifies nitroreductase (NTR)-1/SN39166 as promising new enzyme/prodrug combination for use in CDEPT, with significant therapeutic efficacy demonstrated in 2D monolayers, 3D cultures and in a subcutaneous in vivo model (tumour growth delay of 88% compared to untreated control animals ($P < 0.001$)). In addition to SN39166, we have demonstrated that NTR-1 can also metabolise positron emission tomography (PET) substrates suitable for non-invasive vector visualisation, and a range of commonly used antibiotics, providing an additional safety feature. This multi-functional ability is a distinct advantage for the clinical development of CDEPT, as imaging of therapeutic gene expression and therefore vector spread will be an essential component of patient monitoring in this context. Furthermore, we have established that C. sporogenes endospores are able to cross the blood-brain barrier and have subsequently developed a luciferase-expressing orthotopic rat glioma model to test this novel enzyme/prodrug combination in a more clinically relevant and accurate setting with an organ-specific tumour microenvironment.

P111

Generation of a library of WT1-specific T Cell receptors (TCR) for TCR gene edited T Cell therapy of acute leukemia

ABSTRACT WITHDRAWN

P112

Low-affinity Her2-specific CAR-T cells for the treatment of breast cancer

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Despite the impressive results of CAR-T cells in hematologic malignancies, the outcome of CAR-T cells in patients with solid tumors remains poor. A key question is how to increase CAR-T cell efficacy while avoiding toxicity. Here, we hypothesized that modifying T cells with a low-affinity CAR may be a safe therapy for the treatment of patients with Her2-positive breast cancers. Using a real-time cytotoxicity assay we show that low-affinity Her2-CART cells can efficiently eliminate tumors expressing high or moderate levels of Her2, even at low effector: target ratios. By contrast, low-affinity Her2-CART cells did not kill cells that are negative or low for Her2 expression. Similarly, Her2-CART cells were only able to proliferate and release cytokines when cocultured with tumors expressing high or moderate levels of Her2. These results suggest that low-affinity Her2-CART cells may avoid on-target-off-tumor toxicities but raise the question of whether lowering CAR affinity may result in tumor escape due to overgrowth of tumor cells with low antigen expression. To address this concern, we modified triple negative breast tumor cells to express high, moderate or low levels of Her2 by using a panel of promoters and mixed these cells to generate a model of heterogeneity of Her2 expression. Using this model, we show that tumor cells that are negative or low for Her2 can be eliminated by low-affinity Her2CAR-T cells when cocultured with Her2-positive tumor cells. Experiments to understand the mechanism behind this observation and to analyze the therapeutic profile of low-affinity CART cells in vivo are ongoing.

P113

Enhanced antitumor efficacy of armed oncolytic adenovirus-loaded menstrual blood-derived mesenchymal stem cells in combination with peripheral blood mononuclear cells

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Among the variety of strategies designed to improve the limited antitumor efficacy observed after systemic oncolytic adenovirus (OAdv) administration in clinical trials, the use of mesenchymal stem cells (MSCs) as cell carriers for oncolytic adenovirus is of special interest because of their natural tumor tropism and immunomodulatory properties. The activation status of the immune system of the patients and the inflammatory profile of the MSCs were suggested to play a role in treatment outcome. We have evaluated the effect of OAd infection on menstrual blood-derived mesenchymal stem cells (MenSCs) at the level of their immune profile, their behavior in the presence of autologous or allogeneic human PBMCs, and their impact in antitumor efficacy in vitro and in vivo. Infection by OAdv induces MenSCs immunophenotypic profile changes, generating a pro-inflammatory environment in co-cultures with allogeneic PBMCs, mainly mediated by monocyte activation, and resulting in the activation of both T-cells and Natural Killer cells (NK cells). Combination of allogeneic PBMCs and OAdv-loaded MenSCs present an enhanced antitumor efficacy both in vitro and in vivo, with monocytes and NK cells playing an important role in this efficacy. Finally, the combination of MenSCs with OAdv-cBiTE, an OAdv expressing an epidermal growth factor receptor (EGFR)-targeting bispecific T-cell engager (cBiTE), enhances the antitumor efficacy compared to MenSCs loaded with the unarmed virus.

P114

Alphavirus-driven expression of IFNgamma as a potential strategy for programming of tumour macrophages

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Tumour microenvironment (TME) and tumour associated immune cells, like tumour associated macrophages (TAM), play essential role in cancer development. The remodelling of the intratumoural cytokine status can re-programme TAM activity and enhance their anti-cancer properties. Alphaviral vectors are perspective candidates for cancer gene therapy as delivery agents of therapeutic cytokines. In this study, we investigated the ability of alphavirus-derived IFN-gamma to activate mouse macrophages to M1 tumoricidal phenotype in presence of TLR2/TLR1 ligands. The efficacy of alphaviral IFNgamma therapy was investigated in two in vivo approaches – subcutaneous (i) and orthotopic (ii) 4T1 mouse breast cancer model. The Semliki forest virus encoding mouse IFNgamma (SFV/mIFNgamma) was injected intratumorally with and without TLR2/TLR1 ligands. SFV/mIFNgamma therapy showed significant tumour growth inhibition in orthotopic model independent on the presence of TLR2/TLR1 ligands. FACS analysis of homogenized tumour tissues revealed therapeutic changes in immune cell composition after injection of SFV/mIFNgamma comparing to SFV/Luc vector as a control: reduced number of TAM cells (detected by analysis of CD11b, F4/80, CD206, MHCII and intracellular iNOs and Arginase expression) and increased number of CD8+ cytotoxic T cells. The obtained results confirm the high potential of alphavirus-based delivery of IFNgamma for therapeutic programming of tumour immune cells. This project was supported by LZP grant No Lzp-2018/1-0208 ''Functional programming of tumor-associated macrophages with viral immunotherapy vectors in breast cancer model''.

P115

Cellular viroimmunotherapy as a treatment to spontaneous canine supratentorial gliomas

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Virotherapy has a great potential in cancer treatment as oncolytic viruses can induce immune-mediated rejection of tumors. However, there are hurdles for efficacy, as systemic administration faces multiple neutralizing interactions in blood. To solve this problem we and others have proposed the use of mesenchymal stem cells (MSCs) as a tumor-homing vehicles to favor the virus reach tumors. We have made a veterinary clinical trial with an improved cellular viroimmunotherapy called Celyvir, using canine MSCs infected with an advanced canine oncolytic adenovirus (ICOCAV17) to treat tumors in canine patients. We have enrolled and treated 9 canine patients presenting high grade gliomas. Our treatment, Celyvir, was well tolerated and no significant adverse effects were observed. Seven dogs survived more than 2 months and then were submitted to an imaging study by MRI (Magnetic Resonance Imaging). Using RAVNO (Response Assessment in Veterinary Neuro-Oncology) criteria based on MRI images, we detected an evident tumor area reduction in 5 dogs (71.4%). Further, we found a clinical benefit with 2 partial responses (28.6%) and 4 stabilization disease (57%). Unfortunatelly 1 patient had a progressive disease. In our study, we did not observe any relation between the tumor reduction nor the mass area with the survival time. We showed oncolitic adenovirus presence in peripheral blood of treated dogs, however, no significant changes were detected in immune populations in peripheral blood analyzed by flow cytometry. Our data indicate that oncolytic viruses loaded in mesenchymal stem cells overcome classical limitations of virotherapies and represent a safe cancer immunotherapy.

P116

Cellular virotherapy increases tumor-infiltrating lymphocytes (TILs) and CD8+ activation in murine immunocompetent models

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Oncolytic virotherapy uses virus designed to selectively replicate and kill cancer cells. In contrast to an intratumoral or intravenous administration, our group uses mesenchymal stem cells (MSC) as cellular vehicles to transport the oncolytic adenovirus to the tumor site. This viroimmunotherapy, named Celyvir, has been already applied to children in a human clinical trial and a veterinary trial with good clinical responses. Despite this promising results, development of more realistic immunocompetent animal models are still necessary for the proper study of the treatment. Here we have developed and validated a murine version of Celyvir (mCelyvir) using mouse MSC infected with the murine oncolytic adenovirus dlE102 in immunocompetent mouse models of renal adenocarcinoma and melanoma. In both models, mCelyvir significantly reduced tumor growth by 50%

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and induced changes in number and activation of tumor-infiltrating lymphocytes (TILs). In conclusion, our cellular virotherapy shows antitumor activity based on the activation of the immune system.

P117

Use of Placental MSCs and their exosomes as theragnostic agents for cancer treatment and diagnostic

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INTRODUCTION: The Na/ I symporter gene (hNIS) is expressed in the thyroid and allows the accumulation of iodine from the diet, to form T3 and T4 hormones. Moreover, it is widely used (i) as a reporter gene for molecular imaging (when the positron emitter isotope is I124 for PET or Tc99 for SPECT) or (ii) as a therapeutic gene for cancer therapy, mediated by the accumulation of I131. An unresolved challenge is how to direct this gene specifically to the tumoral area. Previously, our group demonstrated the migratory capacity of placental mesenchymal stem cells (MSCs), carrying an adenovirus-hNIS to tumors, with good results as theragnostic tool. However, as hNIS is expressed at the placental tissue (because transfers iodine to the foetus from the maternal blood), here we decided to study whether placental MSCs and their exosomes (1) express hNIS endogenously and therefore transfers the imaging and therapeutic potentials when administered with radioactive iodine (2) are capable to reach tumoral areas when they are intravenously injected due to the tumoral tissues extravasation. RESULTS/ SUMMARY We proved that human placenta MSCs and their exosomes have endogenous expression of NIS, migrate specifically to the tumour and their endogenous expression of NIS is enough to image both cells or exosomes in vivo, and their accumulation caused significant therapeutic effect combined with 131I. This highlight the use of endogenous NIS expression as therapy but also to trace new metastatic nodules. FUNDING This work has been funded by AECC, Universidad Francisco de Vitoria, ISClll and IACS

P118

Combinatory therapy of the oncolytic adenovirus ONCOS-102 and checkpoint inhibitor resulted in abscopal anti-tumor effect in a humanized NOG mouse model of melanoma

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Melanoma is an aggressive type of skin cancer with incidence increasing globally. Surgery is effective in early stage melanoma, however despite the introduction and widespread use of check point inhibitors (CPIs), melanoma patients with distant metastasis only have a 10–30% 5-year survival rate. Hence, combinations of existing and experimental anti-cancer agents are being evaluated. ONCOS-102 is an oncolytic adenovirus armed with human GM-CSF and an Ad5/3 chimeric capsid. It has shown to be well tolerated in phase I study (NCT01598129) wherein it induced antitumor immunity, infiltration of CD8+ T cells to tumors, and up-regulation of PD-L1 suggesting that ONCOS-102 could serve as an immunosensitizer in combination with CPIs. In-vivo synergism of these two immune modalities has already been demonstrated. Now we have evaluated the effect of intra tumoral ONCOS-102 in combination with intra venous pembrolizumab (CPI) on non-injected lesions in a humanized NOG (hu-NOG) mouse model. We demonstrated abscopal effect in this model with a dosing schedule beginning CPI concurrently with ONCOS-102 followed by only CPI treatment. In conclusion, the data from this study further support the therapeutic potential of ONCOS-102 in combination with checkpoint inhibitors for the treatment of malignant cancer diseases.

P119

Pretreatment immunoscore and an inflamed tumour microenvironment are associated with efficacy in patients with refractory large B cell lymphoma treated with axicabtagene ciloleucel in ZUMA-1

ABSTRACT WITHDRAWN

P120

Analysis of T-cell vector integration sites for a murine gamma-retroviral vector encoding the anti-CD19 chimeric antigen receptor used in the production of axicabtagene ciloleucel

ABSTRACT WITHDRAWN

P121

CD19 and CD22-directed biespecific CAR for B-cell Acute Lymphoblastic Leukemia

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CD19-directed chimeric antigen (Ag) receptor (CAR)-T cells have shown impressive rates of clinical response in refractory/relapse (R/R) B-cell acute lymphoblastic leukemia (B-ALL), the most common cancer in children. However, CD19- relapses are still common. A combinational targeting of multiple Ags represents a potential strategy to overcome relapses associated to such immune escape, so we developed a bispecific CAR containing binding domains for CD19 and CD22 in tandem (CD22/CD19 CAR) and we cloned it in a pCCL lentivector (pCCL.EF1a.scFvCD22scFvCD19.CD8TM.41BB.CD3zeta.T2A.GFP). Lentiviral particles were produced and used to transduce primary pre-activated human T cells (anti CD3/CD28 plus IL7 and IL15). CAR transduction was successfully detected in human T cells by FACS using GFP expression and CD19 and CD22 chimeric proteins. Using CRISPR/Cas9-edited CD19+CD22+, CD19+CD22-, CD19-CD22+ and double KO SEM cells we confirmed in in vitro cytotoxic assays the specificity of the CD22/CD19 CAR. In vitro (using NALM6, SEM and primary B-ALL samples) and in vivo (using NALM6 and SEM) cytotoxic assays revealed that CD22/CD19 CAR achieves very similar cytotoxic potency than CD19 CAR but with significantly lower in vitro production of the pro-inflammatory cytokines IL-2, INF-γ and TNF-α. In conclusion, our CD22/CD19 CAR exerts cytotoxic activity comparable to CD19 CAR but with a significantly lower production of the pro-inflammatory cytokines which may have major implications in lowering the rates of cytokine release syndrome toxicity. Our results are preliminary and further experimental work is underway to assess whether the CD22/CD19 CAR can contribute to delay/prevent long-term (CD19- or CD22-) relapses.

P122

TEM-GBM-001 study: A phase I/IIa dose escalation study evaluating the safety and efficacy of autologous CD34+ enriched hematopoietic progenitor cells genetically modified for human interferon-α2 in patients with GBM and an unmethylated MGMT promoter

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Glioblastoma (GBM) remains the most common malignant primary brain tumour in adults. Prognosis is poor, likely related to a highly immunosuppressive tumour microenvironment (TME). The TME in GBM is mainly composed of tumour associated macrophages (TAMs) & microglia. A subset of tumour-infiltrating macrophages characterized by expression of the angiopoietin receptor Tie2 (TEMs) have features of M2-TAMs, promote tumour angiogenesis & are infrequently found in normal organs. Tie2 is significantly upregulated upon tumour homing. Gene therapy technology has allowed TEMs to be used as carriers for the local and tumour restricted release of interferon-α (IFN). IFN has anti-tumour effects, inhibits angiogenesis & modulates the immune system. Cell-based delivery of IFN by TEMs is expected to provide efficacy, taking advantage of pleiotropic anti-tumour effects & avoiding tolerability issues associated with systemic IFN treatment. A Phase I/IIa study in Milan (INCB and OSR) is evaluating this therapeutic approach (Temferon) in 21 patients with GBM & unmethylated MGMT promoter. Eligible patients are identified immediately after first surgical resection. Post-screening, harvesting of HSPCs occurs, followed by 6 weeks of radiotherapy. Non-myeloablative conditioning consists of BCNU & thiotepa followed by administration of non-manipulated HSPCs and Temferon. In Part A of the study, 3 cohorts receive escalating doses of Temferon. After Part A, a single dose of Temferon will be studied in 12 patients (Part B). Part A is ongoing with the first 3 patients recruited (June 2019) and Temferon is scheduled for administration. An update on progress and preliminary data will be provided.

P123

Antitumour immune activation by "artificial neoantigen"-presenting exosomes derived from the genetically modified cells

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Mutations in the tumour genome can produce neoantigens, which may generate an effective immune responses. However, most tumours have only non-mutated over- or aberrantly expressed self-antigens which elicit weak immune responses. To overcome this disadvantage, we developed a novel "artificial neoantigen strategy". We transformed the tumour cells by in vivo transfection with a plasmid encoding a strong bacterial antigen, early secretory antigenic target-6 (ESAT-6) from Mycobacterium tuberculosis. It induced high antitumour efficacy in mice and dogs. ESAT-6 protein produced in the transfected cells would be degraded into epitopes, and finally presented on the cell membrane in the context of MHC molecules as "artificial neoepitopes". Those cells would secrete exosomes having ESAT-6 epitope/MHC complexes (ESAT-Ex). ESAT-Ex would most likely modulate immune responses. In the previous study, we prepared ESAT-Ex from cultured B16 melanoma cells which had been transfected with an ESAT-6 gene. Injection of ESAT-Ex into B16-bearing mice evidently suppressed tumour growth. Here we investigated the mechanism of antitumour immunity activation by ESAT-Ex. ESAT-Ex was similarly prepared from the ESAT-6-transfected B16 cells. DCs were derived from mouse bone marrow cells. When the DCs were treated with ESAT-Ex, they showed enhanced CD80/86, and IL-12 presentation. Injection of the ESAT-Ex-stimulated DCs exhibited significantly improved antitumour activity in tumour-bearing mice. Such "artificial neoepitope"-presenting exosomes efficiently activated DCs, and evoked antitumour cellular immunity. They are expected as novel cancer vaccines.

P124

Innovative strategy to potentiate oncolytic adenovirus immunotherapy in high-risk neuroblastoma

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Neuroblastoma (NB) is the most common extracranial solid tumour of childhood. Despite advances in multimodal therapy, children with high-risk (HR) NB have a poor prognosis and new therapeutic approaches are needed. Immunotherapy based on Oncolytic Adenovirus (OA) represents an important immunotherapeutic strategy but its anti-tumour efficacy in patients is suboptimal. Our preliminary studies on viral amplification revealed a correlation between the reduced number of new viral particles (VP) and the expression of Heparanase (HPSE), an enzyme responsible for modelling the extracellular matrix by degradation of the heparin-sulfate, one of the main constituents of viral-capsids. We demonstrated, by immunohistochemistry and qPCR, a significant expression of HPSE in 32 HR-NB compared to normal tissues ($p < 0.001$). This data was confirmed in a public database (https://hgserver1.amc.nl/cgi-bin/r2/main.cgi), showing a negative correlation between HPSE expression and outcome. CHLA-255 and SKNAS NB cell lines show high levels of HPSE and a significant resistance to OA compared to HPSEneg tumours ($p < 0.05$), therefore lentiviral viruses encoding different sh-HPSE have been used to silence the two lines. Silencing by itself induces a strong inhibition of HPSE expression and impaired proliferation in vitro and in vivo. Notably, reduced expression of HPSE leads to a decreased tumour progression and a significant reduction of metabolic activity and exosome production. Following OA infection, the silenced cells produce a greater amount of VP compared to wild-type ($p = 0.001$), show a block of autophagy and a down-regulation of pro-tumoural genes. In conclusion, HPSE modulation is a promising strategy to implement viral oncolytic therapies in patients with NB.

P125

Novel approach for treatment of pediatric high-grade gliomas through the combination of oncolytic adenoviruses and gene therapy encoding a BiTE directed to the EphA2 tumor antigen

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High-grade gliomas (pHGG) are amongst the most common malignant neoplasms of childhood. Unfortunately, outcome remains dismal, urging innovative therapeutic approaches. Immunotherapy with Oncolytic Adenovirus (OA) is a promising strategy but its efficacy is suboptimal. We aimed at improving the antitumor efficacy by combining the OA and a gene-therapy with the Bispecific T-cell Engager (BiTE) directed towards the erythropoietin-producing human hepatocellular carcinoma A2-receptor (EphA2), conveyed by a replication-incompetent adenoviral vector (EAd). We demonstrated, by immunohistochemistry and qPCR, the EphA2 expression in 100% (21/21) of pHGGs, its intensity correlating significantly with worst outcome. We tested the transgene amplification, after co-infection, on two HGG cell lines (U373, U87), by qPCR and FACS, confirming a significantly enhanced production in OA+Ead vs EAd alone ($p < 0.01$ and $p < 0.001$, respectively). Notably, the FACS analysis of the co-infected tumor cells after 5 days of coculture with T-cells showed a significantly increased apoptosis with the engagement of T cells (U373: 82,13% +5,02%; U87: 35,53% +5%), as compared to the OA (U373:70,28 + 3,53%; U87: 3,78% +1%). To obtain the tumor eradication, we activated T cells by an anti-CD28 antibody (αCD28), revealing a further apoptosis enhancement and an increase of activation markers of T cells. Finally, preliminary data on a subcutaneous HGG mouse model showed that the combinatorial approach improves tumor control significantly. In conclusion, the combinatorial approach is able to amplify the production of the BiTE and to determine a significant, tumor-redirected activation of T cells, resulting in an effective tumor control, further amplified in the presence of αCD28, representing a promising innovative treatment.

P126

Topological assessment of mouse embryonic stem, cancer and somatic cells via AFM and SEM: a biophysical approach study

ABSTRACT WITHDRAWN

P127

HVJ-E in Combination with CXCL2 Suppressed Murine Melanoma through Neutrophil-mediated anti-tumor effect

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HVJ-E activates anti-tumor immunity mainly through RIG-I/MAVS pathway, but is independent of Toll-like receptor (TLR) pathways. To investigate whether HVJ-E combined with TLR agonist can synergistically induce anti-tumor activity, we injected HVJ-E with or without poly I:C (TLR3 agonist) into mouse melanoma tumor model. The combination treatment of HVJ-E and poly I:C induced synergistic anti-tumor effect compared to standalone treatments. To determine the mechanism of this anti-tumor effect, we analyzed cytokine expression in each treatment group and found that neutrophil chemotactic CXCL2 was produced in tumors with poly I:C or combination treatment, but not HVJ-E treatment. Tumor associated neutrophils (TANs) are traditionally considered to favor tumor development, and can be further classified into N1 (anti-tumor) and N2 (pro-tumor) phenotypes. In our results, poly I:C did not increase N1 ratio, but recruited more neutrophils into tumors. HVJ-E treatment showed no increment of neutrophils in tumors but polarized neutrophils to N1. Therefore, co-treatment of poly I:C and HVJ-E increased both overall numbers and N1 phenotype of TANs. Neutrophil depletion and CXCL2 neutralization decreased the synergistic effects of the combination treatment. Hence, HVJ-E combined with poly I:C induced neutrophil-mediated synergistic anti-tumor effect. Due to the high toxicity potential of poly I:C, we combined HVJ-E with CXCL2, rather than with poly I:C. The combination of HVJ-E treatment with recombinant CXCL2 or CXCL2 pDNA suppressed tumor growth. In summary, we have shown that (1) N1 neutrophil polarization is a new function of HVJ-E-induced anti-tumor effect, and (2) the potential application of CXCL2 on cancer therapy.

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P128

RNA replacement based on trans-splicing ribozyme for cancer theranostics

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Group I intron-based trans-splicing ribozyme enables to sense and reprogram target RNA into gene of interest. Previously, we proposed hTERT-targeting trans-splicing ribozyme downstream therapeutic suicide gene for cancer therapy. Here, we optimized the specific ribozyme for highly efficient antitumor activity with less off-target effect for theranostics application. We enhanced the intracellular expression of the ribozyme at transcriptional/post-transcriptional level and improved tumor selectivity introduced microRNA target site of the ribozyme. Then, systemic administration of adenovirus encoding our refined ribozyme achieved great anti-tumor efficacy and improved ability to specifically target tumor without hepatotoxicity in vivo. In addition, noninvasive imaging modalities were successfully employed to monitor both how well a therapeutic gene was expressed inside tumor and how effectively a ribozyme therapy took an action against tumor. Collectively, the advanced therapeutic ribozyme and its image-aided evaluation system may lead to the powerful strategy for successful clinical translation and the development of clinical protocols for cancer therapy.

P129

Adeno-associated virus vectors (AAV) transduce human primary neurospheres and slice cultures of glioblastoma multiforme

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Glioblastoma multiforme (GBM) is the most malign brain tumor. Tumor cells with stem cell characteristics are involved in resistance development towards standard treatment (i.e. radiation, chemotherapy). Adeno-associated virus vectors (AAV) are promising vectors for gene therapy. Here, transduction efficiency of a novel AAV serotype 2 based capsid variant (AAVv) in comparison with AAV serotype 2 (AAV2) and 6 (AAV6), carrying GFP as a transgene within a self-complementary vector genome, was systematically analyzed in human GBM neurospheres and tissue slice cultures (TSC). Fresh primary GBM samples were cultured as tissue slice on a membrane at air-liquid interface. In parallel, tissue was dissociated and seeded in a 24 well-plate for neurosphere formation. Transduction efficiency was analyzed by confocal microscopy at day 5 followed by immunohistological analysis. Vector copy numbers were analyzed by qPCR. While as expected, there was some patient-to-patient variability in transduction efficiencies, in absolute values, an identical ranking was observed in terms of GFP expression within neurospheres: AAVv > AAV6 > AAV2. This ranking was confirmed by qPCR analysis. Cells stained positive for stem-like cell markers (i.e. SOX2, OLIG2, Nestin, CD44) also showed GFP expression in neurospheres and TSC. In perspective, these model systems represent interesting tools to genetically manipulate GBM cells within a tissue context and AAVv represents a promising vector for gene transfer in GBM.

P130

Long-term systemic expression of a new PD-1 blocking antibody from an AAV vector provides antitumor activity without toxicity

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Immune checkpoint blockade using monoclonal antibodies (mAbs) able to block PD-1/PD-L1 axis has become a promising treatment for cancer. However, this therapy requires repetitive systemic administration of high mAbs doses, often leading to adverse effects. We have generated a novel mAb against PD-1 (aPD1) able to block the PD-1/PD-L1 interaction for both mouse and human molecules. We cloned the aPD1 gene into an AAV vector downstream of different promoters (CMV, CAG, EF1alpha and SFFV) and analyzed its expression in cells from rodent (BHK) and human origin (Huh-7). aPD1 was expressed at high levels in vitro reaching 5–20 micrograms/ml with all promoters except SFFV, which showed much lower levels. In vivo expression of aPD1 was evaluated in C57BL/6 mice after intravenous administration of AAV8 vectors. aPD1 serum levels increased steadily along time, reaching a maximum of 1 microgram/ml two months after treatment with the vector having the CAG promoter (AAV-CAG-aPD1), and without any evidence of toxicity. In order to test the antitumor potential of this vector, mice that had received AAV-CAG-aPD1, or saline as negative control, were challenged with colon adenocarcinoma cells (MC38). Interestingly, AAV-CAG-aPD1 treatment was able to prevent tumor formation in 30% of mice, in contrast to 8% in the saline group, leading to a significantly higher survival. These data suggest that continuous and relatively low level mAb expression from a long-term expression vector could have antitumor effects with low toxicity.

P131

AKT and JUN are differentially activated in mesenchymal stem cells after infection with human and canine oncolytical adenoviruses

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There is increasing evidence about the use of oncolytic adenoviruses (Ads) as promising immunotherapy agents. We have previously demonstrated the clinical efficiency of using mesenchymal

stem cells (MSCs) as cellular vehicles for oncolytic Ads, human ICOVIR5 or canine ICOCAV17, respectively. Considering the better clinical outcomes of canine Celyvir, in this study we searched for differences in MSC cellular responses to Ad infection that may help understand the mechanisms leading to higher antitumoral immune response. In other primary cell types Ads infection activate the NF-κB pathway and interferons and pro-inflammatory cytokines secretion. However, our findings indicates that human ICOVIR5 activates AKT and JUN in both human and canine MSCs whereas canine ICOCAV17 does not, suggesting that ICOCAV17 induces a more limited host response in MSCs which may be related to the better clinical outcome. This mechanism of action would be imitated by selecting specific human MSC in base to their limited host response after Ads infection.

P132

An immunocompetent mouse model of human neuroblastoma

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Xenograft tumor animal models are critical in the study of tumorogenesis in vivo. However, the use of immunodeficient mice is limited in its informativeness since immune system cells are important for tumor formation and growth. The aim of this study was to create a xenograft tumor model in C57BL/6 immunocompetent mice (males) by administration of genetically modified neuroblastoma cells overexpressing fluorescent protein Katushka2S (SH-SY5Y-Katushka2S). SH-SY5Y cell were genetically modified using recombinant lentivirus LV-Katushka2S. This study was performed in the accordance with approved ethical standards and current legislation (the protocol was approved by the Committee on Biomedical Ethics of KFU (No. 3, 03/23/2017)) and with support by RFBR grant 18-34-00738. A xenograft model was obtained by injecting SH-SY5Y-Katushka2S (7.5×105) under the meninges into somatosensory cortex area of 6-week old male mice. Tumor growth and metastasis were being evaluated for 60 days every 4 weeks using IVIS-Spectrum (Caliper Life Sciences, USA). The proliferative activity was evaluated by IHC staining of mouse brain tissue sections with anti-Ki67 antibodies using LSM 810 META confocal microscope. Numerous small clusters of SH-SY5Y-Katushka2S disseminated in the cerebral cortex, ventricular area and cerebellum were detected. A high mitotic index (83%) showed that SH-SY5Y-Katushka2S cells continued to proliferate, therefore, host immune system was not able to completely eliminate them. Thus, the xenotransplantation of human neuroblastoma cells in the brain of immunocompetent mice was successful. The obtained data may contribute to the further development of xenograft models which can be used for testing new anticancer drugs.

P133

Human mesenchymal stem cells as tumor-homing cellular carriers of retroviral replicating vectors for cancer gene therapy

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Mesenchymal stem cells (MSCs), which possess the ability to migrate to tumors and contribute to forming tumor stroma, are being investigated as a promising vehicle for delivery of anticancer agents, including viral vectors. In this study, we evaluated the ability of MSCs to deliver retroviral replicating vectors (RRVs), which have shown highly promising results for gene therapy of cancer in both preclinical and clinical studies. All of 3 different subtypes of human MSCs derived from adipose tissue (hMSC-ad), bone marrow (hMSC-bm) and umbilical cord (hMSC-uc) infected efficiently with RRV, but produced virus progeny less efficiently than tumor cells. We then assessed tumor cell tropism of these MSCs toward mesothelioma cells, using a Transwell plate migration assay. All of these MSCs showed significant migratory activity toward the mesothelioma cell targets. Furthermore, when co-cultured, RRV were transmitted efficiently from MSCs to mesothelioma cells, thereby achieving high levels of tumor cell transduction. Additionally, treatment with 5-fluorocytosine (5-FC) prodrug after MSC-mediated delivery of RRV expressing the yeast cytosine deaminase prodrug activator gene resulted in potent cytotoxicity to malignant mesothelioma cells in vitro. Furthermore, intratumoral RRV transmission in vivo from MSCs to mesothelioma cells was confirmed following MSC injection via tail vein (subcutaneous tumors) or peritoneally (peritoneally-disseminated tumors) by biomolecular imaging and flow cytometry. Notably, hMSC-ad and hMSC-uc showed much higher tumor transduction efficiency compared to hMSC-bm. These data indicate the potential utility of tumor-homing MSC to deliver RRV more efficiently and further enhance the efficacy of RRV-mediated gene therapy for systemic and metastatic cancers.

P134

Screening of reporter gene expressing human adenovirus types with regard to their use in oncolytic/cancer gene therapy against HPV associated cancers

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As Adenoviral vectors based on Adenovirus (AdV) serotype 5 have limitations for in vivo applications, we explored 21 different GFP- and luciferase reporter gene expressing AdV-serotypes for their ability to transduce HPV positive cervical- as well as head and neck cancer cell lines. 24 hours post transduction reporter gene expression levels were quantified for each serotype and cell line by measuring luminescence and fluorescence, using a multi-plate reader. AdV14 showed higher transduction rates compared to the commonly used AdV5. AdV3 showed comparable transduction rates as AdV5, whereas other serotypes did not enter efficiently. AdV14 and AdV3 are promising candidates for the conversion into efficient and specific vectors for the treatment of HPV related epithelial tumours. Moreover, we conducted cell viability assays to identify potential tumour-cell killing effects of specific AdV-serotypes. 48 hours post transduction we saw decreased tumour-cell viability when infected with specific AdV-serotypes. We then selected those serotypes and analysed their replication competence within the HPV positive cancer cells, to investigate their possible oncolytic ability. AdV14 replicated its genome to a higher than

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AdV5, but also AdV3, 21 and AdV35 showed strong replication in HPV positive cervical cell lines, whereas other serotypes did not replicate. We believe that these AdV-serotypes are suitable candidates for the conversion into future vectors with enhanced transduction and replication efficiencies in HPV positive cells that might improve oncolytic or tumour gene-therapeutic treatments of HPV related cancers.

P135

Adult peripheral blood and umbilical cord blood NK cells are good sources for effective CAR therapy against cd19 positive leukemic cells

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B-cell hematologic cancers such as leukemia and lymphoma are common forms of pediatric and adult cancers worldwide. Acute Lymphoblastic Leukemia (ALL) is the most common cancer among children with a prevalence of 20–25% of all cases. The survival rate for these patients at 5 years is 79.2%, but it is still an incurable disease in many patients. Chimeric Antigen Receptor (CAR) T cells therapy has arisen as a new alternative to conventional therapies in order to treat advanced refractory cancers. A potential problem could be the need of using allogenic T cells when is not possible to perform an apheresis to the patient, as allogenic T cells carry a risk of graft versus-host disease (GVHD). Natural killer (NK) cells fight against neoplastic cells without previous sensitization. Moreover, allogenic NK cells exhibit a potent graft versus leukemia (GVL) effect without causing GVHD. Because of these characteristics, allogenic NK cells from adult peripheral blood (AB) and cord blood (CB) are an attractive source for cancer immunotherapy. We transduced AB NK cells and CB NK cells with a lentiviral vector containing CAR-CD19 with similar transduction efficiency (45–50%). Moreover, we performed functional assays, including degranulation and cytotoxicity, against hematological cancer cell lines and primary cells from CLL patients that express CD19 cell surface receptor, with the purpose of demonstrating the specific killing efficiency of these CAR-CD19 transduced NK cells. In summary, AB NK cells and CB NK cells are promising cell sources to consider for CAR-based immunotherapy, due to the low risk of GVHD.

P136

Modifications in the RGD motifs of an oncolytic adenovirus (ISC301) increases its antitumor efficacy by a higher intratumoral activation of the immune system

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Oncolytic virotherapy uses virus designed to selectively replicate and kill cancer cells. Their antitumor effect is the result of the lytic effect and the intratumor stimulation of the immune system. Here we have developed and validated a new oncolytic adenovirus, named ISC301, based on the oncolytic adenovirus ICOVIR-5. ISC301 include RGD motifs in H1-loop of the fiber knobs, but it have been delete the RGD motifs in the penton bases. In our mice models, two days after systemic administration, the antitumor efficacy of ISC301 was significantly increased compared to ICOVIR-5 in an immunocompetent mouse model of adenocarcinoma. In the same tumor model, an increased activation of the NF-kB pathway was observed in vitro and in vivo in tumors treated with ISC301 compared to those treated with ICOVIR-5, resulting also in increased number of tumor-infiltrating lymphocytes (TILs). In conclusion, the new oncolytic adenovirus ISC301 presents higher antitumor efficacy than the previous ICOVIR-5, which seems to be based on a higher intratumoral activation of the immune system.

P137

CRISPR-mediated rearrangement generation of t(4;11) in human prenatal and perinatal hematopoietic stem/progenitor cells

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Rearrangements of the MLL gene are associated with aggressive acute leukemia. The most common MLL-rearrangement is MLL-AF4 which is the hallmark genetic abnormality of infant t(4;11) pro-B ALL, it also has one of the lowest mutation rates among cancers. It is associated with poor prognosis and displays a very brief latency, raising the question of how this disease evolves so rapidly. Despite worldwide efforts, progress about its aetiology, pathogenesis and cellular origin remains unresolved. In order to contribute to these unresolved questions, we have applied a CRISPR/Cas9-mediated genome editing strategy to recreate the t(4;11) translocation in HSPC isolated from different ontogeny stages including fetal liver and cord blood derived HSPCs. The genome edited HSPCs at distinct developmental stages have been functionally assayed to address whether t(4;11) initiates leukemogenesis on its own or whether secondary cooperating hits are required for an overt leukemia. These pioneering studies will reveal a precise understanding on the impact of such chromosomal rearrangements on leukemogenesis, and provide a humanized disease model, offering a platform for new treatment strategies.

P138

Elucidating transcriptional roles in the regulation of IMP3 expression for developing promoter-driven targeted therapy in hepatocellular carcinoma

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Carcinogenesis of hepatocellular carcinoma (HCC) is a multi-step and complex process mainly caused by activation of oncogenes or deregulation of tumor suppressor genes. It is therefore important to identify novel HCC-associated genes in order to provide the underlying molecular mechanisms in the pathogenesis of HCC. Here, we identify IGF2 mRNA-binding protein 3 (IMP3) using cDNA microarray in HCC patients and further demonstrate the upregulated levels of IMP3 mRNA in HCC tumors. Meanwhile, high levels of IMP3 significantly correlate with poor overall survival in patients with HCC. Also in line with our studies, the trait of bad overall survival is also observed in a cohort of HCC patients from Kaplan Meier plotter. Besides, in vitro cell model shows that knockdown of IMP3 greatly inhibits HCC cell proliferation and anchorage-independent tumor growth. Interestingly, IMP3 protein is strongly expressed in the tumor compared to their paired adjacent non-tumorous liver specimens of HCC patients and liver tumor of HBxTg mice, suggesting that regulation of IMP3 overexpression is probably activated by the transcriptional regulation. By luciferase reporter assays, we find that approximate 1-kb upstream region of IMP3's transcription start site is critical for its expression. HCC cells expressing cytosine deaminase driven by 1-kb promoter region are sensitive to 5-FC, indicating that IMP3 promoter is potentially developed for a novel selective gene-directed enzyme prodrug therapy (GDEPT). Further investigation will provide the regulatory mechanisms for IMP3 expression, a GDEPT based on IMP3 promoter, and a useful diagnostic marker in prediction of outcome and prognosis in HCC.

P139

Intravital visualization of magnetic nanoparticles in eukaryotic cells using dark-field microscopy

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In contrast with routine methods for detecting nanoparticles, dark-field microscopy (DFM) allows obtaining full-color, clear images of magnetic nanoparticles (or another type of nanomaterials), which can be located both on the surface and inside of eukaryotic cells. DFM is a fast, simple, and effective method of visualizing nanoparticles. The goal of our study was to detect the localization and define the degree of magnetic nanoparticles (MNPs) aggregation both inside and on the surface of Hep3B cell culture (human hepatocellular carcinoma, ATCC, USA) using enhanced dark-field microscopy. The cells were cultured under standard culture conditions (5% CO2 at 37 °C) with MNPs (0.05 mg/ml) for 24 hours. Then the Hep3B cells were washed with buffer (PBS) and put on slides without fixation. Dark-field and hyperspectral images were collected using the Olympus BX51 vertical microscope (Japan) equipped with the CytoViva® (USA) amplified darkfield condenser, a DAGE CCD camera, and a 100× magnification objective lens. Images were analyzed using Exponent 7 imaging software (Dage-MTI). As a result, dark-field images of magnetic nanoparticles both inside and on the surface of the Hep3B cell culture were obtained with the ability to estimate the degree of aggregation of magnetic nanomaterials. Thus, enhanced dark-field microscopy can be used to visualize a wide range of nanoparticles. This work was performed according to the Russian Government Program of Competitive Growth of KFU, funded by the subsidy (project 16.2822.2017/4.6) allocated to the KFU for the state assignment in the sphere of scientific activities and funded by RFBR 18-34-00306 project.

P140

Deletion of Vaccinia virus genes involved in the blocking of TRIF-IRF3-pathway and its effect on Th1-polarized antitumor immune responses

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Recently, oncolytic vaccinia viruses (VACV) have shown their potential to produce clinically effective cancer treatments. The reasons for this clinical success are not only the direct destruction of infected cancer cells, but also the activation of an antitumor immunity caused by the release of Danger-Associated Molecular Patterns (DAMPs) and Tumor-Associated Antigens (TAAs). DAMPs induce the overcoming of tumor immunosuppression and TAAs can be uptaken by antigen presenting cells and initiate a potent antitumor adaptive immune response. For activating a robust antitumor immunity, it has been demonstrated that a Th1-polarization of the response is preferred, and such polarization can be achieved by an activation of the TRIF-IRF3 pathway, as demonstrated by the use of polyI:C as an adjuvant in cancer vaccination. However, strains of VACV used to date as oncolytic drug candidates endode several proteins involved in the blocking of this pathway and the subsequent secretion of type I interferons, which limits elicitation of an antitumor immunity. By deleting the genes involved in TRIF-IRF3 pathway blocking, we aim for a virus that fully maintains the capacity to replicate in and kill cancer cells, but at the same time has an increased capacity to activate Th1 antitumor immune response. Our studies include monitoring of viral replication, both in vitro and in vivo, quantification of TRIF-IRF3 pathway activation after infection, and their effect on antitumor immune responses and overall antitumor activity in mouse tumor models.

P141

The novel oncolytic lentiviral vector expressing IFNβ and pseudotyped with the measles virus HF glycoproteins displays therapeutic efficacy as a gene therapy-based approach for multiple myeloma

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In the context of developing a novel therapeutic approach for Multiple Myeloma (MM), we generated an IFNβ-expressing lentiviral vector (LV), pseudotyped with the measles virus HF glycoproteins and assessed it in vitro in H929 and JJN3 myeloma cell lines. Production of IFNβ/HF and control GFP/HF LVs was carried out by transient co-transfection of HEK-293T cells. Vector titration and vector copy number per cell (VCN/cell) were determined by flow cytometry and qPCR, respectively. Cell

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survival and cytotoxicity were evaluated using Trypan blue exclusion and CCK-8 colorimetry assays, respectively. Apoptosis was estimated by Annexin-V staining. IFNβ secreted by IFNβ/HF-transduced cells was determined by ELISA, while its paracrine manner of action was evaluated by a Transwell® assay. Lastly, serum neutralizing antibody activity was evaluated by flow cytometry on JJN3 cells transduced with GFP/HF, previously incubated with MM or normal donors' serum. IFNβ/HF exhibited a mean transduction efficiency of 29.5% (H929) and 23.1% (JJN3), at a mean VCN/cell of 1.6 and 1.2, respectively. IFNβ/HF-transduction at MOI 1, led to a dramatic 90.6% and 95.2% reduction of cell survival in H929 ($p=0.01$) and JJN3 ($p=0.04$) cells, respectively. Regarding apoptotic effects in H929 and JJN3 cells, a marked 90.3% ($p \leq 0.001$) and 74.1% ($p \leq 0.001$) increase was documented. IFNβ secreted by the transduced cells, was capable of significantly decreasing the viability of untransduced cells, while the neutralizing antibody barrier of both MM and normal donor serum was confounded at a dilution of 1:64. Collectively, our data suggest that IFNβ/HF LV represents a promising novel therapeutic approach for MM.

P142

Synergistic anticancer therapeutic effect of sorafenib and microRNA-21 inhibitor in Hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Unfortunately, most patients present with advanced disease and have limited treatment options due to lack of effective therapies. Sorafenib is an oral multi-kinase inhibitor used to treat advanced HCC. Although sorafenib has improved the survival time by a few months, it still has a variety of side effects like dermatological, digestive and cardiovascular toxicity. Moreover, sorafenib resistance often develops. MicroRNAs (miRNAs) bind to the 3'-UTR of target genes and thereby repress translation of them. Aberrant expression of miRNAs is closely associated with initiation and progression of pathophysiologic processes including diabetes, cardiovascular disease, and cancer. We found that miR-21 is highly expressed in the HCC cell lines and the livers of patients with HCC. Also, we demonstrate for the first time that suppression of miR-21 activity in combination with sorafenib results in significant inhibition of tumor growth in HCC xenograft model. Our findings suggest that miR-21 plays an important role in promoting HCC tumorigenesis and that inhibition of miR-21 could be beneficial for the treatment of liver cancer.

P143

Influence of physiologically active agents on polyplex-mediated systemic gene delivery to melanoma tumours

ABSTRACT WITHDRAWN

P144

Evaluating CAR-T cells efficiency against solid tumors models

ABSTRACT WITHDRAWN

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P145

AML-derived extracellular vesicles transmit immunomodulatory potential

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Trophic factors including extracellular vesicles (EVs) secreted by AML cells have recently been described as potent modulators instructing the leukemic niche. We observed that AML-derived EVs but not the donor cells and not their secreted/soluble factors (sol.F.) spread an immunomodulatory capacity capable of inhibiting T cell proliferation. AML cell lines (HL-60, OCI-AML3, MOLM-14, KG-1) were cultured under static vs. dynamic (2D vs. 3D) conditions, in ambient air as compared to organotypic reduced oxygen environment in defined particle-depleted media. Tunable resistive pulse sensing indicated a mean particle release ranging from 2x108–2x109 per ml/48h with a mean diameter of app. 150 nm (range: 50 – 750 nm). Large scale crude EV propagation was done by tangential flow filtration (TFF) to obtain >100x particle enrichment. An additional TFF purification step was introduced to separate sol.F. from AML-EVs, followed by size exclusion chromatography (SEC) or ultracentrifugation (UCF) to obtain virtually pure EVs (protein <1.0 mg/mL with UCF and <0.5 mg/ml with SEC). AML-EV morphology was confirmed by electron microscopy and identity by immunoblotting and flow cytometry. Bead-based EV surface profiling showed hematopoietic and EV-specific markers. Single EV flow cytometry revealed calcein-positive events indicating the presence of intact EVs in our preparation. We further observed that 4/4 AML-EV preparations but only 1/4 AML cell lines and 0/4 secreted factor fractions inhibited T cell mitogenesis. Additional functional tests are currently underway. These data show that AML-EVs but not the parental cells or leukemia-derived sol.F. display a previously unexpected immunomodulatory capacity indicating novel targets for therapeutic intervention.

P146

The effect of co-culturing of MSC and SH-SY5Y and cisplatin treatment on proliferative activity and caveolin-1 mRNA expression

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Caveolins are the main components of caveolae, which are involved in many cellular functions, including endocytosis, cholesterol transport, signal transduction and tumour suppression. We investigated the interaction of MSCs derived from human bone marrow and human neuroblastoma cells SH-SY5Y in co-culture as well as the effect of cisplatin (CDDP) on cell proliferation and Cav1α, Cav1β mRNA expression. Study was performed in the accordance with approved ethical standards and current legislation (the protocol was approved by the Committee on Biomedical Ethics of KFU (No. 3, 03/23/2017)) and with support by RFBR grant 18-74-10044. To create co-culture, cells were mixed at 1:1 ratio, CDDP (10 μg/mL) was added. After 72 hours of incubation MTT-test was performed. The proliferative activity of co-culture was significantly lower after CDDP-treatment, however higher in comparison with treated SH-SY5Y. Cav1α expression level in MSCs after co-culturing demonstrated decrease, in SH-SY5Y after co-culturing - increase. Cav1β expression level in MSCs after co-culturing was lower than in control, in SH-SY5Y after co-culturing were observed increase in comparing with control. Rac1 mRNA was increased in MSCs after co-culturing and increased after CDDP treatment, SH-SY5Y treated with cisplatin demonstrated increase. Thus, the increase of caveolin-1 expression in SH-SY5Y after CDDP treatment in co-culture and monoculture can confirm the magnification of the Cav1-dependent invasive, migratory and metastatic properties of cancer cells. Also, the significant decrease of Cav1 expression in MSC after co-cultivation with SH-SY5Y may demonstrate the decreasing of differentiation ability of MSC, which indicates the recruitment of MSC by tumour cells.

P147

The combined action of cisplatin and Physcion increases the ROS level in the lung and pancreas cancer cells

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Cisplatin is one of the commonly used antitumor drugs for the treatment of different cancers. One of the mechanisms of cisplatin cytotoxicity is associated with oxidative stress initiation. Increased level of the reactive oxygen species (ROS) detected in many types of malignancies enhances the cells proliferative activity, but its excessively high level can lead to apoptosis. Thus, one of the promising therapeutic strategies for cancer treatment is the regulation of intracellular ROS levels by targeted drugs. Physcion is an inhibitor of 6PGD enzyme which catalyzes the intracellular antioxidant NADPH formation. In this study, performed according to the Russian Government Program of Competitive Growth of Kazan Federal University, the combined action of the cisplatin and Physcion on the ROS level and viability of lung (H1299) and pancreatic cancer cell lines (AsPc-1) is described. In AsPc-1and H1299 cells, it was shown the treatment with Physcion increased the level of ROS up to 1.3 and up to 1.5 times, respectively. At the same time, the combined action of Physcion with cisplatin caused a growth in the ROS level in these cells up to 1.7 and up to 2,5 times, respectively. Moreover, IC50 of the cisplatin combination with Physcion decreased 1.6 times in the AsPC-1 cells and 2.2 times in H1299 cells compared to cisplatin monotherapy. The synergistic effect of cisplatin and Physcion allegedly is associated with a ROS balance shift towards increase directing tumor cells to apoptosis. Thus Physcion could be a promising drug for cancer treatment improving.

P148

Hyperspectral system imaging for detection of cancer cells

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In recent years, hyperspectral system imaging (HSI) has been used as a tool for the detection of nanomaterials in biological matrices. Here we present using the HSI for obtaining of hyperspectral images in a complex and highly dispersive environment, such as cell cultures. This information is necessary to develop an effective strategy for the detection of cancer cells. HSI records the scattering of light in the visible and near-infrared (VNIR, 400–1000 nm) regions in each pixel of the image field. Using the HSI it is possible to find out the spectral profile that is unique to the each studied object. We studied human colon carcinoma (HCT-116) and human prostate adenocarcinoma (PC3) cell lines. Thus, we collected a scattering intensity database that provided the spectra of cancer cells. The spectral profile for HCT-116 showed a bimodal peak at 550 nm and 675 nm. For PC3, the spectrum showed a band with a maximum at 600 nm and with a noticeable shoulder at 675 nm. The spectral profile for different cancer cells is significantly different, that allows for optical and spectral differentiation. The work was done at the expense of subsidies allocated as part of the state support of KFU in order to increase its competitiveness among the world's leading scientific and educational centers, and through funding under the state 16.2822.2017/4.6 and MD-6655.2018.4. Also, the work was partially carried out with the financial support of the Russian Foundation for Basic Research and the government of the RT grant № 18-44-160001.

P149

Induction of cell death in human melanoma cell lines by the combination of p14ARF plus interferon-β gene transfer

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In our cancer immunotherapy approach, we aim to induce both cell death and an anti-tumour immune response upon gene transfer. Previously, we have shown that combined p19Arf (functional partner of p53) and interferon-β (IFNβ, pleiotropic cytokine) gene transfer resulted in elevated levels of cell killing associated with markers necroptosis, immunogenic cell death (ICD) and immune activation in a mouse model of melanoma. Here we present a critical advance in our understanding of how this approach impacts human melanoma cells. We have constructed non-replicating Ad5 vectors with constitutive expression of either human p14ARF or IFNβ and applied these viruses to the UACC62 and SK-Mel-29 cell lines. For both, elevated levels of killing were encountered upon combined gene transfer as compared to single gene treatment as measured by the accumulation of hypodiploid cells, activation of caspases 3/7 and annexin-V staining. Strikingly, pan-caspase inhibition using Z-VAD-FMK peptide blocked cell death in UACC62, but not SK-Mel-29, indicating a non-apoptotic mechanism of cell death in the latter case. In situ gene therapy of s.c. UACC62 tumours in nude mice revealed superior inhibition of progression when four doses of combined gene therapy were applied, an effect that could be extended with additional virus injections. While combined p14ARF+IFNβ gene transfer is beneficial for cell killing and control of tumour progression, much remains to be studied with regard to the mechanism of cell death and immune activation in response to our gene therapy approach in human cells. Supported by the Sao Paulo Research Foundation (FAPESP), CNPQ and CAPES.

P150

Antitumor properties of artificial microvesicles from mesenchymal stem cells overexpressing TNF-related apoptosis inducing ligand

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Almost all human cells release extracellular vesicles (EVs), spherical micro- and nanoparticles, which are separated from the cell surface and participate in intercellular communication. EVs are a promising tool for the delivery of bioactive molecules for therapeutic purposes. One of the promising cytokines with anti-cancer properties is TNF-related apoptosis-inducing ligand (TRAIL) which is able to selectively induce apoptosis in malignant but not normal cells. The ideal cell type for the production of EVs is human mesenchymal stem cells (MSCs) as they exhibit a homing behaviour to tumor niches and MSC-isolated EVs retain the ability of the parental cells to migrate toward tumor sites. Genetic modification of MSCs with TRAIL gene and the subsequent production of EVs from them can be a promising approach for cancer treatment. This study was performed in the accordance with approved ethical standards and current legislation (protocol approved by the Committee on Biomedical Ethics of KFU (No.3, 03/23/2017)) and supported by grant MK-236.2019.4. In this study, MSCs with TRAIL overexpression were treated with cytochalasin B to increase the yield of EVs. Cytochalasin B-induced artificial microvesicles (CIMVs-TRAIL) were positive for CD44, CD90 and CD105 MSC surface markers, but CD29 and CD73 expression was significantly decreased (about 10%). TRAIL expression in CIMVs was confirmed by qPCR and Western blot. Using TEM and flow cytometry CIMVs-TRAIL were shown to be mostly 50–200 nm in diameter which is comparable with natural EVs. CIMVs-TRAIL exhibited significant antitumor activity in SH-SY5Y tumor cells culture in vitro.

P151

Cancer-derived exosomes loaded with ultrathin palladium nanosheets for targeted bioorthogonal catalysis

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The transformational impact of bioorthogonal chemistries has inspired new strategies for the in vivo synthesis of bioactive agents through non-natural means. Among these, Palladium (Pd) catalysts

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have played a prominent role in the growing subfield of bioorthogonal catalysis by producing xenobiotics and uncaging biomolecules in living systems, and new exciting Pdcatalyzed reactions and applications continue to emerge. However, delivering catalysts selectively to specific cell types still lags behind catalyst development. Towards this goal, we have developed a bio-artificial device consisting of cancer-derived exosomes loaded with Pd catalysts by a novel method that enables the controlled assembly of Pd nanosheets directly inside the vesicles. This new hybrid system mediates Pd-triggered dealkylation reactions in vitro and inside cells and displays preferential tropism for their progenitor cells. The use of Trojan exosomes to deliver abiotic catalysts into designated cancer cells creates the opportunity for a new targeted therapy modality: exosome-directed catalyst prodrug therapy, whose first steps are presented herein with the cell-specific release of the recently approved anticancer drug panobinostat.

P152

WEE1 and CHK1 gene silencing using Polypurine Reverse Hoogsteen Hairpins

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We investigated the inhibition of CHK1 and WEE1 genes, using Polypurine Reverse Hoogsteen hairpins (PPRHs). PPRHs are gene silencing molecules, composed by two mirror repeat polypurine domains, linked by a pentathymidine loop and bound by intramolecular reverse-Hoogsteen bonds. Different PPRHs were designed, 3 directed to WEE1 and 4 to CHK1. All PPRHs were tested individually at 100 nM in HeLa cells in cell viability assays. Five PPRHs reduced cell viability around 80% and were tested in PC3 cell line with similar results. We observed a dose-dependent effect in reducing cell viability when using HpWEE1Pr-T and HpCHK1I1-C. In addition, time-course assays, showed that HpWEE1Pr-T and HpCHK1I1-C were cytotoxic with only 6 hours of cell incubation at 100 nM. After 20 hours of incubation with HpCHK1I1-C and HpWEE1Pr-T, there was an increase in apoptotic cell population of 3-fold. The treatment of HpWEE1Pr and HpCHK1I1-C reduced mRNA levels of their target genes between 1.6-2-fold. Moreover, we observed a decrease of 50% WEE1 protein levels after 24 h incubation of HpWEE1Pr-T and a decrease of 50% of CHK1 protein levels after 15 h of incubation of HpCHK1I1-C. In addition, we observed the formation of two splice variants of CHK1 mRNA after 24 h of incubation with HpCHKI1I1-C. In conclusion, Inhibition of WEE1 and CHK1 genes using PPRHs results in decreases in mRNA and protein levels and increases in apoptosis. Moreover, HpCHK1I1-C promotes the appearance of new splicing variants. Thus, PPRHs can be used to inhibit genes involved in replicative stress as anti-cancer gene therapy.

P153

Production of mouse mesenchymal stem cell lines with Luciferase and Katushka2s reporter gene expression for bioluminescence imaging

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Mesenchymal stem cells (MSCs) are a major component of the tumor microenvironment and play a key role in promoting tumor progression. MSCs have been shown to exhibit a homing behavior toward tumor sites that makes it perspective vehicles for anticancer agent delivery, particularly chemotherapy drugs. The ffLuc-encoding gene cloned from the Photinus pyralis is the most studied and well characterized bioluminescent reporter gene. In this study mouse MSCs (mMSCs) that express the luciferase reporter gene (ffLuc) or Katushka2S were produced. mMSCs were isolated by enzymatic digestion with collagenase. This study was performed in the accordance with approved ethical standards and current legislation (the protocol was approved by the Committee on Biomedical Ethics of KFU (No. 3, 03/23/2017)) and with support by RFBR grant 18-34-00738. The cells were largely positive for MSC surface markers (CD44, CD90, CD29, CD105, CD73 and Sca-1) and negative for hematopoietic stem cell markers. Recombinant lentiviruses LV-ffLuc and LV-Katushka2S were produced by co-transfection of the HEK293T packing cell line. The viral titer was determined by flow cytometry of cells transfected with lentiviruses carrying Katushka2S. mMSCs were transduced with recombinant lentiviral vectors encoding ffLuc or Katushka2S gene. Resulting mMSCs-ffLuc cell line was selected with puromycin for 10 days. The relative intensity and stability of the firefly luciferase signal in mMSCs-ffLuc were analyzed by using ONE-Glo™ Luciferase Assay System. Obtained mMSCs-ffLuc cell line with a stable luminescent signal will be further primed with various chemotherapeutic drugs and its antitumor properties will be analyzed in vitro.

P154

Replicating retroviral vectors spread on the human retinoblastoma SNUOT-Rb1 cells and induce cell death

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A couple of viruses have been developed as oncolytic virotherapy to kill infected tumor cells directly. However, a couple of problems of oncolytic virotherapy such as early viral clearance by host immune system and frequent attenuation of the viral infectivity raised the need of new tools for virotherapy. As a promising tool for cancer treatment, replicating retroviral vector (RRV) is known to have high selectivity into tumors and stability in gene transfer because RRV can replicate only in dividing cells and has extremely low immunogenicity. Retroviral-mediated transfer of the herpes simplex virus type 1 thymidine kinase (HSV1-TK) gene or yeast cytosine deaminase (yCD) gene into glioma followed by treatment with prodrug has been widely used for glioma gene therapy clinical trials. One of the most common childhood cancer, Retinoblastoma (Rb), is an intraocular tumor that grows rapidly and poses a threat to sight and life. In this study, we examined the potential of a split-RRV system encoding HSV1-TK and yCD gene for retinoblastoma gene therapy. We, here, show the feasibility of RRV for retinoblastoma gene therapy.

P155

Development of lipid nanoparticles for the mRNA-mediated cancer immunotherapy

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ABSTRACT WITHDRAWN

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Dissipation of the transmembrane mitochondrial potential in malignant tumor cells by water-soluble polyol-methanofullerene

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The subject of our study is the water-soluble methanofullerenol C60[C9H10O4(OH)4]6 (synthesized in the Institute of Organic and Physical Chemistry, Kazan, Russia), which earlier was shown to uncouple OXPHOS and respiration in eukaryotic Yarrowia lipolytica cells. Here, we report that methanofullerenol C60[C9H10O4(OH)4]6 dissipates transmembrane mitochondrial potential (Ψm) in tumor cells with high mitochondrial activity. The Ψm measurements were performed at 48 h cell culture time point using a ratiometric vital cationic JC-1 fluorochrome on a Guava Millipore flow cytometer. Normalized mitochondrial potential Ψm was expressed in arbitrary units (a.u.) as the ratio of red and green fluorescence signals generated by aggregates and monomers of JC-1, respectively. We tested the influence of methanofullerenol on Ψm in four different malignant human cell lines: SNB-19 (glioblastoma, $\Psi m = 5{,}88 \pm 0{,}47$ a.u), HCT-116 (colorectal carcinoma, $\Psi m = 7{,}66 \pm 1{,}77$ a.u.), MCF-7 (breast adenocarcinoma, $\Psi m = 2{,}73 \pm 0{,}13$ a.u.) and SH-SY5Y (neuroblastoma) ($\Psi m = 0{,}10 \pm 0{,}003$ a.u.). The treatment with methanofullerenol (4 and 40 μmol, 2 hours) in a concentration-dependent manner downregulated mitochondrial potential in SNB-19 cells by 70%, in HCT-116 cells by 50% and in MCF-7 cells by 30%. The methanofullerenol had no effect on SH-SY5Y cells with largely depolarized mitochondria. At the methanofullerenol concentration of 40 μmol the drop in Ψm was accompanied by a decrease in the generation of the superoxide anions in mitochondria. The results obtained justify the rationale for further studies of methanofullerenol C60[C9H10O4(OH)4]6 as a biological response modifier applied in a combination with chemotherapeutic antitumor drugs.

P157

SLC34A2 as a potential prognostic marker of triple-negative breast cancer patients' survival

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Triple-negative breast cancer (TNBC) remains the most aggressive subtype among breast cancers in particular because of the lack of targeted therapies. In spite of the variable patients' response to treatment and a high recurrences rate, chemotherapy remains a conventional treatment for TNBC. Thus, the identification of new prognostic markers for TNBC management is a relevant subject. In this study, we aimed to evaluate the sodium-dependent phosphate transporter NaPi2b, as a molecular marker for TNBC patient's survival prognosis. The mRNA and protein expression of NaPi2b (SLC34A2) were evaluated in formalin-fixed paraffin-embedded tumors of 39 TNBC patients by real-time qPCR and immunohistochemical (IHC) staining of tissue microarray sections, respectively. As a reference gene, the ACTB was used. The NaPi2b was considered as «overexpressed» if the value of mRNA expression was higher than the median in qPCR analysis, and if more than 10% of cells were stained with «++» or «+++» intensity in ICH analysis. Using RStudio, the Kaplan-Meier and Log-rank tests were carried out for survival analysis. It was shown that overexpression of NaPi2b protein in the tumors is associated with a more prolonged overall survival of TNBC patients ($p\text{-value} = 0{,}034$). But any correlations were not observed on the transcriptional level. This finding could be related to the crucial role of NaPi2b regulation exactly on the translational level. Based on this study results, performed according to the Russian Government Program of Competitive Growth of Kazan Federal University, the NaPi2b could be considered as potential prognostic TNBC marker.

P158

Comparative analysis of mechanical properties of non-malignant and cancer cells

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Despite the development of new methods for study of carcinogenesis, the mechanism of the formation of metastasis is still not completely clear. The mechanical properties of cells play an important role in the malignancy development and spread of cancer cells. Change in cell stiffness is one of hallmark of

metastasis formation. Therefore, mechanical properties of cells are one of the important indicators for assessing the physiological state of cells. Atomic force microscopy (AFM) provides accuracy in determining the elasticity of cells. In this study we employed three cell types, fibroblasts, human lung carcinoma cell line (A549) and prostate cancer (PC3). Using the atomic-force microscope Dimension Icon, operating in PeakForce Tapping mode, we obtained the morphological visualization of studied cells. This mode of operation allows detecting the mechanical properties of the sample, including its elasticity. It was found that cancer cells are softer than fibroblasts most likely due to changes in the cytoskeleton that occur in cells during malignancy development. The elasticity of the A549 and PC3 cells was approximately the same. A clear correlation between cell rigidity and their metastatic potential was not shown. The work was done at the expense of subsidies allocated as part of the state support of Kazan (Volga Region) Federal University in order to increase its competitiveness among the world's leading scientific and educational centers, and through funding under the state 16.2822.2017/4.6 and partially carried out with the financial support of the Russian Foundation for Basic Research No. 18-34-20126 (mol_a_ved).

P159

Analysis of Rac1, Bcl2, Cav1α, and Cav1β gene expression of neuroblastoma cells, mesenchymal stem cells and human peripheral blood mononuclear cells after co-cultivation

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The tumor microenvironment plays a key role in the processes of tumor growth and the development of therapeutic resistance. We investigated the effect of the cisplatin on the expression of mRNA Bcl-2, Cav1α, Cav1β, Rac1 of mesenchymal stromal cells isolated from bone marrow (BM-MSC), neuroblastoma SH-SY5Y cells and human peripheral blood mononuclear cells (PBMC) in co-culture. Study was performed in the accordance with approved ethical standards and current legislation (the protocol was approved by the Committee on Biomedical Ethics of KFU (No. 3, 03/23/2017)) and supported by RFBR grant 18-74-10044. To create a co-culture SH-SY5Y (modified by lentivirus encoding green fluorescent protein), BM-MSCs and PBMCs were mixed in a 1:1:1 ratio. After 72 hours of co-cultivation cisplatin was added (10 μg/ml). After additional 72 hours of incubation with cisplatin cells were separated by flow cytometry according to the fluorescence spectrum and size. The expression level of Bcl-2, Cav1α, Cav1β, Rac1 was determined by quantitative PCR, using 18S rRNA as a reference gene. The expression of Bcl-2 increased in the BM-MSC and decreased in PBMC. The expression of Bcl-2 in SH-SY5Y had no significant changes. Cav1α expression decreased in SH-SY5Y and increase in PBMC. Cav1β expression decreased in BM-MSC and SH-SY5Y, but increased in PBMC. Rac1 expression decreased in PBMC and SH-SY5Y, in BM-MSC increased after co-cultivation. The data may indicate the active participation of PBMC in the processes of inhibition of tumor cells growing, reducing their metastatic potential, as well as in suppressing the tumor-supporting properties of BM-MSC.

P160

Artificially cloaked viral nanovaccine

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Virus-based cancer vaccines are nowadays considered among the most promising approaches in the field of cancer immunotherapy, despite the observation that the majority of the immune responses they elicit are against the virus and not against the tumor. In contrast, targeting tumor associated antigens is effective, however the identification of these antigens remains challenging. Here, we describe ExtraCRAd, a novel multi-vaccination strategy focused on an oncolytic virus artificially wrapped with tumor cancer membranes carrying tumor antigens. We demonstrate that ExtraCRAd displays increased infectivity and oncolytic effect in vitro and in vivo. We show that this novel nanoparticle platform controls the growth of aggressive melanoma and lung tumors in vivo both in preventive and therapeutic setting, creating a highly specific anti-cancer immune response. In conclusion, ExtraCRAd might serve as the next generation of personalized cancer vaccines with enhanced features over standard vaccination regimens, representing a novel way to target cancer.

P161

Expansion of genetically engineered regulatory T cells *in vitro* and *in vivo* via signaling through a synthetic, small-molecule-controlled IL-2 receptor

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Deficits in regulatory T cell (Treg) number and function are central to the pathogenesis of autoimmune disease. Here we describe the production, expansion, and in vivo function of gene-edited Tregs (edTregs). edTregs are generated through conversion of CD4-positive T cells into regulatory-phenotype T-cells via CRISPR/Cas9-mediated engineering of the FOXP3 gene. The engineering process results in over-expression of the native FoxP3 gene from a strong, heterologous promoter, and confers a stable Treg-like phenotype on the gene-edited cells. To support efficient engraftment and persistent suppressive capacity, our approach further comprises co-introduction of a synthetic IL-2 receptor activatable by rapamycin, thus giving an orthogonal, titratable, proliferative signal for edTreg expansion. We demonstrate gene editing of 25–50% of CD4-positive T cells and rapamycin-driven preferential expansion of edited cells to >85% purity in a clinically translatable process. edTregs display cell-surface markers such as IL2R-alpha, CTLA-4, and IL7R-alpha in a manner indistinguishable from thymic Tregs. In contrast to stimulation of unedited cells, edTregs do not produce appreciable IL-2 and have lower TNF and IFN-gamma production. Following adoptive transfer into immunodeficient NSG mice, edTregs protect against experimentally induced xenogenic graft-versus-host disease and show improved persistence in vivo with rapamycin treatment. Rapamycin-inducible edTregs represent a

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novel alternative to expanded polyclonal Treg therapy for autoimmune disease.

P162

Engineering of highly functional and specific transgenic T cell receptor (TCR) T cells using CRISPR-mediated in-locus insertion combined with endogenous TCR knockout

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Adoptive transfer of T cells genetically modified to express tumor-specific TCRs for the treatment of cancer has garnered considerable attention in recent years. However, current approaches have certain limitations and safety concerns. Specifically, lentiviral or retroviral insertion of a transgenic TCR (tg-TCR) generates random integration events at multiple loci and can result in supra-physiological expression levels. Mispairing with endogenous TCRs, if not removed, can also lead to unwanted specificity and low yield. Using CRISPR/Cas9, we identified guide RNAs (gRNA) targeting TRAC and TRBC1/2 loci with high indel frequency and specificity. Additionally, we identified a TRAC guide that yielded high insertion rates using adeno-associated virus templates. Combinations of efficient activation methods, T cell culture, gene editing, and electroporation conditions resulted in loss of >98% endogenous TCR α and β chains while reaching >70% insertion rates of tg-TCRs without further purification. The edited tg-TCR T cells expanded comparably to non-edited T cells and exhibited low levels of exhaustion. Functional characterization of Wilm's Tumor 1-specific tg-TCR T cells suggests that TCR insertion into the TRAC locus results in functionally reactive T-cells with robust on target activity. Transgenic T cells generated with our methods for deleting endogenous α and β chains showed significantly improved tg-TCR expression levels and greatly reduced mispairing as compared to cells that were edited only for TRAC. TCR replacement using CRISPR/Cas9-mediated genome editing could lead to improved tg-TCR-T cell therapies for the treatment not only of hematological cancers, but also of solid tumors.

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P163

Allogenic CAR.CD19 natural killer cells: a new immuno-gene-therapy "off-the-shelf" in the treatment of acute lymphoblastic leukaemia

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Use of chimeric antigen receptors (CAR) represents a promising approach to enhance NK-cell function against acute lymphoblastic leukemia (ALL). Like T cells, NK cells can be genetically modified to express CARs and represent an ''off-the-shelf'' product, potentially eliminating the challenges of patient-specific products that plague current CAR-T cell therapies. We developed a feeder- and FBS-free manufacturing approach, resulting in a 3.2-log expansion after 20 days of culture. Peripheral blood derived NK cells (PB-NK) preserve ADCC activity and show a high percentage of CD56+ CD57- cells (85% ± 13%), as well as very low percentage of PD-1 expression inhibitory receptor. After genetic modification with retroviral CAR construct, NK cells display a stable in vitro CAR.CD19 expression (38% ± 15%) and show significant anti-leukemia activity towards CD19+ tumour cell lines (7% ± 9%, 16% ± 30% and 22% ± 16% of residual 221, DAUDI and BV173 cells, respectively). Importantly, CAR.CD19-NK cells exert high anti-leukemia activity towards primary leukemia blasts from patients with B-cell precursor ALL. The advantage of NK cellular adoptive platform over the more conventional T cell-based one was also proved in in vivo xenogenic mouse model. In particular, a single CAR.CD19-NK cell systemic infusion was capable of inducing significant tumor control in immune-deficient leukemia-bearing mice, compared to NT-NK cells. Moreover, employing double infusions of effector cells, the OS of mice treated with CAR.CD19-NK cells was superior than that of mice receiving CAR.CD19-T cells in which we observed a human IFN-γ and TNF-α plasma level elevation.

P164

Engineering BCMA-CAR T cells to overcome CD19-negative relapse in B cell non-Hodgkin lymphomas

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Genetic engineering of T cells with chimeric antigen receptors (CARs) has recently emerged as a promising therapeutic strategy to treat cancer patients. Currently approved CD19-specific CAR-T cell therapies have demonstrated remarkable curative potential, achieving 40% of complete response rates for relapsed or refractory non-Hodgkin lymphoma (NHL). However, CD19-negative disease at time of relapse has been described as a mechanism of treatment failure, underlining the need for alternative targets. Thus, we developed novel CAR-T cells against B cell maturation antigen (BCMA), and investigated their potential for the treatment of CD19-negative relapsed NHL. In this study, an anti-BCMA single chain variable fragment was cloned into a third-generation lentiviral vector, which was incorporated to a CAR backbone containing 4-1BB or CD28 costimulatory domains, a CD3ζ chain with a P2A element and tdTomato reporter to track the engineered T cells. To model the CD19-negative relapse observed in patients, CRISPR-Cas9 technology was used to establish a CD19-knockout Ramos lymphoma cell line (R19KO). Efficacy of BCMA CARs was validated in vitro against R19KO cells expressing a GFP-firefly luciferase reporter. Finally, we compared CD19-BBz, BCMA-BBz and BCMA-28z CAR-T cells in an in vivo relapse model in which NSG mice were injected with R19KO. As expected, CD19 CAR-T cell treatment resulted in lymphoma progression. Treatment with both BCMA CAR-T cells demonstrated successful anti-lymphoma activity, while more durable responses were conferred by the BCMA-BBz CAR. In summary, our data suggest BCMA-CAR

T cell therapy as a potential strategy for the treatment of NHL, regardless of previous CD19-targeted therapy.

P165

$\gamma\delta$ CAR-T cell therapy for bone metastatic castrate resistant prostate cancer

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Bone metastasis is a frequent complication in advanced prostate cancer, with the resultant lesions significantly contributing to patient morbidity and mortality. We report a study of the antitumour and bone protecting effects of the combination of $\gamma\delta$ T cells, expressing a chimeric antigen receptor (CAR), with zoledronate (Zometa®). The premise of this study is that zoledronate, a bisphosphonate prescribed to inhibit osteoclast activity, can indirectly induce activation and chemotaxis of T cells expressing a Vγ9Vδ2 T-cell receptor. Based on this interaction, we tested the ability of human primary $\gamma\delta$ T cells expressing an anti-Prostate Stem Cell Antigen (PSCA) CAR, to eradicate C4-2B prostate tumors in bone. In vitro, $\gamma\delta$ CAR-T cells displayed more potent cytotoxicity than $\alpha\beta$ counterparts. Moreover, cytotoxicity and cytokine secretion were enhanced by zoledronate, achieving completed eradication of tumour cells, indicating that the activity of the endogenous Vγ9Vδ2 T-cell receptor is preserved in $\gamma\delta$ CAR-T cells. To test the impact of $\gamma\delta$ CAR-T cells in vivo, NSG mice were intratibially injected with PSCA/luciferase-expressing C4-2B cells. Tumours were allowed to establish for 10 days and then randomized into control or $\gamma\delta$ CAR-T groups. Subsequent bioluminescent imaging indicated a rapid and significant ($p = 0.0006$) regression of tumours in the $\gamma\delta$ CAR-T cell group, leading to increased overall survival ($p = 0.0002$). Ex vivo bone morphometry analysis also demonstrated the significant protective effect of $\gamma\delta$ CAR-T associated bone disease. Collectively, these results support the use of $\gamma\delta$ CAR-T cells, in combination with zoledronate, as a novel immunotherapy for bone metastatic prostate cancer.

P166

TEM8/ANTXR1-targeting CAR-T cells as a therapeutic modality for the pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies. Although patients with metastases from colorectal or breast cancer have reported improved outcomes with the advent of new chemotherapeutic agents, there has not been much increase in survival of patients with pancreatic cancer. Therefore, a better therapeutic option is urgently required. By analyzing tissue samples from 104 PDAC patients at stage I, II, III or IV, we found that the expression of tumor endothelial marker (TEM8/ANTXR1) was significantly overexpressed. Therefore, it would be a potential target for immunotherapy. Here, we designed CAR-T constructs using anthrax protective antigen (PA63) that is a ligand of TEM8/ANTXR1, instead of typical scFv derived from monoclonal antibodies. After expressing PA63-CAR in human primary T lymphocytes, PA63-CAR-T cells underwent degranulation and released IFNγ in response to TEM8/ANTXR1-positive primary PDAC cells as well as to MIAPACA-2 and PANC1 tumor cell lines in vitro, but not against TEM8/ANTXR1-negative melanoma tumor cells. Furthermore, injection of PA63-CAR-T cells into tumor-bearing mice significantly delayed subcutaneous tumor growth. In conclusion, we identified TEM8/ATNXR1 as a novel target for CAR-T therapy in pancreatic cancer. This is the first study to show that PA63-CAR-T cells could provide an effective clinical treatment for TEM8/ANTXR1-positive primary and metastatic tumors in pancreatic cancer patients. Since TEM8/ATNXR1 was also known to be overexpressed in other solid tumors such as breast cancer and colorectal cancer, the PA63 CAR-T therapy can be extended to other solid tumors.

P167

Engineering NK cells to express nanobody-based Chimeric Antigen Receptors (CARs)

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Nanobodies are the recombinant variable domains derived from heavy chain antibodies that naturally occur in llamas and other camelids. A key advantage of nanobodies over the pair of variable domains of conventional antibodies is their better solubility. This makes nanobodies particularly suited as binding domains for chimeric antigen receptors (CARs). The ectoenzyme CD38 is overexpressed by multiple myeloma and other hematological malignancies and is a potential target for immunotherapy. Using nanobodies that bind to three distinct epitopes of CD38, we engineered retroviral vectors encoding for CARs in which the nanobody is linked via the hinge of IgG4 to the transmembrane domain of CD28 and the ITAMs of CD28, 4-1BB and CD3ζ. We stably expressed these CARs in the human NK cell line NK92. NK cells expressing CD38-specific nanobody-based CARs specifically lysed CD38-expressing Myeloma cells independently of the bound epitope, whereas NK cells expressing control nanobody-based CARs did not. Moreover, NK cells expressing CD38-specific nanobody-based CARs showed little if any cytotoxicity against target cells in which the CD38 gene had been inactivated using CRISPR/Cas9. Similarly, in primary bone-marrow cells retrieved from multiple myeloma patients NK cells expressing CD38-specific nanobody-based CARs significantly reduced the number of viable Myeloma cells. Our results demonstrate that functional CD38-specific CAR NK cells can be generated using nanobody derived binding domains. These CD38-specific nanobody-based CAR NK cells hold promise as new 'off-the-shelf' therapeutic tools for the therapy of multiple myeloma.

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Broadening the applicability of antigen-specific T-cell immunotherapy by CRISPR-CAS9 inactivation of the glucocorticoid receptor

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Infections after allogeneic hematopoietic stem cell transplantation (allo-HSCT) represent potentially fatal complications. While pharmacotherapy often fails, adoptive immunotherapy (AI) with pathogen-specific T cells (pSTs) is a promising alternative. However, T cells perform suboptimally under steroids, the first-line treatment of transplant-associated complications, thus depriving the most susceptible to infections patients of the potential benefits of AI. We explored the impact of dexamethasone (DEX) on T cells and developed a CRISPR/Cas9 system to genetically disrupt the glucocorticoid receptor (GR) and confer resistance to steroids, towards the development of steroid-resistant pSTs. A 3-day DEX exposure impaired the proliferation of CD3/CD80-pulsed primary T cells and induced apoptosis and the transition from a central to an effector memory phenotype, while upregulatedPD-1 and CTLA-4 over the ''no-DEX'' condition. To inactivate the GR, T2 cells transduced with lentiviral vectors encoding Cas9 and 10 different gRNAs (targeting GR at a transcription start site or exons 2–5) in pooled libraries or separately, were incubated±DEX. In DEX's presence, cells edited with two gRNApoolsor8/10single gRNAs presented similar proliferation as the untreated cells, suggesting functional DEX-resistance.GR disruption was confirmed by western blotting. Among tested gRNAs, the optimal gRNA will be selected on the basis of high GR disruption efficiency andlowoff-target activity. Overall, we provide a series of gRNAs disrupting the GR and conferring resistance of T cells to steroids. We endeavor to ultimately generate steroid-resistant pSTs, enabling the most vulnerable to infections patients as those post allo-HSCT and under steroids, to benefit from AI.

P169

CAR-Tregs for the treatment of Systemic Lupus Erythematous

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Systemic Lupus Erythematosus (SLE) is a paradigmatic autoimmune disease, characterized by an aberrant immune response against nuclear antigens. Regulatory T cells (Tregs) represent a subset of T lymphocytes able to exert immune suppressive functions. In the context of SLE, Tregs are dysfunctional, being unable to control the disease. Chimeric Antigen Receptors (CARs) are chimeric molecules capable of redirecting T cell specificity against target antigens, greatly enhancing T cell activity. The aim is to employ CAR technology to redirect and enhance Treg activity in order to exploit their immune suppressive capabilities to control SLE manifestations. We expanded Tregs from peripheral blood of Healthy Donors using anti-CD3/CD28 magnetic beads in presence of IL-2 and rapamycin. We transduced the cells using a LV encoding for a second generation anti-CD19 CAR obtaining a good efficiency of transduction. CAR-Tregs suppressed the proliferation of autologous Peripheral Blood Mononuclear Cells when co-cultured together in presence of a polyclonal stimulus. CAR-Tregs showed antigen-specific immune suppressive capabilities when co-cultured with autologous B cells, being able to completely abolish B cell proliferation. To test their capabilities to suppress an antigen specific conventional T cell response, we co-cultured CAR-Tregs together with conventional anti-CD19 CAR-T cells and a CD19+ cell line. CAR-Tregs demonstrated to be able to suppress conventional CAR-T cell response, improving tumor cell proliferation. We efficiently generated a CAR-Treg based strategy that can be employed in SLE. Now we plan to further validate CAR-Treg functionality in an in vivo SLE mouse model.

P170

A promising approach of GD2-specific chimeric antigen receptor (CAR) T cells with CD28/4.1BB costimulatory domains for treatment of GD2+ sarcomas

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Genetically modified T cells expressing chimeric antigen receptors (CARs) represents a new class of therapeutics that has shown encouraging results for the treatment of some types of cancer. The disialoganglioside GD2 is an important potential target for CAR T cells due to its overexpression in many tumors, including Rhabdomyosarcoma (RMS), Ewing's Sarcoma and Osteosarcoma (OS). We set up a preclinical model of RMS (RD cell line) and OS (143B cell line) to assess anti-tumor activity of CAR T cells using a γ-retroviral vector carrying a third generation GD2-specific CAR (GD2.CD28.4-1BB.z), currently used in our phase I/II clinical study for patients with High Risk and/or relapsed/refractory Neuroblastoma (NCT03373097). The cytotoxic activity was assessed in vitro by co-culture assays, in which GD2.CAR-T cells demonstrated a significant tumor control compared to untransduced (NT) T cells against both RD (6.02% ± 9.40% vs 66.07% ± 13.86% of residual tumor, p = 0.0005) and 143B (13.2% ± 12.71% vs 69.25% ± 10.00% of residual tumor, p = 0.00005). GD2.CAR-T cells confirmed this potent anti-tumor activity even when plated at high ratios of tumor/CAR-T cells (16:1) for both RD (58.06% ± 23.98% vs 84.55% ± 7.73%, p = 0.02) and 143B

(38.26% ± 23.18% vs 75.64% ± 11.54%, p = 0.003). In our RMS mouse model, GD2. CAR-T cells showed both a superior anti-tumor activity and persistence compare to NT T cells, which resulted in a significant reduction of tumor bioluminescence after 50 days (5,7x109 vs 2.6x107, p = 0.05) and increased overall survival (40% vs 0% at day 75, p = 0.023). In conclusion, these data show that our GD2.CAR-T cells could represent an effective approach for the treatment of GD2-positive pediatric sarcomas.

P171

Gene editing: Paving the way for accelerated clinical development of adoptive cell immunotherapies

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Precision genome engineering requires technologies that allow efficient and reproducible delivery of DNA, mRNA and RNP-based reagents into a range of primary cells and stem cells. In addition, clinical gene editing requires a transfection platform that is GMP-compliant and scalable to accommodate billions of cells in a single transfection. Here we share data on gene deletion and correction in primary cells and cell lines following transfection of CRISPR/cas9 and zinc finger nuclease molecules using MaxCyte's clinically validated flow electroporation platform. Data on efficient modification of induced pluripotent stem cells, knock-down of checkpoint inhibitors in T cells and correction of point mutations in hematopoietic stem cells are included along with examples of process scalability. Finally, we include data on multiplex editing and gene knockout in T- and NK-cells.

P172

Specificity and efficacy of second generation CAR T-cells against CD44v6 antigen in primary HNSCC cells

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The standard-of-care for patients with head and neck squamous cell carcinoma (HNSCC) still results in relapse in at least 50% of patients within the first five years. Thus, immunotherapy including chimeric antigen receptor (CAR) T-cell therapy has the potential to serve as novel treatment option. Among a rather limited number of potential target antigens for CAR T-cells on HNSCC is CD44v6, a CD44 splicing variant. CD44v6 has been the target for monoclonal antibody-based phase I/II studies in human and used to direct CAR T-cells against human AML blasts. In order to assess the importance of a CD44v6 CAR against HNSCC, we first analysed 33 primary HNSCC lines for their CD44s and CD44v6 expression by flow cytometry and noticed that CD44s and CD44v6 were expressed homogeneously on most cell lines. No cell line was CD44s- and CD44v6+. When co-cultivating HNSCC cell lines with different CD44v6 and CD44s antigen densities with a 2nd generation CD44v6 CAR lentivirally expressed in primary human T-cells, we observed that CD44v6 CAR T-cells eliminate malignant cells very efficiently, even when less CD44v6 is present on the cells. CD44s+ cells that did not express CD44v6 were not lysed. To further validate the specificity of the CD44v6 CAR to isoform 6 encoded by exon 11, we knockout two cell lines with high CD44s and CD44v6 expression and then singly re-expressed either CD44s or CD44v6. Cytotoxicity of CD44v6 CAR T cells only occurred on the latter cells. Thus, CD44v6 CAR T-cells are excellent effector cells against CD44v6+ primary HNSCCs.

P173

Towards engineered pluripotent stem cell-derived non-allogeneic, antigen-specific T cells

ABSTRACT WITHDRAWN

P174

Comparing the functionality of redirected T cells in HBV immunotherapy using chimeric antigen receptors or T-cell engaging antibodies

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Chronic hepatitis B affects 255 million humans worldwide putting them at high risk to develop liver cirrhosis or hepatocellular carcinoma. Nucleos(t)ide analogues are the current treatment but they do not achieve virus elimination because of their inability to target the nuclear persistence form of the hepatitis B virus (HBV), the covalently closed circular DNA (cccDNA). A curative treatment will have to target HBV persistence that drives liver cancer development. To cure HBV and target HBV-associated liver cancer, an immune therapeutic approach will be needed to activate HBV-specific T cell responses. HBV-specific effector T cell responses can be generated by T-cell redirection using either chimeric antigen receptors (CAR) or T-cell engager antibodies. To evaluate the functionality of both approaches, we redirected T cells towards HBV-infected cells by utilizing bi-/tri-specific antibodies or CAR-transduced T cells using the same scFv binding the small HBV envelope protein (S) to target infected hepatocytes. Design of antibodies and CAR combined the domains for simultaneous targeting HBV-envelope proteins and activating T cells via CD3/CD28 signalling. Both tools were compared with respect to their ability to activate T cells, to kill HBV envelope protein expressing hepatocytes, to elicit antiviral activity and reduce cccDNA. We demonstrate that both approaches, bispecific antibodies and S-CAR grafting, activated T cells and showed antiviral effects on infected hepatocytes. However, S-CAR transduced T cells were more effective than antibody-engaged ones within the first 96 hours of co-culture indicating that activation via bispecific antibodies takes a longer time than direct activation by intracellular CD3/CD28 signalling.

P175

Antigen-specific targeting of triple negative breast cancer using Cytokine-Induced Killer cells redirected with monoclonal antibodies

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Cytokine-Induced Killer (CIK) cells are ex vivo expanded T cells with NK cell phenotype. They are an attractive approach for cellular immunotherapy, as they are capable of recognizing tumor cells without the need of antigen-specific priming and can be efficiently and rapidly expanded in vitro. We recently reported that CIK cells have a relevant expression of FcγRIIIa (CD16a), which can be exploited in combination with clinical-grade monoclonal antibodies (mAbs) to redirect their cytotoxicity in an antigen-specific manner. In this study, we evaluated the efficacy of this combined therapy in in vivo mouse models of triple negative breast cancer (TNBC), an aggressive tumor that occurs in approximately 15% of all breast cancer patients. Here, CIK cells injected together with Cetuximab (CTX) induce a significant delay in tumor growth in mice implanted with TNBC patient-derived tumor xenografts (PDX) or injected with MDA-MB-231 cell line. Remarkably, the combined treatment almost completely abolish metastatic spreading to the lungs. We confirmed this relevant finding in a mouse model where the primary tumor was surgically removed before the treatment, as it occurs in clinical setting for the treatment of patients. The growth of metastases in the lungs and lymph nodes was significantly delayed by the combined treatment and mice had a significant better survival. Taken together, these data highlight the potentially of the CIK cell therapy combined with tumor-specific antibodies as a promising treatment for TNBC metastases. This promising strategy could be further improved and extended to different types of malignancies.

P176

A combinatory CAR relying on T-cell signaling via endogenous CD3 elicits paramount tumor antigen sensitivity via retrovirally, ivt-RNA-, or CRISPR/Cas9 NHEJ/HDR-treated human T-cells

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Chimeric antigen receptor (CAR) based cellular immunotherapy of cancer revealed curative efficacy of liquid tumors. As a perspective, we proposed a novel CAR design that in the style of a T-cell receptor (TCR) associates with the endogenous CD3 components to confine T-cell activation to the limiting resources of signaling and costimulatory molecules such as CD3z, CD28, and 4-1BB. This may more closely relate to physiological T-cell signaling thereby minimizing detrimental hyperactivation and ''off-target''-reactivity. As model tumor antigen, we used the tight-junction protein Claudin 6. In several CAR protein scaffolds the antigen-recognizing fragment was fused to the constant domains Ca/Cb of a TCR which mediate association to CD3z2, CD3de (Ca) and CD3ge (Cb). The tandem order of identical V-domains on either Ca/Cb-chain of a heterodimer (ie VH-VH-HuCa + VL-VL-HuCb) accomplished a salient interchain affinity to create a robust CAR/CD3-complex. These by either retroviral transduction or ivt-RNA electroporation reprogrammed CAR T-cells exhibited superior characteristics in cytokine secretion (IFNg ELISA/ELISPOT), cytotoxicity (Luciferase-, spheroid-, impedance-measurements), and proliferation (CFSE) against ivt-RNA antigen titrated target cells. Since Ca/Cb-designed CARs have to compete with endogenous TCRs for incorporation into the native CD3 complex and secondly, they might cross-activate the endogenous TCR ending up with autoreactivity, the latter were deleted by virtue of CRISPR/Cas9-NHEJ utilizing ivt-RNA Cas9 and synthetic gRNAs targeting the Hu TRAC locus in electroporation. This intervention improved CAR expression and reactivity against Cl6-positive SKOV3. We also confirmed tumor lysis by CRISPR/Cas9-HDR treated human T-cells via genomically inserting a DNA template of the 2A-linked combinatory CAR.

P177

LVs development for a fine-tuned regulation of CARs in T cells

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Despite the remarkable success of anti-CD19-CAR-T therapies, there are still important issues that compromise the safety (cytokine release syndrome, neurologic toxicity, B-cell aplasia) and efficacy of the therapy (exhausted cells/poorly regulated CAR expression). To overcome these challenges, we aim to develop lentiviral vectors (LVs) that can lead to 1) inducible and/or 2) TCR-like CAR-T cells. For the first strategy, we showed that the LentOnPlus-Is2-LV, previously generated by our group, is a good platform to regulate transgene expression in T cells in vitro and in vivo without the requirement of transactivators. The system exhibited very low leaking and very good sensitivity to Doxycycline (<1pg/ml in vitro and 1ug/ml in jelly for in vivo). Based on that, we produced inducible 3rd generation CAR T cells (iCARIs2-T cells), that lysed specifically CD19+ cells only in the presence of Dox. However, iCARIs2-LVs require further optimization due to low titer, which we are trying to improve by reducing the size of the IS2 insulator. For the second objective, we have designed a panel of chimeric promoters based on Wiskott-Aldrich (WAS), LCK, CD247, CD4 and B2-microglobulin (B2M) loci. All the chimeric promoters drive GFP expression following the TCR expression profile upon TCR engagement, highlighting the outperforming behavior of the B2M. We have constructed LV-B2M and LVs-WAS expressing 3rd generation anti-CD19-CAR to investigate whether these LVs achieve TCR-like expression of CARs in T cells and whether they are more efficient and/or have longer survival compared to EF1-α promoter-driven CAR-T cells.

P178

Assessment of efficacy and safety of universal αCD19-CAR T cells

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Current adoptive immunotherapy strategies use autologous T cells expressing a Chimeric Antigen Receptor (CAR) against CD19+ B cells malignancies. Beyond the impressive benefit, time and quality of manufacturing T cells from the refractory patients constitutes a critical limitation for a successful therapy. Allogenic universal CAR-T cells can overcome the histocompatibility problem and can be generated from healthy donors to treat several patients. Targeting TCR can avoid graft versus host disease (GVHD) and lead to a less exhausted population. In this line, we have generated primary αCD19-CAR T cells that are 70–90% deficient for the TCR using Cas9/sgRNA ribonucleoprotein without affecting to the phenotype and the proliferation capacity. Both WT and TCRKO-αCD19-CAR-T cells showed good efficacy and selectivity in vitro. Using this platform, we investigated the potential benefits of inducible TCRKO-CAR T cells and of TCRKO-CAR T cells expressing the CAR following the TCR expression pattern. TCRKO T cells expressing the CAR in a TCR-like fashion displayed specific lytic activity and a less exhausted phenotype. On the other hand, iTCRKO-CAR T were able to control type B leukemia in vitro only in the presence of doxycycline. CRISPR/Cas9 technology could encounter undesired side effects on T cells due to off-targets and on-target repair mechanims. We didn't find any significative cleavage in six predicted off-targets in silico. However, we found large deletions on the target site of up to 4 kb. At this point, we are generating mouse models to study the efficacy and safety of these TCRKO-αCD19CAR T cells in vivo.

P179

Development of an efficient transduction protocol for CAR-19 in cord blood derived-NK cells

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Immunotherapy treatment infusing autologous modified chimeric antigen receptor (CAR)-T cells has grown over the last years leading to the development of more than 200 clinical trials infusing CAR T cells to treat haematological malignancies. Nonetheless, NK modified with a CAR represent also a potential source of immune cells due to several advantages that they present in comparison to T cells, such as the property of not inducing graft versus host disease (GVHD) development in the patient. Moreover, cord blood-derived NK cells (CB-NK) are NK cells that could be used as an ''off the shelf product'' from allogenic cord blood units while T cells have to be autologous to avoid GVHD. However, CB-NK are not so easily transduced as T cells. Therefore, we aimed to optimize different expansion and transduction protocols for CB-NK using lentivirus. First, we compared EF1-a and MSCV promoters, and observed that MCSV increased CB-NK transduction in comparison to EF1-a. Then, we compared two protocols for CB-NK expansion; one based on the co-culture of CB-NK with K562-based artificial APCs expressing membrane bound IL-21 ''Clone 9''with addition of IL-2 for 14 days, and a second protocol based on NK MACS medium (Miltenyi) with IL-2 and IL-15 addition. Whereas the first protocol achieved a higher CB-NK expansion, the second one obtained more potent CB-NK and a slight higher transduction. Finally, we tested retronectin, polybrene and vectofusin-1 as transduction reagents, and observed that vectofusin-1 provided with the best transduction and cell viability, achieving transduction rates for a CAR19 higher than 50%.

P180

Defective localization with impaired tumor cytotoxicity contributes to the immune escape of NK cells in pancreatic cancer patients

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Tumor infiltrating lymphocytes (TILs), found in patients with advanced pancreatic ductal adenocarcinoma (PDAC), are shown to correlate with overall survival (OS) rate. Although majority of TILs consist of CD8+/CD4+ T cells, the presence of NK cells and their role in the pathogenesis of PDAC remains elusive. We performed comprehensive analyses of TIL, PBMC, and autologous tumor cells from 80 enrolled resectable PDAC patients to comprehend the NK cell defects within PDAC. Extremely low frequencies of NK cells (< 0.5 %) were found within PDAC tumors, which was attributable not to the low expression of tumor chemokines, but to the lack of chemokine receptor, CXCR2. Forced expression of CXCR2 in patients' NK cells rendered them capable of trafficking into PDAC. Furthermore, NK cells exhibited impaired cell-mediated killing of autologous PDAC cells, primarily due to insufficient ligation of NKG2D and DNAM-1, and failed to proliferate within the hypoxic tumor microenvironment. Importantly, these defects could be overcome by ex vivo stimulation of NK cells from such patients. Importantly, when the proliferative capacity of NK cells in vitro was used to stratify patients on the basis of cell expansion, patients whose NK cells proliferated <250-fold experienced significantly lower DFS and OS than those with ≥250-fold. Ex vivo activation of NK cells restored tumor trafficking and reactivity, hence provided a therapeutic modality while their fold expansion could be a potentially significant prognostic indicator of OS and DFS in such patients.

P181

PureQuant real-time PCR-based assay for quantitative determination of immune cell identity and purity

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1: Thermo Fisher Scientific

Significant progress has been made in harnessing the power of the immune system, in particular, T cells, to treat certain kinds of cancers. One of the key challenges in developing immune cells as therapeutic agents is the accurate estimation of their identity and purity. Current methods used for the characterization of T cell types and other immune cell products rely on flow cytometry. Flow cytometry can accurately estimate CD8+ T lymphocytes and other surface markers, but the method is challenging to implement in a cGMP manufacturing environment posing logistical challenges such as requirement for live cells, variability leading to difficulty in standardization and high throughput. In addition, cytometric methods are not accurate for specific intracellular targets that positively identify Regulatory T (Treg) cells and T Helper 17 (Th17) cells. Therefore, there is an emerging need for alternative assay methods that address these challenges. Methylation state is known to be unique for specific cell types and can thus be used as an identifier in a heterogeneous population of cells. Capitalizing on these differences in methylation patterns, we developed assay kits that quantify the percentage of Treg and Th17 by detecting methylation status of FoxP3 and IL17A via qPCR of bisulfite converted genomic DNA. In contrast to flow analysis, sample requirement is minimal, and the assay works with fresh/frozen cells or genomic DNA. The combination of accuracy, low sample requirement and flexibility provides an ideal measurement system for confirmation of identify and purity of T cell types critical for therapeutic applications.

P182

Highly efficient and specific multiplexed gene editing in T cells using enhanced zinc-finger nucleases (ZFNs) enables strategic engineering of allogeneic T cell immunotherapies

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Off-the-shelf immunotherapy with allogeneic CAR-T cells could help overcome some intrinsic challenges of autologous cell therapy such as product consistency, scalability and affordability. Successful engineering of allogeneic T cells requires two key components: highly efficient and specific nuclease activity, and an effective single-step multiplexed gene editing process. Our allogeneic T cell product profile has 3 key attributes: 1. TCR/CD3-negative, achieved by ZFN-mediated knock-out of TRAC; 2. HLA class I-negative, achieved by ZFN-mediated knock-out of β2M; 3. Expression of CAR/TCR, achieved by AAV-mediated gene insertion at the disrupted TRAC locus. We have demonstrated consistent modification of multiple genes at >90% in T cells. Importantly, our next generation ZFNs achieve both, efficient and highly specific gene editing, with minimal-to-no off-target activity in cells where on-target editing is >90%. Safe and efficient editing is critical in multiplexing to generate a higher proportion of final cells with all the desired edits. In our process, we simultaneously disrupt TRAC/β2M and achieve targeted insertion of GFP at >90%. We have also multiplexed TRAC/B2M-ZFNs with ZFNs targeting checkpoint genes (CISH) for strategic product design. We have shown that we can achieve a compound efficiency of 76%, where >76% of cells contain all 4 desired edits, when treated with multiple reagents in a single-step process (TRAC, β2M and CISH knock-out at efficiencies of 93%, 96%, 93%, respectively, and GFP insertion into TRAC at 91%). These industry leading capabilities of ZFNs enable the development of safe engineered allogeneic T cell products for application in oncology, autoimmunity or infectious diseases.

P183

Combining ROR1, CD5 or CD19 targeted chimeric antigen receptors reduces off-tumour toxicity while maintaining lysis against double-positive mantle cell lymphoma cells

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Mantle cell lymphoma (MCL) is an aggressive mature B-cell lymphoma, that responds well to initial treatment, but frequently relapses and has a bad prognosis. Chimeric antigen receptors (CARs), combining antigen recognition and T-cell activation in a single molecule, are a promising novel therapeutic approach for CD19+ malignancies. However, 2nd generation CARs show high on-target off-tumour cytotoxicity, due to the presence of CD19 on all healthy B-cells. To increase the specificity of CAR T-cells towards MCL blasts and reduce off-tumour effects, the signalling domains (zeta, CD28, CD137) were split to separate CARs (Split-

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CAR) targeting combinations of ROR1, CD5 or CD19 as target antigens, that are frequently co-expressed in MCL.1st, 2nd & 3rd generation CARs were lentivirally (co)expressed in primary human T-cells. CAR T-cells were co-cultured overnight with various MCL cell lines and lysis of target cells was determined by flow cytometry. While the cytotoxicities of 1st, 2nd and 3rd generation CD19 CARs were similar, 1st generation ROR1 and CD5 CARs showed reduced lysis compared to their 2nd or 3rd generation counterparts. For proof-of-principle studies, co-stimulatory CD19 or CD5 CARs were co-expressed in T-cells with 1st generation CD5 or ROR1 CARs. Co-expression of these CAR combinations in T-cells resulted in specific lysis of double-positive MCL cells, while single-positive cells, including autologous T-cells, were not/less efficiently killed. This elaborate Split-CAR approach allows to largely limit the on-target off-tumour toxicities for ROR1, CD5 and CD19 as specific target antigen combinations for MCL blasts.

P184

Optimizing CAR-T process development by utilizing a novel small scale stirred tank bioreactor

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1: Sartorius Stedim Biotech

With the commercialization of promising Chimeric Antigen Receptor T cell (CAR-T) therapies, cellular immunotherapy has gained significant momentum in the field of advanced therapies. Currently, shake flasks or spinner flasks for suspension processes are widely used in the process development of advanced therapies. However, these cell expansion approaches are labor-intensive and require a high level of highly skilled operator manipulation. Additionally, they often offer a low level of cell culture environment monitoring and control resulting in challenges when scaling up the process to state-of-the-art GMP production platforms. To address this, the ambr® 250 modular system provides a cost-effective and rapid way to evaluate critical process parameters (CPPs) whilst maintaining critical quality attributes (CQAs) to ensure safe and consistent cell culture expansion. It also allows to introduce monitoring and control of CPPs in an automated way to better understand the process and aid the much needed process development in the field of cell-based therapies. Ultimately, it provides a robust and automated scale down model tool that will ease the process scale up. Here we demonstrate the utility of the ambr®250 modular and the newly designed unbaffled single impeller vessel as a process evaluation tool for the expansion of CAR-T cells.

P185

Development of robust T-cell expansion system and single-cell analysis of their expanded cells

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Gene-modified T cells, such as CAR-T and TCR-T, have been widely developed in cancer immunotherapy. Although remarkable clinical efficacy is obtained by using CAR-T or TCR-T cells, T-cell expansion method has been still explored to prepare clinically effective cells. Since stem-cell memory like T-cell (Tscm) fraction is said to be effective for cancer immunotherapy, it is important that a large number of T cells containing high proportion of Tscm cells are obtained by the T-cell expansion. We have already reported T-cell expansion method using RetroNectin® (RN) with anti-CD3mAb (RN stimulation), in which a large number of CCR7+CD45RA+ T cells, indicating naïve T-cell markers, can be prepared. We have also developed new culture medium that can be proliferated T cells even in the absence of plasma/serum. The new medium were compared to commercially available other medium in the presence or absence of human serum. As a result, new medium showed robust T-cell expansion in both conditions, even though one of other medium couldn't be proliferated T cells in serum-free condition. The T cells expanded by RN stimulation using new medium contained higher proportion of CCR7+CD45RA+ T cells. Since most of those CCR7+CD45RA+ cells also expressed Tscm markers, CD27 and CD95, it was considered these cells have Tscm phenotypes. Further, preliminary result of single-cell RNA and TCR Seq analysis using ICELL8™ system suggested that RN stimulation could be expanded various type of T cells with little bias. We are currently conducting detailed analysis about the properties of the expanded cells.

P186

Advancing adoptive cellular therapies using non-viral-based engineering

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1: MaxCyte

Excitement over recent promising breakthroughs in autologous cellular therapies has been tempered by the manufacturing costs associated with viral-based gene delivery methods and by safety concerns associated with random integration of viral vectors. As an alternative to viral-based gene delivery, we describe the use of clinically validated, scalable electroporation for delivering mRNA, transposons, and ribonuclear proteins to primary T cells, natural killer cells, hematopoietic stem cells and induced pluripotent stem cells. We include data to show efficient expression of chimeric antigen receptors and T cell receptors via mRNA delivery and transposon-mediated gene insertion into T cells and NK cells. We also show highly efficient gene editing following transfection of CRISPR/Cas9 and zinc finger nuclease reagents into T cells, NK cells and iPSCs. Finally, we highlight key attributes of the MaxCyte platform, such as reproducibility and scalability, while including data from actual case studies of cellular therapies that are being developed using the MaxCyte cell engineering platform.

P187

Investigating Optimal Storage Conditions in the Manufacture of CAR-T cells

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1: UCL

Cryopreservation of CAR-T cells is currently considered as an important step in the treatment of cancer patients. The current techniques for the cryopreservation of CAR-T cells cause intracellular damage, resulting in cell death and reduced potency. Moreover, the addition of these steps prior to the administration of these cells into the patients adds to the logistical complexity and cost. Hypothermic storage is currently being used to preserve the quality of stem cells and human tissue. We, therefore, hypothesize an alternative solution utilizing hypothermic storage

conditions for preserving chimeric antigen receptor (CAR)-T cells. CAR-T cells were expanded for 10 days before exposure to 37oC, 33oC, and 24oC. Experiments were compered to cells cryopreserved in liquid nitrogen. Following 7 days of storage, the cells were resuspended in complete RPMI media with the addition of IL2 (100IU). Cell viability, apoptosis rate, and killing potency were examined. The results indicated no statistical significance between viability upon cell recovery from storage, cell growth following storage, and the percentage of killing the target (Nalm-6) after storage. This method simplifies the transportation of live cells in long-distance for up to 24 hours that can maintain cells in good viability. Furthermore, these data collectively suggest that adopting a hypothermic storage approach can preserve the function of CAR-T cells whilst avoiding the deleterious effects of DMSO and high costs associated with subzero shipping.

P188

A new approach to arm T cell therapies with conditional, transgenic payload outputs

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1: Chimera Bioeignieering

Current chimeric antigen receptor (CAR) T cell therapies are clinically efficacious against several B cell malignancies, but are less effective at eliminating solid tumors. A key contributor to this observed lack of efficacy is the tumor microenvironment (TME) that is erected by solid tumors to impose immunosuppressive physical and chemical barriers to T cell function and survival. To break TME-driven immunosuppression, attempts have been made to arm CAR-T cells with the ability to produce immunomodulatory payloads that target features of the TME. By their nature these immunomodulators are frequently toxic when systemically delivered, and due to a limited repertoire of existing programmable gene regulators, systemic delivery of these immunomodulators by armed CAR-T cells is common. We have characterized a novel, post-transcriptional gene regulatory node that strictly governs effector outputs in human T cells. We have applied this gene regulation to our lentiviral vector designs, and have achieved the goal creating enhanced CAR-T therapeutics that conditionally produce tightly controlled tumor-proximal immunomodulatory outputs only upon tumor engagement. In preclinical models, our enhanced CAR-T cells demonstrate superior efficacy relative to unarmed CARs, and robustly restrict payload delivery to the tumor.

P189

A new transduction enhancer accelerates CAR-T production

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The promising cellular immunotherapies using genetically reprogrammed immune cells such CAR-T cells or TCR-T cells have led to advanced treatments for hematological cancers. However, these costly multi-step manufacturing therapies exceed $400k dollars per patient. Gene transfer of CARs and TCRs is a crucial manufacturing step, often relying on lenti- or retroviral vector with low transduction rates. A range of genetic transfer enhancers is used to improve transduction. Until now, the coating of cultureware with recombinant fibronectin has been shown to provide increased enhancement. However, the coating procedure is cumbersome with time-consuming liquid handling steps performed in a cleanroom environment. Here we describe Protransduzin™, a novel peptide that can enhance transduction of CARs in human leukapheresis-derived CD3 lymphocytes by up to 24% (over control) dependent on culture system and multiplicities of infection. A side-by-side comparison of Protransduzin™ with recombinant fibronectin and an additional, commercially available histidine-rich amphipathic peptide, showed that Protransduzin™ is at least as potent as the adhesion molecule but more potent than the other peptide. Since Protransduzin™ can be added directly with the viral particles, it can be used to streamline CAR-T cell production. Moreover, in connection with mini bio-reactors, it is possible to do a large scale production by allowing transduction and expansion in one vessel. Using our optimized protocol, hands-on time can be reduced along with the contamination risk arising from repeated liquid handling steps. In conclusion, using Protransduzin™ reduces CAR-T cell production costs and is a significant step forward towards making cellular therapies more affordable.

P195

Dual functioning, novel chromatin insulators incorporated in lenti-viral vectors (LVs) for safer and more effective hematopoietic stem cell (HSC) gene therapy

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Despite the unequivocal success of HSC gene therapy (GT), limitations still exist including the genotoxicity risk and variegation/silencing of gene expression. Non-human origin chromatin insulators (CIs) proposed as a means to minimize vector-mediated genotoxicity (enhancer-blocking CIs, EB-CIs) and transgene silencing/variegation (barrier CIs, BA-CIs), are not devoid of limitations. Recently, human, small-sized CIs have been identified as robust EB-CIs reducing genotoxicity (Liu M, Nat.Biotech 2015). We here, aimed to test whether ≤284bp EB-CIs (A1, A2, B4, C1, E2, F1) in addition to their enhancer-blocking activity, function also as BA-CIs, in order to ultimately identify an ideal, dual functioning CI. CIs were incorporated in reporter LVs (GFP, A1-GFP, A2-GFP, B4-GFP, C1-GFP, E2-GFP, F1-GFP) without negatively affecting titers. Transduced CD34+ cells were cultured in methylcellulose or/and towards erythroid differentiation or/and transplanted into NSG recipients. GFP expression was significantly higher in cells transduced with the insulated A2-GFP-, B4-GFP-, C1-GFP-LVs than the uninsulated vector GFP-LV ($p<0{,}005$). Post erythroid differentiation, B4-GFP-LV expressed higher GFP over the parental vector

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($p < 0,05$). GFP expression and mean fluorescence intensity (MFI) in individual colonies, were higher with B4- and C1-GFP-LV compared to GFP-LV ($p < 0,001$). The increased expression was VCN-independent, given the similar VCN and the significantly higher expression after normalization of MFI per VCN ($p < 0,001$), between the insulated (A1-GFP, B4-GFP, C1-GFP) and uninsulated (GFP) vectors. In vivo, B4-GFP-LV recipients expressed long-term, higher GFP over the GFP-LV recipients ($p < 0,04$). Overall, the novel CIs have no detrimental effect on vector titers and some of them, apart from being powerful EB-CIs, also possess strong barrier activity (B4>C1>A1>A2); features that may lead to the development of safer and better in vivo performing therapeutic LVs.

P196

Wiskott-Aldrich syndrome protein tegulates nuclear body organization, chromatin structure, alternative splicing and cell proliferation

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Wiskott-Aldrich syndrome (WAS) displays immunological dysfunctions and predisposition to cancer. The molecular mechanisms of WAS remain obscure. We generated two isogenic models of WAS based on either patient iPSCs followed by gene correction, or on CRISPR-mediated gene deletion in a wild type iPSC line. Both isogenic models not only recapitulated known phenotypes but also revealed that WASP is important for the organization of the nucleolus, nuclear speckle, PML nuclear body, and heterochromatin. We discovered that WASP interacts with components of the nucleolus and nuclear speckles, including chromatin modifiers and splicing factors. Innate and adaptive immune cells from either iPSC models or peripheral blood of patients share abnormal gene expression signatures particularly in proliferation, epigenetic regulators and alternative splicing. WASP mutations are sufficient to drive an accelerated cell cycle and tumor-promoting splicing changes. Mechanistically, genome-wide binding of WASP significantly overlaps with CpG islands, CTCF binding sites and motifs of hematopoietic transcription factors. Many genes misregulated in WAS, including cell cycle genes and splicing factors, are direct targets of WASP. Genes normally bound by WASP are on average depressed in WAS mutants and show loss of H3K9me3 and gain of H3K27Ac. Furthermore, WASP deficiency leads to a significant loss of CTCF at its binding sites in the ribosomal DNA (rDNA) loci, which causes copy number instability of rDNA, a hallmark of cancer cells. Together, our data show that WASP is a tumor suppressor that is important for the organization and stability of the nuclear genome, and WASP deficiency promote tumorigenesis.

P197

Automation in hemopoietic stem cell gene therapy: results of a head-to-head comparison of a manual vs an automated procedure

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The recent successes in gene therapy for the treatment of rare blood disorders such as primary immunodeficiencies and hemoglobinopathies have highlighted the need to generate robust and scalable manufacturing processes. Here we compare large scale lentiviral transductions of hemopoietic stem cells performed on the CliniMACS Prodigy® to manual processes in terms of efficacy and viability of the end product. In our first series of experiments, we used a low, non-saturating MOI of 30 and the results showed that transduction efficiency on the CliniMACS Prodigy® was significantly increased from an average of 23.3% (manual) to 50.5% (CliniMACS Prodigy®). These results were further confirmed when we employed an MOI of 100 and showed that the average transduction efficiency was 74% from the CliniMACS Prodigy® compared to 54.1% from the manual steps. Moreover, the total viability of the cells cultured on the CliniMACS Prodigy® remained unaffected after two days of cultivation with an average recovery of 105% but with significantly less variability (SD: 15.4) compared to the manual steps (SD: 35.7). Finally there was no difference to the average VCN which was 2.2 from the manual steps and 2.4 from the CliniMACS Prodigy®. Overall, our results indicate that the CliniMACS Prodigy® generates higher transduction rates, combined with high viability compared to the manual process, but most importantly, with significantly lower variability, suggesting that it represents a closed system able to automatically perform complex processes as successfully as when manual handling steps are performed, but with higher predictability, efficiency and with minimal user interaction.

P198

Targeted in-frame deletion of the F8 B domain to restore FVIII function in patient-iPSCs derived ECs

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FVIII protein includes three A domains, one B domain and two C domains. Given that the F8 cDNA is too large to be packaged into AAV capsids, addition of B domain (F8-B) deleted F8 has shown therapeutic effects in animal models and clinical trials. To date 475 mutations have been recorded within F8-B, and more than 90% of those mutations result in premature termination and cause severe haemophilia A (HA). Here we described an in situ genetic correction strategy via an in-frame deletion with CRISPR/Cas9 to restore the function of FVIII in HA patient-iPSCs. We firstly reframed a frameshift mutation in F8-B to restore the F8 transcript and FVIII function via a targeted deletion of a few base pairs using CRISPR-Cas. And then, to extend this strategy to cover all mutations within F8-B, the entire F8-B was deleted. The F8-corrected iPSCs were differentiated into endothelial progenitor cells (EPCs) and endothelial cells (ECs), in which the clotting activity of the corrected FVIII was investigated in vitro and in vivo. The bleeding phenotype was rescued in HA mice after transplantation of the F8-corrected EPCs. Our results demonstrate an

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efficient approach for in situ genetic correction via introduction of an in-frame deletion in F8-B to restore the FVIII function in patient-iPSC derived ECs with potential clinical impact in HA gene therapy. And for the first time, we demonstrated in vitro and in vivo the function of FVIII that is encoded by the endogenous B domain-deleted F8.

P199

Harnessing HLA-TCR interaction and the exhaustion signature for the isolation of tumor-specific T lymphocyte

ABSTRACT WITHDRAWN

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P200

Cardiac patch loading Tβ4-overexpressed mesenchymal stem cells promotes repair of infarcted myocardium by activating epicardium

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Recent studies suggest that the epicardium plays an important role in cardiomyogenesis during development, while it becomes quiescent in adult heart. Thymosin beta 4 (Tβ4) has an effect on activating the epicardium. However, effectiveness of Tβ4 administration is unsatisfactory. Therefore, this study investigated efficiency of activating the epicardium and repairing infarcted myocardium by Tβ4 released from the cardiac patch. After mesenchymal stem cells (MSCs) isolated from bone marrow of rats and mice were transfected with Tβ4, the sustained release of Tβ4 from the cells was examined. For preparing of cardiac patch, the cells transfected with Tβ4 and Flag were seeded on PLACL/collagen membrane. In myocardial infarction models of rats and Wt1CreERT2/+, R26mTmG mice, the patches were implanted on the epicardium of the infarcted region. In rat models, differentiation of the epicardium-derived cells (EPDCs) and the engrafted MSCs towards cardiovascular cells was examined by Wt1 immunostaining and Flag labelling. In transgenic mouse models, the activated EPDCs expressed GFP. At four week or six month after implantation of the patches, cardiac function was improved, and scar area in the infarcted region was reduced significantly. EPDCs were increased in subepicardium and myocardium, and some Wt1+ cells and GFP+ cells expressed CD31, α-SMA or cTnT. Angiogenesis and lymphangiogenesis in the para-infarcted region were enhanced. Flag labelling showed that some engrafted MSCs migrated into subepicardium and myocardium. These results suggest that Tβ4 released from the transfected MSCs in PLACL/collagen patch may effectively attenuate left ventricular remodelling and improve cardiac function by activating the epicardium.

P201

Liver-directed gene therapy with lentiviral vectors achieve normal levels of clotting factor VIII and IX in non-human primates

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Liver-directed gene therapy with adeno-associated viral (AAV) vectors delivering a clotting factor transgene has shown successful results in adults with hemophilia. However, because AAV vectors do not actively integrate into the host cell genome, they are diluted upon cell division in liver growth, thus challenging their proficient use in pediatric patients. In contrast, lentiviral vectors (LV) integrate into the target cell chromatin and are maintained as cells divide. We have developed LV that achieve stable transgene expression in the liver following systemic administration and allow dose-dependent reconstitution of coagulation factor IX (FIX) activity in mouse and dog models of hemophilia B. We recently generated improved phagocytosis-shielded LV, which, upon intravenous (i.v.) administration to non-human primates (NHP), showed selective targeting of liver and spleen and enhanced hepatocyte gene transfer, reaching up to 300% of normal activity of a human FIX transgene, without signs of toxicity or clonal expansion of transduced cells. In order to apply our gene therapy strategy to hemophilia A, we have codon-optimized the coagulation factor VIII (FVIII) transgene and incorporated a DNA fragment encoding a non-structured XTEN polypeptide, known to increase the half-life and expression level of the payload protein into the B-domain region of FVIII (coFVIII-XTEN). I.v. administration of LV-coFVIII-XTEN to NHP resulted in 60–100% of normal circulating FVIII, with immune suppression. These studies support further preclinical assessment and clinical evaluation of liver-directed LV gene therapy in patients with hemophilia.

P202

Role of peripheral blood circulating haematopoietic stem/progenitor cells during physiological haematopoietic maturation and after gene therapy

ABSTRACT WITHDRAWN

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P203

Combination of lentiviral and genome editing technologies for the treatment of sickle cell disease

ABSTRACT WITHDRAWN

P204

In vivo transduction of HSCs in a Fanconi anemia mouse model after mobilization and intravenous injection of an integrating adenoviral vector system

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Fanconi anemia is characterized by an inability to repair DNA damage in hematopoietic stem and progenitor cells resulting in eventual marrow failure. To overcome the challenges and limitations of Fanconi Anemia gene therapy protocols, we developed a minimally invasive and readily translatable in vivo approach. It involves the HSPC mobilization with G-CSF/AMD3100 and intravenous injections of a HDAd5/35++ vector system consisting of a CD46-targeting, helper-dependent adenoviral vector and the hyperactive Sleeping Beauty transposase (SB100x) that mediates integration of the vector-encoded FancA/GFP genes. We tested our approach in a Fanconi anemia mouse model

(hCD46+/+/FancA-/-). We found that G-SCF/AMD3100 mobilization resulted in efficient HSPC mobilization (~10-fold increase of LSKs). At week 6 post transduction with the HDAd-FancA/GFP vector, 120mg/kg cyclophosphamide (CY) was given for in vivo selection of the corrected cells. This resulted in a 60-fold increase in GFP-positive cells, indicating selection of gene corrected HSPCs. Bone marrow mononuclear cells harvested from mice one week after CY treatment, displayed a significantly greater resistance to the DNA cross-linker mitomycin C (MMC) in progenitor colony forming unit (CFU) assays (>3- fold more colonies than in control animals) suggesting a partial phenotypic correction of Fanconi anemia. Two weeks after CY, hematological parameters were fully recovered and a second injection of CY (60mg/kg) was given. Mice will be followed for four more weeks for gene marking and MMC resistance of CFUs and results will be reported. Overall, we present a simplified and cost-efficient platform for gene therapy of Fanconi anemia, which can overcome the current limitations.

P205

Non-genotoxic conditioning CD45-SAP promotes immunological reconstitution in a mouse model of Severe Combined Immunodeficiency caused by RAG1 defect

ABSTRACT WITHDRAWN

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P206

Targeting therapeutic proteins to platelet alpha granules

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Alpha granules (αG) are the most abundant secretory granules present in platelets, and the repositories of effector proteins that are secreted upon platelet activation. It has previously been shown that ectopic proteins such as clotting factor VIII (FVIII) could be sorted into αG for activation-mediated release from platelets promoting improvement of a specific disease phenotype. Here we propose an adjustable model for targeting different therapeutic proteins to platelet αG using 3rd generation self-inactivating lentiviral vectors with αG sorting signals. The vectors are designed with the murine platelet factor 4 (mPF4) promoter restricting expression of transgenic proteins to the megakaryocyte (MK) lineage. Specificity of mPF4 to MKs and platelets was shown in murine bone marrow transplantation experiments and in vitro assays, where low or undetectable transgene expression was observed in other hematopoietic and non-hematopoietic primary cells and cell lines. For the sorting and storage of expressed proteins in αG, proteins are coupled to short peptide sorting signals from the cytokine Rantes and from platelet surface receptors. We have demonstrated that these vectors could effectively sort GFP or FVIII into αG of MKs differentiated from murine Lin- bone marrow cells compared to a non-targeting vector without sorting signals (about 3–4 fold enrichment). Furthermore, efficacy of both the Rantes and platelet surface receptor sorting signal vectors was also demonstrated for MKs and platelets differentiated from primary human CD34+ cells utilizing a GFP reporter gene. Our model therefore creates numerous applications for gene and cell therapy by utilizing platelets as carriers of therapeutic agents.

P207

Transplantation of lymphatic endothelial progenitor cells and sustained release of VEGF-C promote cardiac lymphangiogenesis after myocardial infarction

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Impairment of cardiac lymphatic vessels leads to cardiac lymphedema and dysfunction. Recent studies have indicated that stimulation of lymphangiogenesis may reduce cardiac

lymphedema. However, it is poorly understood whether lymphatic endothelial progenitor cells (LEPCs) contribute to cardiac lymphangiogenesis. The lymphangiogenetic effect of lymphatic endothelial progenitor cells (LEPCs) remains poorly understood. This study investigates effectiveness of delivering LEPCs and VEGF-C with self-assembling peptide (SAP) on cardiac lymphangiogenesis. CD34+VEGFR-3+ LEPCs isolated from rat bone marrow could differentiate into lymphatic endothelial cells after VEGF-C induction. VEGF-C stimulated LEPCs to incorporate into the lymphatic capillary-like structures. The designer functionalized SAP could adhere to the cells and released VEGF-C sustainedly. In the condition of hypoxia and serum deprivation or abdominal pouch assay, the SAP hydrogel protected the cells from apoptosis and necrosis. After intramyocardial transplantation of the SAP hydrogel carried the cells and VEGF-C in rat MI models, cardiac lymphangiogenesis was increased, cardiac edema and reverse remodeling was reduced, and cardiac function was improved significantly. VEGF-C released from the hydrogel induced differentiation and incorporation of LEPCs as well as growth of pre-existed lymphatic vessels. Enhancement of cardiac lymphangiogenesis was beneficial for elimination of the inflammatory cells. These results demonstrate that the combined delivery of LEPCs and VEGF-C with the functionalized SAP promotes cardiac lymphangiogenesis and repair of the infarcted myocardium effectively. This study represents a novel therapy for relieving myocardial edema of cardiovascular diseases.

P208

Induction of DNA methylation in CD34+ cells during *ex vivo* genetic modifications

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DNA methylation is a key epigenetic modification that can durably modify gene expression patterns. In recent publications, we reported that some lentiviral vector (LV) batches, especially integration-defective LVs (IDLV), reproducibly induced genome-wide DNA hyper-methylation in CD34+ cells during the process of ex-vivo transduction, shortly after exposure to the vector. To understand the potential consequences of DNA methylation on the transcriptome of CD34+ cells, we simultaneously investigated DNA methylation levels using a 850K EPIC Illumina chip and concomitant gene expression levels by RNA-seq. Again, certain batches of LV robustly induced DNA methylation and differentially methylated the CpG in the TSS regions of about 200 genes involved in developmental and stem cell pathways. However, RNAseq detected no difference on RNA expression at the time point examined. As a comparison, we also examined the effects of AAV6 on CD34+ cell transduction, since AAV6 is used in genome editing experiments to provide the donor DNA template. Using the same protocol as with LV, transduction of CD34+ cells by AAV6 induced no DNA methylation changes in the cells but resulted in dramatic changes in the transcriptome marked by the induction of P53-related genes. Thus, independently of insertional genotoxicity gene therapy vectors can have potentially significant epigenetic effects on target cells during ex vivo transduction. We are now developing targeted amplicon bisulfite sequencing to identify the molecular mechanisms involved in the DNA methylation effects of IDLVs.

P209

Preclinical evaluation of the pCCLCHIM-p47 lentiviral vector for the gene therapy of p47phox deficient Chronic Granulomatous Disease

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Chronic Granulomatous Disease (CGD) is an inherited primary immunodeficiency disorder caused by defective components of the phagocytic NADPH oxidase, the enzyme responsible for pathogen killing. CGD patients are susceptible to recurrent and often life-threatening infections and suffer from hyperinflammation. A phase I/II clinical trial of lentiviral gene therapy is currently underway for the X-linked form of CGD with promising results. We propose to use a similar strategy to tackle p47phox deficient CGD, caused by genetic defects in the cytosolic p47phox subunit of the NADPH oxidase that represents the most common form of autosomal recessive CGD (25% of CGD cases). To this aim, we have developed a lentiviral vector - the pCCLChim-p47 - that expresses high levels of p47phox in myeloid cells. The current study reports on a comprehensive preclinical safety evaluation of the vector. Here we show that transduction with the pCCLChim-p47 vector of hematopoietic stem and progenitor cells (HSPC) does not affect their homing properties in xenograft models. In vitro immortalization assay and in vivo genotoxicity studies using the p47 knock-out mouse model confirmed that the vector is not mutagenic, and does not alter blood parameters or lineage distribution in hematopoietic organs. Further studies have been conducted in human HSPC taken from p47phox - deficient CGD patients, which showed that the vector is able to restore high levels of p47phox expression and NADPH activity upon granulocytic differentiation. Overall, our study paves the way for the clinical application of the pCCLChim-p47 lentiviral gene therapy.

P210

Targeted gene correction for the treatment of Severe Combined Immunodeficiency caused by mutations in the IL7R gene

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Severe combined immunodeficiency (SCID) is a life-threatening syndrome characterized by a block in T and/or B cell development that has a fatal outcome in children by the second year of life. Mutations in the IL7R gene are responsible for the third most common form of SCID, and the majority of T-B+NK+ cases. Currently, the only available cure for SCID patients has been allogeneic hematopoietic stem and progenitor cells (HSPCs) transplantation. Gene therapy has proved to be a powerful tool to treat rare genetic diseases affecting the hematopoietic system. However, previous attempts to introduce a correct IL7R gene into murine hematopoietic stem cells by retroviral vector transduction has shown that dysregulated IL7R expression can predispose to leukemia. CRISPR-mediated genome editing using Adeno Associated

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virus (AAV) enables the correction of the mutated IL7R locus with a regulated and controlled transgene expression. We were able to detect up to 80% of gene targeting using CRISPR-system and 40% of homologous recombination-mediated genome editing co-delivering an AAV donor with GFP cDNA cassette. As a ''proof of concept'' of our strategy, we could show that the delivery of AAV encoding a codon-optimized IL7R cDNA cassette in IL7R-deficient model cell line restores IL7R expression. We are currently assessing the long-term engraftment and differentiation potential of corrected HSPCs in animal models as well as the safety of our platform by off-target analysis. Our final goal is to up-scale our protocol for clinical readiness and translate it into the next generation of therapeutic tools for IL7R-deficient SCID patients.

P211

A novel lentiviral vector for gene therapy of β-hemoglobinopathies: co-expression of a potent anti-sickling transgene and a microRNA downregulating BCL11A

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β-globin gene mutations lead to adult hemoglobin defects causing β-thalassemia and sickle cell disease. Transplantation of autologous hematopoietic stem cells (HSCs) transduced with lentiviral vectors (LVs) expressing an anti-sickling β-globin transgene (βAS) is a promising curative treatment that is partially effective in patients affected by severe forms of β-hemoglobinopathies. To ameliorate the therapeutic potential of gene replacement approaches, we developed a novel LV expressing βAS and an artificial microRNA (amiR) targeting the fetal hemoglobin (HbF) repressor BCL11A (βAS/amiR-LV). This amiR re-activates the expression of the endogenous γ-globin fetal chains, which could synergize with βAS to improve the clinical course of β-hemoglobinopathies. We inserted the amiR in intron 2 of βAS under the control of β-globin promoter/enhancers, reducing potentially off-target toxicity by limiting its expression to the erythroid lineage. Human erythroid HUDEP-2 cells were transduced with βAS/amiR-LV or a LV containing only βAS (βAS-LV). Neither the insertion of the amiR, nor its position in intron 2 affected βAS expression. BCL11A expression decreased in βAS/amiR-LV-transduced HUDEP-2 leading to γ-globin de-repression, a high proportion of HbF+ cells and elevated γ-globin chain content. We validated these findings in HSC-derived primary erythroid cells. γ-globin de-repression coupled with βAS expression resulted in a 2-fold increase in the total amount of therapeutic β-like globins per vector copy number in βAS/amiR-LV- compared to βAS-LV-transduced cells. These results show that the combination of gene replacement and gene silencing strategies improve the efficacy of current therapeutic approaches and represents a novel treatment for β-hemoglobinopathies.

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Kinetics and composition of haematopoietic stem/progenitors mobilised cells upon G-CSF and Plerixafor administration in transplant donor or patients undergoing autologous gene therapy

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Mobilised Haematopoietic Stem/Progenitor Cells (mHSPC) are becoming a major cell source for autologous HSPC-gene therapy (GT) due to accessibility and number of collected cells. However, there is limited information on the composition and kinetics of responses of distinct HSPC subsets to the mobilizing agents Granulocyte Colony-Stimulating Factor (G-CSF, G) and C-X-C Chemokine receptor type 4 (CXCR4) antagonist Plerixafor (P). We studied HSPC mobilisation kinetics in 19 patients with different inherited disorders who collected autologous mHSPC for the purpose of gene therapy or back up, as well as in 12 adult healthy subjects who donated for allogeneic transplantation. We quantified HSPC subsets in the BM and peripheral blood (PB) at steady state and in the PB after administration of (G) and (G+P), through a high-resolution 30-parameter flow-cytometry analysis. Our data showed enrichment of both primitive and myeloid progenitors upon G, followed by an increase of lymphoid progenitor contribution after P. Interestingly, G+P mHSPC resembled their BM counterpart before mobilization. We also found that primitive mHSPC amount in PB after G+P correlated with primitive HSPC counts before mobilisation in both PB and BM. CXCR4 was differentially expressed among the distinct HSPC subpopulations in BM and PB upon mobilisation procedure. This information will be important to define the best strategy for mHSPC collection for gene therapy and help to identify characteristics influencing haematopoietic reconstitution.

P213

Towards the lentiviral-mediated gene therapy for Glanzmann thrombasthenia

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Glanzmann thrombasthenia (GT) is an autosomal recessive platelet disorder characterized by defects in platelets aggregation due to defective αIIbβ3 integrin expression because of mutations in genes encoding for αIIb or β3 glycoproteins. The disease can result in significant morbidity and life-threatening hemorrhages. Allogenic HSC transplantation represents the only curative treatment for patients with a severe GT phenotype. However, transplant-related complications have limited its application in these patients. To investigate the feasibility of a gene therapy approach for GT, we have generated a self-inactivating lentiviral vector in which the ITGB3 gene is driven by the human glycoprotein 6 promoter (hGP6.ITGB3 LV), to induce a preferential expression of the therapeutic protein in the megakaryocytic lineage. To investigate the efficacy of this LV to restore the function of GT cells, GT-like CD34+ cells were generated by disrupting the ITGB3 gene by means of the use of CRISPR/Cas9 technology, using three different combinations of synthetic guide RNAs. Efficient mutations in ITGB3 exon 3 were generated in nucleofected healthy donor CD34+ cells which abrogated the expression of both CD61 and CD41 in more than 90% of cells differentiated towards the megacaryocytic lineage. The transduction of GT-like CD34+ cells with the hGP6.ITGB3 LV re-expressed CD61 and CD41 in in vitro differentiated megakaryocytes. Our results suggest that the hGP6.ITGB3 LV may enable the efficient and stable phenotypic correction of GT megakaryocytes, suggesting their applicability in the gene therapy of GT patients.

P214

Preclinical development of a haematopoietic stem cell gene therapy for adenosine deaminase 2 deficiency

ABSTRACT WITHDRAWN

P215

Vector genome processing of AAV5-hFVIII-SQ in mouse and monkey livers

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Hemophilia A (HA) is a congenital bleeding disorder caused by deficiency in clotting factor VIII (FVIII) activity. AAV5-hFVIII-SQ (valoctocogene roxaparvovec) is an adeno-associated virus 5 (AAV5)-based gene therapy vector comprising a B-domain-deleted human coagulation factor (F)VIII (hFVIII-SQ) under the control of a liver-specific promoter and is currently being evaluated in phase 3 clinical studies for the treatment of severe HA. Previous studies in AAV transduced mice and non-human primates (NHP) have shown that circularized monomeric and concatemeric episomes are the major DNA species associated with long-term expression of the gene product in the target cell. In order to understand the kinetics of AAV5-hFVIII-SQ genome processing, long-term studies of up to 6 months post AAV5-hFVIII-SQ administration have been conducted in both murine and nonhuman primate models. In the present study, we used drop-phase droplet digital PCR (ddPCR), quantitative PCR and Southern blotting following various nuclease treatments, to characterize and quantify the different molecular forms of AAV5-hFVIII-SQ vector genome in the liver at several time points. These experiments demonstrated formation of monomeric and multimeric circular episomes in the livers as early as one week post dosing. Quantities of circular episomes increased over 6 months with predominantly head-to-tail configuration. In contrast, the non-circular DNA population became more fragmented, and decreased in total quantity. The levels of duplex, circular full-length genomes significantly correlated with the levels of hFVIII-SQ transcripts. In conclusion, long-term (up to 6 months) FVIII expression from AAV5-hFVIII-SQ transduction is associated with formation of circular episomes in the liver.

P216

Restored macrophage function ameliorates disease pathophysiology in a mouse model for very early onset inflammatory bowel disease (VEO-IBD)

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Macrophages, in crosstalk with regulatory T-cells and other immune cells, govern the tight balance between pro- and anti-inflammatory responses in the intestinal system. If this network is disturbed, excessive inflammatory responses can lead to inflammatory bowel disease (IBD). IL-10 signaling is a major regulator of immune tolerance in the intestinal mucosa, and mutations in IL-10 or the IL10-receptor lead to very early onset (VEO) IBD, a life-threatening disease which is often unresponsive to conventional medication. Recent studies demonstrated that defective IL-10R signaling on innate immune cells is a key driver of severe intestinal inflammation in VEO-IBD. These data suggest that the correction of the macrophage defect alone may have therapeutic implications. We here evaluated the potential of hematopoietic stem cell gene therapy in an IL10rb-/- VEO-IBD mouse model and demonstrate that the therapeutic responses closely correlate with gene correction in intestinal macrophages. To further explore this finding, we investigated the therapeutic efficacy of a local macrophage administration by employing a combination of (i) depletion of endogenous hyperinflammatory macrophages followed by (ii) intra-peritoneal administration of wild-type macrophages. This 6-week therapy significantly reduced colitis as shown by decreased frequency of inflammatory macrophages in the lamina propria, improved histology scores of colon sections as well as normalized peripheral blood values in the IL10rb-/- VEO-IBD mouse model. In summary, we show that the correction of the Il10R-defect in macrophages either by HSC gene therapy or a local macrophage-based therapy can ameliorate disease related symptoms and potentially represent a novel treatment approach for VEO-IBD patients.

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P217

Preclinical studies of a gene therapy approach for the bone marrow failure in X-linked dyskeratosis congenita

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X-linked dyskeratosis congenita (X-DC) is a low prevalent inherited bone marrow failure syndrome (IBMFS) caused by mutations in the DKC1 gene. This gene encodes for a ubiquitously expressed nucleolar protein named dyskerin which plays an essential role for the telomerase function and rRNA pseudouridylation. Nowadays, the only curative treatment for the BMF of these patients is hematopoietic stem and progenitor cell transplantation (HSPCT). However, the poor outcome of HSPCT in DC patients, mainly due to the toxicity of conditioning regimes and graft versus host disease, postulate the necessity of developing innovative therapies for the treatment of BMF in DC patients. We have previously described the Genetic Suppressor Element 4 (GSE4), a dyskerin derived peptide which efficiently rescues different phenotypic hallmarks of DC fibroblasts. Based on these results, we constructed a LV carrying the GSE4 sequence and designed a gene therapy approach for the treatment of BMF in X-DC patients. Preclinical studies with healthy donor CD34+ cells were performed, demonstrating the efficient transduction of these cells and the preservation of their clonogenic and NSG mice reconstitution potential. Lentiviral-mediated transfer of GSE4 in hematopoietic cell lines from X-DC patients showed an improved expression of telomerase related genes, as well as an enhanced expansion of transduced cells. These preclinical studies offer in vitro and in vivo data showing no evident toxicity as well as functional restoration in GSE4-transduced X-DC cells, suggesting that the stable expression of GSE4 in HSCs from X-DC patients may restore their repopulating and differentiation properties.

P218

AAV-mediated protein trans-splicing and genome editing for therapy of Haemophilia A

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Haemophilia A (HemA) is the most common bleeding disorder (1:5'000 males) caused by deficiency of clotting factor VIII (FVIII). Of the severely affected cases, nearly half are due to the same F8 intron 22 inversion that results in translocation of exons 1–22. The current treatment is frequent prophylactic FVIII administrations that can be burdensome and predispose to neutralising antibodies development. Thus, gene therapy holds great promise for a single-administration life-long cure. Previous delivery of full-length F8 using AAVs has been challenging due to the gene's large size exceeding their cargo capacity. To overcome this limitation, a protein trans-splicing (PTS) strategy with two AAVs, each encoding a half of F8 flanked by split inteins, was designed. In vitro studies demonstrated the occurrence of PTS by the presence of excised intein but no full-length FVIII was seen likely due to its instability. Therefore, AAV8-F8 inteins were injected into haemophilic mice where FVIII activity test showed slight phenotypic correction. The improvement of the platform's efficiency is currently being investigated. To specifically correct the F8 translocation of exons 1–22, CRISPR/Cas9 combined with homology-independent targeted insertion (HITI) of donor DNA is being adapted. The technology could insert the missing exons downstream of the translocation, thus restoring endogenous F8 expression. As proof-of-concept in vitro, the missing exons plus a reporter were integrated into a template of truncated F8 (exons 1–22 with intron 22), and restored FVIII expression and secretion. Next, HITI will be tested into patient-derived iPSCs and a humanised HemA mouse model presently being generated.

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Development of a new all-in-one *in vitro* safety assay for gene therapy

ABSTRACT WITHDRAWN

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P220

Optimized manufacturing of lentiviral corrected hematopoietic stem cells under GMP conditions

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The optimized GMP manufacturing of genetically corrected cells is particularly relevant in hematopoietic stem cell (HSC) gene therapy approaches. In these procedures, the efficient transduction of the HSCs needs to be compatible with the preservation in the number and function of transduced HSCs, especially in diseases in which the stem cell compartment is compromised such as Fanconi anemia. Here we have investigated the efficacy of transduction in healthy donor CD34+ cells with a preclinical EGFP and a GMP therapeutic lentiviral vector in the presence and the absence of transduction enhancers (TE). The use of two different GMP culture media (X-Vivo-20 and SCGM) was also tested. Although no differences in the viability and the progenitor cell recovery were observed in cells transduced under these different conditions, transduction efficiencies in the pool of CD34+ cells were on average 2–3 fold higher when TEs were used. Increases were more significant when the SCGM was used, as compared to the X-Vivo 20 medium. Similarly, higher transduction efficacies in primitive HSCs (CD34+, CD90+, CD38- cells) were observed when cells were transduced with the SCGM as compared to X-Vivo 20 medium, in both instances in the presence of TEs. Finally, similar transduction efficacies were observed in CD34+ cells that were transduced in the presence of retronectin-coated plates when compared to transductions conducted in non-coating retronectin media. These results will have a significant impact on the development of hematopoietic gene therapy trials with therapeutic lentiviral vectors.

P221

Increasing fetal hemoglobin by genetic editing of sickle cell disease patient hematopoietic stem cells

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Beta-hemoglobinopathies, such as Sickle Cell Disease (SCD) and beta-thalassemia are amongst the most common monogenic diseases with tens of millions of patients globally. They arise from mutations in the beta-globin gene HBB and the only curative treatment is an allogeneic hematopoietic stem cell transplant, which is complicated by limited matching donor availability, severe adverse reactions and excessive costs. Hemoglobinopathy symptoms can also be ameliorated with the increase of fetal hemoglobin (HbF), but pharmacological treatments are sub-optimal. However, naturally occurring beneficial alterations can lead to elevated levels of HbF in adulthood. When this benign Hereditary Persistence of Fetal Hemoglobin (HPFH) syndrome alterations are coinherited with a beta-hemoglobinopathy the disease can be much milder or even symptomless. Several HPFH alterations have been identified. Gene therapy and gene editing tools such as ZFNs, and CRISPR/Cas9 hold much promise for the treatment of genetic diseases and have been previously studied also for the treatment of beta-hemoglobinopathies. The core of the project is to reproduce in hemoglobinopathies patients' Hematopoietic Stem Cells (HSCs) the naturally protective HPFH genomic variants. Here, we have used the CRISPR/Cas9 - based genetic engineering as well as single base editing to introduce beneficial HPFH mutations into hematopoietic cell lines, control and SCD patient HSCs in vitro. After being genetically edited and differentiated to hemoglobin-producing red blood cells, there was a 2-fold increase in the fetal Hb production, compared to the not treated cells. In addition, the differentiation capacity and the genetic profile of the cells did not show significant changes.

P222

LEVIS: a clinical scale, "all in one" T-cell product from non-transplantable cord blood units, targeting infections and leukemic relapse post-hematopoietic cell transplantation

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Leukemic relapse and viral infections account for the majority of treatment failure post allogeneic hematopoietic cell transplantation (allo-HCT). Adoptive immunotherapy with non-genetically engineered virus-specific T cells has been shown to successfully control post-allo-HCT viral infections. However, single-epitope leukemia-specific T-cells (Leuk-STs) targeting the less immunogenic leukemia-associated antigens (LAAs) have met limited clinical success. By adapting our previously developed protocol of Leuk-STs, targeting multiple antigen epitopes by stimulation with overlapping peptides spanning the whole proteins, we aimed to generate an ''all-in-one'', T-cell product from Non-Transplantable Cord Blood Units (NT-CBUs), simultaneously targeting multiple viruses [Epstein Barr(EBV), cytomegalovirus(CMV), BK virus(BKV), adenovirus(AdV)] and LAAs (LEukemia-VIrus-specific T-cells-LEVIs). Matured CD34+-derived dendritic cells from NT-CBUs were stimulated with both leukemic [Wilms tumor protein (WT1) and Preferentially Expressed Antigen in Melanoma (PRAME)] and viral [EBNA1, LMP2 and BZLF1(EBV); IE1 and pp65(CMV); Hexon and Penton(AdV) and Large-T and VP1(BKV)] pepmixes and used to prime autologous T-cells. After expansion, clinically relevant cell numbers were obtained (CD3+cells:1.01 ± 0.2x108). The T-cell products were polyclonal (CD4+:39 ± 2%, CD8+:50% ± 2%), expressing effector memory (CD45RA-/CD62L-:47.36 ± 5%) and effector memory RA markers (CD45RA+/CD62L-:48.50 ± 4%) while containing insignificant amount of naïve and regulatory CD4+/CD25highcells. LEVIS demonstrated high specificity against all targets [spot-forming units (SFC)/2x105 cells: CMV:311 ± 149, EBV:319 ± 111, BKV:245 ± 122, AdV:432 ± 32, Leuk:553 ± 257], similar to that conferred by Leuk-STs alone, suggesting lack of antigen competition by the immunodominant viral antigens. LEVIS were cytolytic in vitro without an exhausted or anergic phenotype [Programmed cell Death protein-1+(PD-1):4 ± 2%; Cytotoxic T-Lymphocyte Associated Protein-4+(CTLA-4):0.2 ± 0.1%]. Overall, ''recycling'' of NT-CBUs may provide an off-the-shelf, single T-cell product, simultaneously fighting viral infections and leukemic relapse post-transplantation.

P223

Complete knockout of the Wiskott-Aldrich syndrome gene impairs phagocytosis and chemotaxis and enhances proliferation in macrophages

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Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disease caused by WAS protein (WASP) mutations. WAS is associated with symptoms including microthrombocytopenia, eczema, autoimmunity and cancer. The molecular mechanism underlying WAS remains elusive. The genotype-phenotype relationship in WAS is complex. There are over 200 mutations that lead to hypomorphic levels or complete loss of WASP, while it is impossible to predict clinical severity based on the mutation alone. To help evaluate phenotype variability due to mutational background of different patients, we developed an isogenic WASP-knockout (WASP-KO) induced pluripotent stem cell (iPSC) model using the CRISPR/Cas9 technique that completely removed the WAS gene. The isogenic iPSC model was differentiated into macrophages, which are reported to be affected by WASP mutations. The results showed complete knockout of WASP did not affect the iPSC pluripotency or efficiency of hematopoietic differentiation from iPSCs. However, it resulted in dramatic morphological changed in the derived macrophages. Importantly, complete knockout of WASP impaired the phagocytosis and migration of macrophages, while promoted their proliferation. Consequently, the cell-cycle inhibitors p16 and p21 were downregulated in WASP-KO macrophages, consistent with their higher proliferation. Additionally, the epigenetic regulator EZH2 and splicing factor SRSF2 are overexpressed in WASP-KO macrophages, which indicates that WASP is involved in epigenetic and mRNA splicing events. In short, we successfully generated a de novo WASP-KO iPSC model, and found that WASP plays important roles in phagocytosis, migration and regulation of cell proliferation. This novel WASP-KO iPSC model can recapitulate WAS disease and can be used in drug screening.

P224

Conditional immortalisation of haematopoietic progenitors via tetracycline-regulated LMO2 expression

ABSTRACT WITHDRAWN

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P225

Development of a fully-closed and automated manufacturing process for ATIR101: Donor lymphocytes depleted of host alloreactive T-cells

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ATIR101 (Kiadis Pharma) is a donor-derived T-lymphocyte enriched leukocyte preparation depleted ex vivo of host-alloreactive T-cells using photodynamic treatment. ATIR101 is administered as an adjunctive immunotherapy after a T-cell depleted haploidentical haemopoietic stem-cell transplantation. ATIR101 is being developed to provide the recipient with donor T-cell based immunity, that will bridge the period of temporal immune deficiency. The current manufacturing process for ATIR101 has many manual and open handling steps. The process is performed by 2–3 operators in a Grade A/B cleanroom environment, adding to operational costs and discomfort for manufacturing operators. For further industrialization of ATIR101, a fully closed and largely automated process was developed which is comparable to the open process currently in use. We have closed and automated each open unit-operation using the Sefia S-2000 platform. Each change is supported by comparability data on cell composition and product quality. The result is a robust process that can be performed by a single operator in a Grade C cleanroom environment. We will present data demonstrating comparable step-yields for washing steps and ficoll-density gradient centrifugation. In addition, we will discuss solutions used to close the t-flask cell cultures, allo-reactive T-cell depletion and final formulation steps in the ATIR101 manufacturing process. The resulting closed and automated process shows comparable performance, yields and product quality attributes to the original open process.

P226

Manufacture of an ATMP for the treatment of X-linked Severe Combined Immunodeficiency (X-SCID)

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X-SCID is a primary immunodeficiency characterised by the absence of T, B and NK cells. Patients with X1-SCID suffer from recurrent and life-threatening infections. Currently, the only curative treatment is haematopoietic stem cell transplant (HSCT). Previous ex-vivo gene therapy clinical trials have been conducted for this disease, using both gamma retroviral vectors (g-RV) and self-inactivating (SIN) g-RV. However, numerous clinical trials for many diseases, including X-SCID have moved to the use of SIN-lentiviral vectors (LVs). Great Ormond Street Hospital is one of the sites for this clinical trial. Here we describe the optimised 3-day manufacturing procedure consisting of several steps: CD34+ selection using COBE and CliniMACS®Plus from the starting material (mobilised peripheral blood stem cells or bone marrow), pre-stimulation culture in the presence of SCF, FLT3L and TPO cytokines, transduction with a SIN LV where the expression of the therapeutic transgene is driven by the EFS promoter, and a final harvest / formulation process where the transduced cells are re-suspended in a commercially available freezing mix and transferred to cryobags. Finally, the cells are cryopreserved using a control rate freezer and stored at LN2 vapour phase. Required QC tests are performed both pre-freeze and post-thaw. The introduction of transduction enhancers (LentiBOOST™ and Protamine sulphate) allows the use of 4-times less vector whilst still achieving enhanced transduction efficiency. A stability study using healthy donor cells has been also conducted demonstrating that the cryopreserved transduced cells are stable for at least 6 months in LN2 vapour phase.

P228

Stable transduction of long-term HSCs under optimized GMP-conditions for the gene therapy of LAD-I patients

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Leukocyte adhesion deficiency type I (LAD-I) is a primary immunodeficiency caused by an impaired neutrophil migration capacity. This phenotype is caused by mutations in the ITGB2 gene that encodes for CD18, the B2 subunit of integrins essential for the arrest and extravasation of leukocytes to inflamed areas. Since the efficacy of hematopoietic gene therapy relies on the stable engraftment of gene-corrected HSCs in treated patients, here we have optimized the transduction conditions of human HSCs with a therapeutic lentiviral vector in which the expression of CD18 is driven by a chimeric promoter incorporating regions of two genes encoding for proteins predominantly expressed in mature myeloid lineages. Healthy donor CD34+ cells, either cord blood or mobilized peripheral blood CD34+ cells were transduced at differences multiplicities of infection with the therapeutic LV. Additionally, cells were transduced in the presence or the absence of different transduction enhancers. A single transduction round of CD34+ cells in the presence of specific combinations of transduction enhancers rendered optimized transduction efficacies with minimal loss in

hematopoietic progenitors. Using relatively low MOIs of 50 i.u./cell resulted in transduction efficacies of 70% and mean vector copy numbers of 2–5 copies/cell. Optimized transduction conditions were then developed under GMP conditions, and transduced cells were transplanted into NSG mice. In these studies, stable repopulation levels of human cells harboring 1–3 copies of the therapeutic vector per cell were observed. These results constitute the final preclinical studies required to initiate the first lentiviral-mediated gene therapy trial in LAD-I patients.

P229

Patient-specific TP53 mutations CRISPR-Cpf1 editing in chronic lymphocytic leukemia by suicide gene delivery

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Patients with Chronic Lymphocytic Leukemia (CLL) harboring TP53 mutations, despite being subjected to burdensome cycles of therapy, do not have many possibilities of healing due to the acquired abilities of TP53-mutated clones to escape the control systems. We present here a suitable example of transformative gene-based medicine using CRISPR/Cpf1. Our system which has been recently patented is based on the locus specific delivery of the suicide gene thymidine kinase (TK) in target cells successfully isolated from TP53 mutated cancer patients. We were able to deliver the lentiviral vector (LV) encompassing the CRISPR/Cpf1 system and TK sequence into engineered HEK293 holding the target mutation. Once the TK has been integrated into the target site we treated cells with ganciclovir (GCV) leading to selective cell death. We were able to detect a very significant cell death in the samples treated with GCV compared to controls. In vivo experiments on NOD/SCID IL2Rg null mice are ongoing. Mice are treated with busulfan a day before the intravenous injection of PBMC isolated from chronic lymphocytic leukemia patients bearing the TP53 point mutation c.548C>G; p.Ser183*. One day post cells injection animals are infected with CRISPR/Cpf1 viral particles and the day after GCV is delivered intraperitoneally. Animals are monitored weekly to detect a decrease in neoplastic B-CLL count. These results show the high efficiency and specificity of our approach which might move to the clinic in the near future.

P230

Getting personal: mutation-specific gene editing and addition strategies for β-thalassemia and beyond

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[Background] Beta-thalassemia as a severe monogenic hematopathology is an ideal target for advanced therapy medicinal product (ATMP) development based on gene addition or gene correction. It is also a frequent testbed for more widely applicable ATMP technology, including the establishment of efficient mutation-specific therapies. [Aim] With focus on the common β-thalassemia mutation, IVSI-110(G>A), this study demonstrates the development of highly efficient mutation-specific gene addition and gene disruption strategies applicable in primary cells. It further extrapolates the applicability of one of our approaches, the disruption of aberrant regulatory elements by designer nucleases, to a large number of disease-causing mutations. [Methods] NHEJ-mediated disruption was performed in erythroid cell lines and patient-derived CD34+ cells, using plasmid transfection, lentiviral vectors or nucleofection of in vitro synthesized TALEN mRNAs and CRISPR/Cas9 RNA-guided nuclease (RGN) ribonucleoprotein complexes. Post-disruption analyses were performed at the level of DNA, RNA, protein and cell-morphology. Additional mutations, subjected to in silico disruption and splice site analyses, were retrieved from molecular databases and systematic literature searches. [Conclusion] Gene addition based on small lentiviral transgenes and functional repair by TALEN and RGN based on target disruption showed exceptional functional correction for the targeted β-thalassemia mutation. Application of the latter with directly translatable (virus- and DNA-free) delivery showed high bulk disruption efficiencies, minimal toxicity and minimal off-target activity on the main the HBB paralog HBD in primary patient-derived cells, and is suitable for a large number of human diseases. Application of the current approaches therefore has great scope for personalized ATMP development.

P231

Fetal liver haemato/vascular progenitor cells as a cell-based therapeutic tool for neonatal haemophilia

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The development of strategies based on cell therapy approaches requires further insights into new stem/progenitor cell populations presenting stable engraftment potential and restoration capacity of the deficient factors causing the disease. We have previously shown that hemato/vascular progenitor cells from the foetal liver (FL) possess higher hematopoietic and vascular endothelial engraftment potential when transplanted to newborn mice compared with adult-derived counterpart progenitor cells (1), constituting likely therapeutic targets for the treatment of

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congenital hemato/vascular related diseases in neonates. The hemophilia A (HA, a deficiency of factor FVIII) and other rare coagulation disorders, including deficiency of factor FV, are caused by mutations decreasing the production of coagulation factors by hematopoietic and/or vascular endothelial cells. We have shown that different FL-derived cell populations produce FVIII (2), suggesting its suitability as cell therapeutic targets for the treatment of hemophilia diseases. Here we will present the results on the phenotypic and functional characterization of FL progenitors, focusing in three aspects: 1) Characterization of vascular endothelial stem cells during development; 2) Determination of coagulation factor production; 3) Analysis of chimeric HA mice transplanted with wild type FLGFP-derived cells at day 1 after birth. These research should be instrumental for the establishment of a preclinical model for HA treatment in neonates. (1) Cañete el all, Stem Cells, 2017. doi.org/10.1002/stem.2494 (2) Serrano et al, Throm & Haem, 2018. doi.org/10.1055/s-0038-1661351

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Treatment of steroid refractory acute graft-versus-host disease with allogenic adipose tissue-derived mesenchymal stromal cells. A multicenter phase I/II clinical trial

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Acute graft-versus-host disease (aGvHD) is a significant cause of morbidity and mortality following allogenic hematopoietic stem cell transplantation. Immunosuppression with corticosteroids forms the basis of first-line therapy, producing responses in less than 50% of patients with aGvHD. Mesenchymal stromal cells (MSC), based on their immunomodulatory properties, may play a key role in the treatment of the disease. MSC suppressive effect on T-cell function has been shown to be only transient. Therefore, the efficacy of MSCs could be increased when administered sequentially. Additionally, preclinical studies suggest that MSCs obtained from adipose tissue show an immunomodulatory potential at least as strong as MSC obtained from bone marrow, and a greater proliferative potential. On this basis, a phase I/II, single arm, open-label, multicentre study to assess the safety and efficacy of a sequential dose infusion (0,7-1 x 106 MSC/kg, days +1, + 4, + 11, + 18) of allogenic adipose tissue-derived MSC in patients with SR-aGvHD (Grade II-IV) was designed and is currently ongoing. After the first ten patients had received the MSCs, an interim analysis was carried out. Twelve serious adverse events were reported, none of which MSCs related. Only one severe infection occurred, deemed unrelated to MSCs, which resolved without sequelae. No suspected unexpected serious adverse reactions occurred. Neither infusion-related toxicity was observed.

P233

StemPro™ HSC expansion medium supports superior expansion of human haematopoietic stem-progenitor cells

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Since numbers of harvested hematopoietic stem cells (HSCs) continues to limit not only laboratory research but also clinical HSC transplantation and gene therapies, development of ex vivo culture systems to expand harvested human hematopoietic stem-progenitor cells (HSPCs) remains a critical translational research quest. To address the barrier that HSCs generally die or differentiate in current ex vivo culture media, we have developed a xeno-free, serum-free medium – StemPro™ HSC Expansion Medium by extensive evaluation of iterative modification of medium constituents.

P234

Impact of selected cell culture media and different HEK293 production cell lines on the expression of a variety of recombinant adeno associated virus subtypes

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Gene therapy is a promising technology to therapeutically address rare diseases caused by genetic disorders. In this context, recombinant adeno associated virus (rAAV) emerged during the last years as the vector of choice for gene therapy. In the present study we investigated the impact of different HEK293 cell lines and several commercially available cell culture media on the upstream yield of a variety of AAV subtypes after transient transfection. All experiments were performed in high throughput micro bioreactors or in small scale laboratory bioreactors. Kinetics of AAV capsid expression monitored over several days post transfection showed different patterns regarding capsid specific ELISA and qPCR. Additionally, we determined to what extend the AAV particles were released into the supernatant or kept in the cytosol of the cells. The outcome of our study was compared against published data.

P235

Human plasma and platelets from neonatal and adult blood express differential sets of regenerative proteins

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Rejuvenation is aiming to revert age-related disease development. In animal models, umbilical cord blood (UCB)-borne factors and immune cells were shown to contribute to rejuvenation. Human platelet lysate (HPL) is frequently used for efficient in vitro cell propagation, but differences between adult and UCB-derived HPL remain partly elusive. To identify candidate regenerative factors we compared protein contents of neonatal and adult plasma and platelets. UCB samples were used to produce neonatal platelet lysate (PL) and aliquots from apheresis platelet concentrates (each n = 9) were employed as adult counterpart. For each preparation, platelet concentration was adjusted to 7–10 x 10^11/L. Plasma supernatants and platelets were obtained by centrifugation, platelet pellets were resuspended in saline. Two freeze/thaw cycles (-30°C/+37°C) induced platelet lysis. The content of 105 protein was analyzed by Proteome ProfilerTM Array, whereas 63 (plasma) and 48 (platelets) proteins were detected. In neonatal plasma we found more proteins with high expression (>200,000 spot density) and thirteen proteins, including GDF 15, PDGF-AA and serpinE1, being significantly elevated compared to adult plasma. Also in neonatal PL a higher number of proteins was detected compared to adult PL, with 20 significantly elevated proteins including VCAM-1, PF4/CXCL4 and EGF. In adult plasma ten proteins and in adult PL three proteins were significantly higher compared to neonatal samples. In conclusion, we detected differential growth factor and cytokine contents of neonatal and adult plasma and platelets indicating distinct regenerative potential. Additional experiments are underway to characterize their specific impact in functional readouts.

P236

Comparison of the CliniMACS Plus and the CliniMACS Prodigy for CD34 enrichment of mobilised peripheral blood stem cells (mPBSC)

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Hematopoietic stem and progenitor cells (HSPCs) can be enriched in mobilised leukapheresis for transplant or ex vivo gene therapy procedures, based on the expression of the cell surface marker CD34. Miltenyi Biotec manufactures two closed processing devices that can be used for CD34+ cell enrichment process: CliniMACS Plus and CliniMACS Prodigy. At Great Ormond Street Hospital for Children NHS foundation trust (GOSH), the CD34+ cell enrichment is typically performed using the COBE 2991 cell processor and CliniMACS plus devices. The COBE® 2991 is used first to perform platelet depletion, volume reduction, incubation with anti-CD34 beads and wash before the selection. The cells are then transferred to the CliniMACS plus to perform the column separation steps. The new CliniMACS Prodigy can complete all these steps in a closed fashion on a single machine using the LP-34 program. Initial beta testing at GOSH revealed design issues. Miltenyi promptly adapted the LP-34 program and required tubing set. We have evaluated the re-issued version 2.2 CliniMACS Prodigy LP-34 program in combination with the revised TS310 tubing set with larger pre-column. We have carried out 3 full scale validations of the LP-34 program with healthy donor mobilised leukapheresis. The selection purity varied from 89.4% to 99.3% and the yield, from 59.5% to 69.1%. These results are within the range expected for the COBE 2991 and CliniMACS plus, indicating the LP-34 program could be implemented in future HSPC gene therapy clinical trials.

P240

Towards the clinical translation of a gene therapy approach for the treatment of familial ALS

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Amyotrophic Lateral Sclerosis (ALS) is an adult-onset motor neuron disease with no cure. We recently developed a gene therapy strategy for familial ALS forms (fALS) caused by mutations in SOD1 gene and toxic gain of function of the encoded enzyme. We designed antisense (AS) sequences that induced human SOD1 (hSOD1) silencing through exon skipping. The AS were fused into the U7 small nuclear RNA particle and delivered in vivo through an AAV10, enabling high transduction in the central nervous system. Upon treatment of SOD1G93A mice (over-expressing hSOD1 with G93A mutation) we obtained the most favorable therapeutic effect to date for ALS and we were awarded the ''1M$ treatment prize'' by the Prize4Life association. The pre-clinical work for clinical translation of this approach is undergoing. Specifically, we compared three administration methods: intravenous (IV), intracerebroventricular (ICV) or combined (co)-IV/ICV. We also performed a dose-escalation study to reduce the amount of vector previously tested. We injected SOD1G93A mice (n = 6/group, sex-balanced) at 50 days of age with the AAV10-U7-hSOD1 at two doses (low and medium). We then selected the most efficient delivery route and injected another group with a third dose (high). We monitored mice for body weight, neuromuscular functions (grip test and rotarod) and survival. We observed a remarkable effect on survival and neuromuscular functions after ICV administration of the high dose, with a mean lifespan increase of 76% in treated mice compared to non-injected controls. These results significantly contribute to the pre-clinical development of the AAV10-mediated exon skipping strategy for SOD1-linked ALS.

P241

LYS-SAF302 gene therapy study in mucopolysaccharidosis type IIIA (mps IIIA) children

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Mucopolysaccharidosis type IIIA (MPSIIIA) (OMIM 252900) leads to infantile-onset neurodegeneration and early death. Though the involvement of multiple organ systems in MPS IIIA is recognized, central nervous system (CNS) manifestations predominate, in particular intellectual disability, progressive loss of acquired skills, behavioral and sleep disturbance. The reduced lifespan is related to progressive neurological deterioration rather than involvement of other organ systems. Gene therapy direct-to-brain administration has a strong rational for such diseases because of the profound CNS component and the blood brain barrier, which limits the passage of therapies administered via peripheral routes. Long-term 5-year data in four MPS IIIA patient treated with an intracranial administration of a first-generation adeno-associated viral (AAV) gene therapy (LYS-SAF301) (Tardieu et al 2014) showed that direct to CNS gene therapy shows potential and is well tolerated. The Phase 2–3 trial uses an optimized gene therapy construct and dose (LYS-SAF302). The study is recruiting 20 patients in US and EU sites. The first patient was treated in February 2019. The principal inclusion criterion is a cognitive developmental quotient (DQ) score >50% based on the Bayley Scales of infant and toddler development third edition (BSID-III). The primary objective is to assess the drug efficacy in improving or stabilizing the neurodevelopmental status of patients compared to the expected evolution based on pooled data from published literature and recently completed natural history data from Lysogene's observational study in 23 patients.

P242

E2F4-based gene transfer improves learning and memory and reverses body weight loss in a mouse model of Alzheimer's disease

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Cell cycle reentry in neurons followed by neuronal tetraploidization (NT) represents an early alteration in Alzheimer's disease (AD) that precedes and recapitulates its neuropathological hallmarks. Forced cell cycle reentry in murine neurons has been shown to induce AD neuropathology, including synaptic dysfunction, neurofibrillary tangle formation, extracellular deposits of beta-Amyloid peptide, gliosis, delayed neuronal cell death, and cognitive deficits. In differentiating neurons, non-pathological NT is blocked by a dominant negative form of E2F4 (E2F4DN), unable to become phosphorylated by p38MAPK in two conserved Thr residues. Therefore, we investigated whether neuronal delivery of E2F4DN could reduce AD-associated NT and prevent disease outcomes in 5xFAD mice, a known model of AD. Here we report that neuronal expression of E2F4DN, as a transgene or by systemic administration of an AAV.PHP.B-E2F4DN vector, abolishes NT in the cerebral cortex. E2F4DN does not cause side effects and prevents both cognitive impairment, evaluated by Y-maze and Morris water maze, and other disease-associated alterations such as paw-clasping behavior and body weight loss. We also show that E2F4 is expressed in cortical neurons from Alzheimer patients, associated with Thr-specific phosphorylation, as evidenced by proximity ligation assay. We propose E2F4DN-based gene therapy as a promising therapeutic approach against AD.

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P243

CRISPR-Cas9-mediated genome editing improves motor deficits and lifespan in a mouse model of Huntington's disease

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Huntington's disease (HD) is a currently incurable and ultimately fatal neurodegenerative disorder caused by a CAG trinucleotide repeat expansion within exon 1 of the huntingtin (HTT) gene, which results in the production of a mutant protein that forms inclusions and selectively destroys neurons in the striatum and other adjacent structures. The RNA-guided Cas9 endonuclease from CRISPR-Cas9 systems is a versatile technology for inducing DNA double-strand breaks that can stimulate the introduction of frameshift-inducing mutations and permanently disable mutant gene function. In the present work, we demonstrate that the Cas9 nuclease from Staphylococcus aureus, a small Cas9 orthologue that can be packaged alongside a guide RNA into a single adeno-associated virus (AAV) vector, can be used to disrupt the expression of the human mutant HTT gene in the R6/2 mouse model of HD following in vivo delivery to the striatum. Specifically, we found that CRISPR-Cas9-mediated disruption of the mutant HTT gene resulted in a ~50% decrease in neuronal inclusions ($P<0.008$) and indel mutations in up to 15% of analyzed HTT transgenes, with no detectable increase in off-target mutations. Crucially, R6/2 mice treated by CRISPR-Cas9 had improved motor function, reduced hind limb clasping, and a ~15% increase in lifespan ($P=0.01$) compared to control animals injected with an AAV vector encoding a non-targeting CRISPR-Cas9 system. These results thus illustrate the potential for CRISPR-Cas9 technology to treat HD and other autosomal dominant neurodegenerative disorders caused by a trinucleotide repeat expansion via in vivo genome editing.

P244

Rett syndrome gene therapy improves survival and ameliorates behavioral phenotypes in MeCP2 null

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Rett syndrome is an X-linked progressive neurodevelopmental disorder affecting approximately 1/10,000 girls. Rett patients experience loss of achieved developmental milestones beginning at 6–18 months. Patients often survive for 40–50 years but experience lifelong disability requiring intense supportive measures and 24/7 care. Rett syndrome is caused by mutations in the X-linked methyl-CpG binding protein 2

(MeCP2) gene, encoding a ubiquitous transcription factor with activating and repressing functions for thousands of genes in the brain. Rodent modeling studies demonstrated re-expression of MeCP2 ameliorates Rett-syndrome–like phenotypes. Therefore, a gene-replacement therapy is a promising strategy. We generated AVXS-201, a self-complementary adeno-associated virus serotype 9 vector expressing the human MeCP2 gene under the control of a minimal MeCP2-promoter. We determined the efficacy in male mice (MeCP2-null and wild-type) and performed safety and dose-ranging studies in male non-human primates (NHPs; Macaca fascicularis). Mice were injected intracerebroventricularly with multiple doses, and behavior and survival were monitored. Weight, blood, and liver parameters of five juvenile NHPs were monitored after lumbar intrathecal injection of AVXS-201. We found that all doses of scAAV9.-MeCP2 tested in MeCP2-null mice increased survival and rescued behavioral symptoms, with multiple doses improving survival by >300%. In NHPs, weight, blood parameters, and liver enzymes remained normal through 18 months post-treatment. No indications of pathology were found in the NHPs sacrificed at 5 weeks post-injection. Overall, our results show good expression of MeCP2 throughout the whole central nervous system after a single injection. AVXS-201 restores MeCP2 expression and is suitable for first-in-human testing.

P245

Gene therapy in a murine model of Niemann-Pick disease type C

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Niemann-Pick disease type C (NPC) is a congenital neurodegenerative disorder caused by mutations in the NPC1 (95%) or NPC2 (5%) gene which involved in cholesterol transport in lysosome. Broad clinical manifestations of NPC1 include liver failure, pulmonary disorder, neurological deficits, and psychiatric symptoms. The condition relentlessly deteriorates and patients with severe phenotype die within several years. We generated a tyrosine-mutant AAV9/3 vector that expresses human NPC1 under a CMV promotor (AAV-CMV-NPC1) and injected into the left lateral ventricle (5 μl) and cisterna magna (10 μl) of Npc1 homo-knockout (Npc1-/-) mice. Each mouse received total 2.7×1011 vector genome at day 3 or 4 of life. AAV-treated Npc1-/- mice (n = 11) marked their average survival more than 28 weeks, while all the saline-treated Npc1-/- mice (n = 11) died within 16 weeks. Saline-treated Npc1-/- mice lost body weight from 5 weeks until death. However, the average body weight of AAV-treated Npc1-/- mice increased until 15 weeks. AAV-treated Npc1-/- mice also showed a significant improvement on the Rota-rod performance. A pathological analysis at 9 weeks showed that cerebellar Purkinje cells were preserved in AAV-treated Npc1-/- mice. In contrast, untreated Npc1-/- mice had the almost total loss of cerebellar Purkinje cells. Our results were better than all previous reports of lateral ventricle injection only or intravenous injection. Broader vector delivery to the central nervous system by combined injection into both the lateral ventricle and cisterna magna induced good results. Our result suggests the feasibility of gene therapy for patients with NPC.

P246

Preclinical evaluation of gene therapy for neurodegenerative diseases caused by inherited deficiency of progranulin

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Families carrying loss-of-function mutations in the gene encoding progranulin (PGRN) exhibit an autosomal dominant inheritance pattern of fatal adult-onset neurodegenerative diseases including frontotemporal dementia, progressive supranuclear palsy, corticobasal syndrome, Parkinson's disease, dementia with Lewy bodies and Alzheimer's disease. Despite their diverse clinical presentations, these patients universally exhibit rapidly progressive brain atrophy and characteristic accumulation of lipofuscin in the lysosomes of neurons. Patients carrying homozygous loss-of-function mutations likewise exhibit lysosomal storage lesions and progressive neurodegeneration, but with disease onset 3–4 decades earlier than patients carrying a single mutation. Gene replacement is an attractive approach for these disorders because PGRN is both secreted and endocytosed, suggesting that a small number of PGRN-expressing cells could supply the protein to the entire central nervous system. We evaluated AAV vector administration into the cerebrospinal fluid (CSF) as a minimally invasive approach to achieve widespread gene transfer and restore PGRN expression in the brain. In PGRN-deficient mice, AAV delivery into the CSF resulted in robust PGRN expression and reduced lysosomal storage lesions and microgliosis throughout the brain. In nonhuman primates, delivery of AAV vectors to the CSF via intra-cisterna magna injection achieved CSF PGRN concentrations markedly exceeding those of normal human subjects. AAV-mediated PGRN expression was remarkably well tolerated in nonhuman primates, with no evidence of dose-limiting toxicity even at vector doses that induced supraphysiologic PGRN expression. These findings support further development of gene therapy for neurodegenerative diseases associated with PGRN deficiency.

P247

A novel AAV-based miQURE gene therapy for SCA3

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Spinocerebellar ataxia type 3 (SCA3), is a fatal neurodegenerative disorder characterized by brainstem and cerebellar atrophy. Clinical manifestations include progressive gait ataxia with involvement of cranial nerves. Expansion of a CAG trinucleotide repeat in the ataxin-3 gene (ATXN3) causes the accumulation of aberrant, toxic ataxin-3 protein in brain regions located in the posterior fossa. Lowering expression of the ATXN3 gene should alleviate mutant ataxin-3 toxicity. A non-allele-specific ATXN3 silencing approach was investigated using proprietary, next-generation miQURE™ technology. Artificial therapeutic

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microRNAs were engineered to target various regions of the ATXN3 gene (miATXN3) and packaged into AAV (AAV-miATXN3). The AAV-miATXN3 candidates were tested for target engagement and potential off-target activity in neurons differentiated from induced pluripotent stem cells (iPSCs). Small RNA sequencing showed efficient guide strand processing without any passenger strands. The AAV-miATXN3 candidates strongly reduced ATXN3 mRNA in iPSCs-neurons. In vivo reduction of mutant ataxin-3 was tested in a SCA3 knock-in mouse model by intraventricular, intracisternal and cerebellar intraparenchymal AAV-miATXN3 injections. Intracisternal AAV-miATXN3 administration resulted in the most effective reduction (up to 65%) of mutant ataxin-3 protein in cerebellum and brainstem. Next, proof-of-concept of pathology improvement upon ataxin-3 lowering was shown in a lentiviral SCA3 mouse model. Intra-striatal administration of AAV-miATXN3 resulted in a strong reduction of mutant ataxin-3 production and prevention of toxic ataxin-3 aggregation. In conclusion, intracisternal administration of AAV-miATXN3 significantly lowers mutant ATXN3 protein in primary sites of SCA3 neuropathology. These results provide evidence to further investigate the distribution, efficacy, tolerability, and safety of AAV-miATXN3 in larger animals.

P248

Klotho gene therapy as a treatment for Alzheimer's disease

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Deficiency in Klotho (KL) gene cause systemic ageing and shortened lifespan in mice, neurodegeneration, memory deficits and increased oxidative stress in brain. Conversely, KL overexpression is related to increased lifespan, memory and cognitive capacities, and reduced oxidative stress in brain. It was reported that KL levels are lower in cerebrospinal fluid (CSF) of Alzheimer's disease (AD) patients compared to healthy adults. Our group reported that the membrane (mKL) and the secreted (sKL) variants of KL have a different expression profile in mouse brain. Moreover, sKL decreased faster in AD mice than in control mice. Recently, we observed that KL levels are already reduced in CSF of AD patients with moderate cognitive decline and that both KL isoforms have different expression profile in brain of AD patients and healthy donors. Therefore, KL is considered a neuroprotective and anti-ageing factor that could be involved in the progression of AD. In order to study the effect of KL overexpression in AD, AAV9 vectors coding for sKL or mKL were administered into CNS of 3xTg-AD model mice through stereotaxic injection. Behavioural tests were performed to study the effect on anxiety, motor performance and cognitive capacities. The effects on AD physiopathology were evaluated through biochemistry and immunohistochemistry in CNS samples. Expression of the transgene and the endogenous forms were also analysed. Beneficial effects on mouse behaviour and AD features would demonstrate the potential of KL as a therapeutic molecule for treating neurodegenerative diseases through gene therapy strategies.

P249

Broad and specific expression of Glial-Derived Neurotrophic Factor (GDNF) in muscles as gene therapy strategy for Amyotrophic Lateral Sclerosis

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Glial cell line-derived neurotrophic factor (GDNF) is a powerful growth factor that can protect motoneurons in vitro and in vivo. However, systemic administration of GDNF is associated with side effects like decreased weight and reduced activity. One of the aims in gene therapy is the restriction of transgene expression to the target tissues to avoid secondary effects. Nevertheless, treatment of neuromuscular diseases may require both, specific but generalized muscle or motoneuron transduction. To specifically target most skeletal muscles in the superoxide dismutase 1 mouse bearing the human G93A mutation (SOD1G93A), a model of amyotrophic lateral sclerosis (ALS), we intravenously injected AAV2/8 coding for the mouse GDNF under the control of the muscle-specific human desmin promoter. Wild-type and SOD1 mice were treated before the onset of the disease as a preventive strategy. Muscle-specific GDNF expression delayed the disease onset in SOD1 animals by preserving the neuromuscular function from 12 to 20 weeks of age in both male and female mice, as assayed by electrophysiological and rotarod tests. The number of innervated neuromuscular junctions and of surviving spinal motoneurons significantly increased, together with reduced glial reactivity in treated SOD1 mice. Moreover, activation of the PI3k/Akt/ERK signaling pathways in the treated animals suggests a mechanism of protection from excitotoxicity and inflammation and the activation of cell survival pathways by GDNF in this model. Importantly, no adverse secondary effects were detected, highlighting the potential of this strategy in ALS therapy.

P250

Intrathecal gene therapy significantly extends lifespan of the Twitcher mouse and the Krabbe dog models

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Krabbe disease is a lysosomal storage disease caused by mutations in the gene encoding the enzyme galactosylceramidase (GALC), which is responsible for the degradation of galactosylceramide and galactosylsphingosine (psychosine).

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Toxic accumulation of psychosine results in demyelination in the central (CNS) and peripheral nervous systems (PNS). The most common presentation is early infantile with disease onset by 6 months of age and progression to death by 2 years of age. We previously demonstrated that administration of neurotropic AAV in the cerebrospinal fluid (CSF) of large animals via the cisterna magna shows efficient transduction of most motor neurons, sensory dorsal root ganglia neurons, and scattered cortical neurons, thus providing sources of enzyme to cross-correct both CNS and PNS. We studied CSF administration of AAVhu68 encoding human or canine GALC in the Twitcher mouse and the Krabbe dog model respectively. We established a dose response in newborn Twitcher mice injected in the lateral ventricle with a median survival of 130 days at the highest dose of $1x10^{11}$ gc ($6.7x10^{11}$gc/g brain). GALC levels in brain and serum were supraphysiological without toxicity and mice demonstrated improved neuromotor function and myelination in CNS and PNS. When newborn CSF administration was followed by bone marrow transplant in postnatal day 10 conditioned mice, survival was extended to >300 days in the absence of overt symptoms. In presymptomatic Krabbe dogs, a single cisterna magna injection of AAV.cGALC at $3x10^{13}$ gc provided phenotypic correction, survival increase, nerve conduction normalization and improved brain MRI, demonstrating the scalability of our approach.

P251

Safe and sustained elevation of sulfamidase after administration of AAV9-sulfamidase to the CSF of dogs: Seven year-follow up

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Mucopolysaccharidosis Type IIIA (MPSIIIA) is a rare autosomic recessive Lysosomal Storage Disease (LSD) caused by deficiency in sulfamidase, sulfatase involved in heparan sulfate (HS) degradation. Undegraded HS accumulates in lysosomes, resulting in severe progressive neurodegeneration with relatively mild somatic pathology. Patients usually die within the first decades of life. We previously demonstrated in MPSIIIA mice that the intra-cerebrospinal fluid (intra-CSF) administration of AAV9 vectors encoding sulfamidase could mediate whole-body disease correction through transgene expression throughout the CNS and liver. Here, we evaluated the long-term expression and safety of this gene therapy approach in a large animal model. Healthy Beagle dogs received an AAV9 vector encoding canine sulfamidase (AAV9-cSulfamidase). Transgene expression was followed for ~7 years through measurement of sulfamidase activity in CSF. Safety was evaluated through biochemical and hematological parameters, magnetic resonance imaging of the brain and spinal cord, and abdominal ultrasounds. A full neurological evaluation was also performed. Liver biopsies were obtained to study persistence of vector and transgene expression in liver. Seven years after administration, in situ hybridization assay for detection of sulfmidase mRNA expression in the CNS and liver was assessed. A single intra-CSF AAV9-cSulfamidase administration to dogs, at a clinically relevant dose, resulted in long-term stable increase in sulfamidase activity in CSF, in the absence of any safety concerns. Altogether these results demonstrate the long-term safety and sustained elevation of sulfamidase after intra-CSF AAV9-cSulfamidase gene transfer and support the clinical translation of this therapeutic approach for the treatment of MPSIIIA and other LSD with CNS involvement.

P252

High efficiency of CRISPR/Cas9 gene editing of the p.(Thr158Met) MECP2 mutation in mitotic and post-mitotic cells

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Rett syndrome is one of the most common causes of intellectual disability in girls, resulting in severe cognitive and physical disabilities. The classic form is caused by mutations in the transcriptional regulator MeCP2. Effective therapies are not currently available and the need for tight regulation of MeCP2 expression for proper brain functioning makes gene replacement therapy risky. Using CRISPR/Cas9-based gene editing we successfully corrected one of the most common MECP2 mutation hotspots, c.473C>T-p.(Thr158Met), in patient cells. A two-plasmid system was developed suitable for delivery with Adeno-Associated Viral vectors (AAVs). Mutation-specific sgRNA and donor DNA were selected and cloned together with a double mCherry/EGFP reporter system. Cas9 flanked by sgRNA recognition sequences for auto-cleaving was cloned in a second plasmid. Editing efficiency was validated in cells from four unrelated patients with the c.473C>T-p.(Thr158Met) mutation. For one patient, fibroblasts were used to derive induced Pluripotent Stem Cells (iPSCs) and iPSC-derived neurons. NGS analysis on fibroblasts and iPSC-derived neurons demonstrated an exceptionally high editing efficiency in all patients, with up to 80 % of Homology-Directed Repair and a very low percentage of indels. Preliminary infection experiments demonstrated a good AAV9 co-infection in iPSC-derived neurons, in line with the efficiency of this serotype in targeting neuronal cells. To further validate efficacy and safety, in vivo experiments with EGFP-expressing AAv9 were performed in the Mecp2-T158M mouse are ongoing. The described approach is patented at University of Siena and ISPRO (application n. 102018000020230).

P253

A route of administration study of BBP-812, an AAV9-based gene therapy for the treatment of Canavan disease, in juvenile cynomolgus macaques

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Canavan Disease (CD) is a rare pediatric leukodystrophy caused by aspartoacylase deficiency. The disease is characterized by elevated N-acetylaspartic acid, the substrate of aspartoacylase. Patients present with a lack of psychomotor development and most die by the age of ten. We are developing BBP-812, an AAV9-based gene therapy containing a codon optimized human Aspa transgene, to introduce functional aspartoacylase into CD patients. To understand the optimal dosing route for BBP-812 we conducted a study in juvenile cynomolgus macaques comparing; intravenous infusion (IV: doses of 1.8E13, 6E13, and 1.8E14 vg/kg), intrathecal lumbar injection (5E12 vg total), or unilateral intracerebroventricular injection (5E12 vg total). Animals were sacrificed three and eight weeks after dosing. During the in-life phase, animals were monitored for changes in hematology and both serum and urine chemistries. Biodistribution was measured throughout the brain and spinal cord as vector genomes per diploid cell by droplet digital PCR and transgene RNA was assessed by qRT-PCR. There was a transient increase in ALT and AST in the highest dose IV treatment group at day 3 which returned to normal without intervention by day 8 post dosing. All other markers of hematology, as well as serum and urine chemistries were unchanged. Biodistribution analysis demonstrated that all three routes of administration achieved transduction throughout the spinal cord. However, only animals receiving IV delivered BBP-812 showed transduction throughout the deep brain structures critical for the treatment of CD. This work supports the continued development of BBP-812 by intravenous infusion for the treatment of CD.

P254

Whole brain delivery of an instability-prone Mecp2 transgene corrects behavioral and molecular pathological defects in mouse models of Rett syndrome

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Rett syndrome (RTT) is an incurable neurodevelopmental disorder caused by mutations in the gene encoding for methyl-CpG binding-protein 2 (MECP2). Gene therapy for this disease presents inherent hurdles since MECP2 is expressed throughout the brain and its duplication leads to severe neurological conditions as well. However, the recent introduction of PHP.eB, an engineered capsid with an unprecedented efficiency in crossing the blood-brain barrier upon intravenous injection, has provided an invaluable vehicle for gene transfer in the mouse nervous system. Herein, we use PHP.eB to deliver a novel instability-prone Mecp2 (iMecp2) transgene cassette which prevents supraphysiological Mecp2 protein levels in transduced neural tissues by increasing RNA destabilization and inefficient protein translation of the viral Mecp2 transgene. Intravenous injections of the PHP.eB-iMecp2 virus in symptomatic male and female Mecp2 mutant mice significantly ameliorated the disease progression with improved locomotor activity, coordination, lifespan and normalization of altered gene expression and mTOR signaling. Remarkably, PHP.eB-iMecp2 administration did not resulted in severe toxicity effects either in female Mecp2 mutant or in wild-type animals. In contrast, we observed a strong immune response to the transgene in treated male Mecp2 mutant mice that was partially overcome by immunosuppression. Overall, PHP.eB-mediated delivery of the iMecp2 cassette provided widespread and efficient gene transfer maintaining physiological Mecp2 protein levels in the brain. This combination defines a novel viral system with significant therapeutic efficacy and increased safety holding important clinical implications for RTT.

P255

Restoring neuronal cholesterol efficiently rescues ALS mouse model

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Defects in brain cholesterol metabolism contribute to neurodegenerative diseases, such as Alzheimer's disease (AD), Huntington's disease (HD) and Spinocerebellar ataxias (SCAs). Beside contributing to myelin compartment, cholesterol is a critical component of membranes where it plays key structural and functional roles. Cholesterol cannot cross the blood brain barrier (BBB) and is synthesized in situ. Cholesterol 24-hydroxylase (CYP46A1) ensures the conversion of cholesterol into 24S- hydroxycholesterol that can freely cross the BBB. This key neuronal enzyme of brain cholesterol metabolism plays a crucial role in maintaining brain cholesterol homeostasis and is a major neuronal stress response in conditions with oxidative stress, like ageing or toxic protein accumulation. We previously demonstrated that CYP46A1 overexpression after AAV-CYP46A1 delivery, efficiently rescued clinical and neuropathological hallmarks of mouse models of AD, HD and SCA3. CYP46A1 gene therapy is able to restore deficient autophagy process in these diseases and clearance of misfolded protein aggregates. We thus hypothesized us that CYP46A1 could be a relevant therapeutic target for amyotrophic lateral sclerosis (ALS) to improve stress response, clearance of toxic aggregated proteins like SOD1, synaptic transmission, inflammation and finally neuronal survival. We demonstrate that AAVi-CYP46A1 improves the severe SOD1G93A ALS mouse model. We demonstrate a significant and prolonged motor rescue of animals treated pre or post-symptomatically, compared to untreated animals. Functional and histological rescue as well as survival data will be presented. Evaluation of this therapeutic strategy is ongoing in another model of ALS.

P256

SINEUPs: a new antisense, long non-coding RNA-based platform to increase endogenous protein levels for therapy

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SINEUPs represent a new platform to increase endogenous protein levels of target mRNAs for therapeutic purposes. They are antisense long non-coding RNA (lncRNAs) that stimulate translation of sense mRNAs. Their activity depends on the combination of two domains: the overlapping region, or binding domain (BD), dictates SINEUP specificity, while an embedded inverted SINEB2 element acts as effector domain (ED) controlling the enhancement of mRNA translation. Their modular structure can be employed to artificially engineer their BD and design synthetic SINEUPs to specifically enhance translation of virtually any target gene of interest. They usually increase target protein expression of 2–3 fold, thus avoiding massive over-expression. Moreover, they are active only on cells that express target mRNAs, thus limiting the side effects. As representative examples, SINEUP-GDNF RNA increases endogenous GDNF protein levels both in-vitro and in-vivo, when injected in the mouse striatum. Furthermore, 6 months after injection, SINEUPs-GDNF protects mice in a neurochemical model of PD. SINEUP-frataxin RNA increases endogenous frataxin protein levels restoring mitochondrial activity in Freidreich's Ataxia patient's cells proving SINEUPs as a therapeutic strategy for haploinsufficiencies.

P257

Gene transfer rescues established phenotypes in a mouse model of Rett syndrome

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Mutations in the X-linked gene MECP2 result in Rett syndrome (RTT) which is characterized by severe motor and cognitive impairments. We and others have shown that neonatal delivery of AAV9/hMECP2 to Mecp2-/y mice extended survival and ameliorated RTT-like phenotypes. However, the potential impact of gene therapy on symptomatic Mecp2-/y mice is unknown. The aim of this study was to assess the ability of vector-derived transgene to rescue advanced RTT-like phenotypes in mice modeling Rett syndrome. A functional human MECP2_e1 minigene (ΔNIC) was packaged in a scAAV-PHP.B vector and injected intravenously into symptomatic 8 weeks old Mecp2-/y mice at either 1x1012 vg/mouse (low dose) or 2x1012 vg/mouse (high dose). Injected mice were monitored for survival, body weight and RTT-like phenotypes. Mecp2-/y mice treated with the low dose showed 100% survival and normalization of the bodyweight during the 40-week study period. In comparison, vehicle-treated Mecp2-/y mice had a median survival of 11 weeks. Importantly, most established RTT-like phenotypes were improved/stabilized after injection. Incidence of apneas, a prominent feature in RTT, was significantly ($p < 0.001$) reduced compared to vehicle-treated Mecp2-/y mice. In contrast, Mecp2-/y mice treated with the high dose showed modest survival improvement with no effect on the RTT-like phenotypes. This data shows, for the first time, that many RTT-like phenotypes are reversible following vector-derived MeCP2 expression, but it also indicates a narrow therapetic dosage window. Optimizing vector designs that incorporate regulatory elements to control levels of MeCP2 expression are likely to enhance the safety profile in RTT gene therapy.

P258

AAV-mediated delivery of a novel anti-BACE1 VHH reduces Abeta in an Alzheimer's disease mouse model

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Single domain antibodies (VHH) are potentially groundbreaking therapeutics, with important biological value for treatment of several diseases, including neurological disorders. Despite their promises, VHH have not been widely used in the central nervous system (CNS), as it is hard to establish and maintain therapeutic levels, both because of their restricted penetration across the blood-brain-barrier and their rapid clearance from the parenchyma. We proposed a gene transfer strategy based on adeno-associated virus (AAV) vectors to deliver VHH directly into the CNS, ensuring continuous production at therapeutic levels. As a proof-of-concept, we explored the potential of AAV-delivered VHH to inhibit BACE1, a well-characterized target in Alzheimer's disease. First, we generated a panel of VHH targeting BACE1. One of them, VHH-B9, showed high selectivity for BACE1 and efficacy in lowering amyloid beta ($A\beta$) production in an in vitro APP cleavage assay and primary neuronal cultures. We then went on to demonstrate significant reductions in $A\beta$ levels after AAV-based delivery of VHH-B9 into the CNS of a mouse model of cerebral amyloidosis. These results constitute a novel therapeutic approach for neurodegenerative diseases, which is broadly applicable to a range of CNS disease targets.

P259

Improving the ability of hematopoietic stem cells to generate a microglia-like progeny upon transplantation

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Hematopoietic stem and progenitor cells (HSPCs) contribute to the turnover of brain resident myeloid populations upon transplantation in recipients receiving a proper conditioning. Transplanted HSPCs home to the brain, engraft locally and give

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rise to a mature progeny that shares transcriptional, morphologic and functional features with microglia. In the context of metabolic and neurological diseases, engrafted cells can act as vehicles for therapeutics to the brain as well as critical modulators of the environment towards neuroprotection. However, the impact of this approach is affected by the slow pace of reconstitution of resident myeloid cells/microglia by the transplanted HSPCs progeny, as compared with the rapid progression of the neurological disease. In order to fasten this process, we identified a key factor that may enhance the ability of hematopoietic cells to generate microglia-like progeny upon transplantation. In the murine setting, we demonstrated that i) transplantation of Gene X haploinsufficient HSPCs leads to more robust and faster microglia reconstitution as compared to wild type HSPCs; ii) in competitive transplantation setting, haploinsufficient cells prevail over wild type HSPCs in the repopulation of hematopoietic organs and brain of recipients. Moreover, haploinsufficient cells rapidly acquire a mature microglia-like morphology and phenotype, suggesting also a qualitative advantage over wild type HSPCs. We are now elucidating the biological mechanism underneath this phenomenon.

P260

AAV-mediated gene editing as a potential therapeutic approach for C9ORF72-linked ALS/FTD

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A hexanucleotide repeat expansion (GGGGCC or G4C2) in the first intron of chromosome 9 open reading frame 72 (C9ORF72) is the most common genetic origin for both Amyotrophic lateral sclerosis (ALS) and Frontotemporal dementia (FTD), two different neurodegenerative diseases with overlapping molecular hallmarks. The available treatments are dramatically ineffective in slowing disease progression, highlighting the need for more efficient therapeutic strategies. It was in this context that we proposed to develop and test two new experimental treatments, both consisting of a highly targeted gene editing strategy, using the CRISPR-Cas9 tool. One approach focuses on expressing SaCas9 through an AAV delivery vector together with a pair of gRNAs that cut in the flanking regions of the hexanucleotide expansion, excising the repeats and allowing the re-ligation of the gene. Our second strategy is designed to mutate the region upstream of the expansion, disrupting important regulatory elements for the transcription and RAN translation of the G4C2 repeats. So far, we successfully cloned SaCas9 and two U6-driven gRNAs in a single AAV expression vector and demonstrated the efficacy of our two systems in a human cell line, achieving robust gene editing without affecting the expression of WT C9ORF72. Using AAV9 as a delivery vehicle, we were able to rescue in vitro hallmarks of this disease in two patient-derived models, and observed a promising therapeutic effect in vivo, using a recent BAC mouse model. Our proof-of-concept study might provide the basis for a more targeted and efficient therapy for C9ORF72-ALS/FTD than what is currently available.

P261

Adeno-associated virus serotype 9 (AAV9) antibodies in patients with spinal muscular atrophy (SMA) screened for treatment with onasemnogene abeparvovec

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Onasemnogene abeparvovec (formerly AVXS-101), an AAV9-based survival motor neuron 1 (SMN1) gene-replacement therapy (GRT), improved outcomes in SMA type 1 patients in a phase 1/2a study. Here, we report AAV9 antibody titers in SMA patients screened for the onasemnogene abeparvovec/AVXS-101 clinical trials and the managed access program (MAP). SMA patients (biallelic SMN1 loss, 1–3xSMN2) 0–60 months of age were screened to determine eligibility for a one-time intravenous onasemnogene abeparvovec dose (SPR1NT [phase 3, NCT03505099], START [phase 1/2a, NCT02122952], STR1VE [phase 3, NCT03306277], STR1VE-EU [phase 3, NCT03461289]), or intrathecal AVXS-101 dose (STRONG [phase 1/2a, NCT03381729]). AAV9 antibody titers were considered exclusionary if they were persistently >1:50. As of June 10, 2019, of 36 mothers screened in SPR1NT, 11 asymptomatic newborn patients (ages: 0–6 weeks) were consequently tested, 2 of which had exclusionary antibody titers. In STR1VE, 0/25 symptomatic patients (ages: 0–6 months) screened had titers >1:50. In STR1VE-EU (Europe), 5/40 symptomatic patients screened had exclusionary titers. Similarly, only 1/16 symptomatic patients screened in START was excluded due to titers >1:50. In the MAP, 0/48 patients screened (ages: 0.5–25 months) had exclusionary titers. As of March 29, 2019, 3/34 of the older patients screened in STRONG (ages: 6–60 months) had persistently elevated titers. Screening results from the onasemnogene abeparvovec/AVXS-101 clinical trials and MAP show that 5.5% (11/199) of SMA patients screened had exclusionary AAV9 antibody titers. Levels of AAV9 antibody titers should not affect the ability of most SMA patients to receive AAV9-based GRT.

P262

Cerebellar transduction of astrocytes as gene therapy strategy for Megaloencephalic Leukoencephalopathy with subcortical cysts (MLC)

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With the aim to achieve global and robust gene expression in the cerebellum, we tested different delivery routes of administering viral vectors using an AAVrh10 vector driving the expression of GFP under the regulation of the GFAP promoter, specific for astrocytes. We found that subarachnoid injection in the CSF was the most efficient route in transducing Bergmann's glia, severely affected in Megaloencephalic leukoencephalopathy with subcortical cysts (MLC), a neurological disorder cursed with macrocephaly, motor functions deterioration, cerebellar ataxia and mental decline. It is a type of vacuolating myelinopathy characterized by swollen brain with diffuse white-matter abnormalities and subcortical cysts. AAVrh10 coding for MLC1 under the control of the GFAP promoter was injected in the cerebellar subarachnoid space of MLC KO and WT animals at 2 months of age, before the onset of the disease, as a preventive approach. We also tested a therapeutic strategy by injecting the animals at 5 months, once the disease has been already stablished, or at 15 months, when it has progressed to a more severe pathology. Western-blot, quantitative RT-PCR and immunohistochemistry showed MLC1 expression in the cerebellum and extremely reduced myelin vacuolation, a hallmark of the disease, in treated 8- and 18-month old animals. Moreover, GlialCAM, an Ig-like adhesion molecule, and the chloride channel CLC2, both implicated in MLC, restored its localization in Bergmann glia after treatment. These results may have implications for gene therapy to treat MLC patients as well as for other diseases affecting motor function and ataxias.

P263

Delivery of the SCN1A into the brain using High-Capacity Adenoviral vectors for the treatment of Dravet Syndrome

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Dravet Syndrome (DS) is a severe encephalopathy with infantile onset, characterized by refractory seizures, increased risk of sudden death, as well as mental, behavioural and motor manifestations. In up to 90% of cases the genetic defect consists of mutations in one of the SCN1A alleles, leading to haploinsufficiency. SCN1A encodes the alpha subunit of a voltage-dependent Sodium channel (Nav1.1), which is critical for the function of inhibitory interneurons in the brain. Taking into account the complexity and size of the SCN1A coding region (6 Kb), gene transfer of this gene into the brain requires vectors with high cloning capacity and stability. To this aim we have developed a High-Capacity (Helper-Dependent) Adenoviral vector expressing SCN1A under the control of a constitutive promoter. We provide proof of concept that this vector is stable and mediates potent transgene expression in vitro and in vivo. Therapeutic effect is currently evaluated in a SCN1AA1783V knock-in mouse model of DS, which presents a full repertoire of disease manifestations. The vector was administered by stereotaxic injection in the brain. Electrophysiology was studied using intracranial electrodes in freely moving mice. So far we have observed good tolerance to the treatment, and reduction of epileptiform discharges in the electroencephalography recordings. Our preliminary results indicate that gene transfer of SCN1A using High-Capacity adenoviral vectors is safe and potentially efficacious for the treatment of DS.

P264

Evidence that prevalent SMG6 insertions are not associated with abnormal clinical findings in patients treated with Lenti-D autologous hematopoietic stem cell gene therapy for cerebral adrenoleukodystrophy

ABSTRACT WITHDRAWN

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P265

AAV-mediated expression of antisense oligonucleotides for the treatment of C9orf72-ALS

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Viral vectors derived from Adeno Associated Virus (AAV) represent a valuable tool for gene therapy of neurodegenerative diseases. Amyotrophic lateral sclerosis (ALS) is the most common motor neuron (MN) disorder in adults, characterized by progressive degeneration of both upper and lower MNs leading to paralysis and death. While the majority of ALS cases are sporadic, about 10% of forms are familial (fALS). Recently, we developed an AAV10-mediated gene therapy strategy for fALS linked to SOD1 mutations. Using the small nuclear RNA U7 carrying antisense (AS) oligonucleotides we silenced the expression of toxic SOD1 through exon skipping prolonging survival of SOD1G93A mice. In light of these previous results, we are now developing a similar AAV10-based gene therapy approach for C9orf72-linked ALS, the most frequent genetic cause of fALS (40% of cases), in order to counteract the RNA foci formation and toxic dipeptides translation, protecting C9 protein production. We have produced Lentivirus and AAV plasmids carrying the U7 RNA and expressing different AS sequences against the C9 repeats. We assessed their silencing efficacy in immortalized fibroblasts and spinal MNs-iPSC, both derived from C9 patients. We have observed reduction of RNA foci in the transduced cells with the lentivirus expressing AS against C9 and we are now evaluating the effects on dipeptides and C9 protein. In parallel, we are characterising the C9 BAC mouse model (FVB/NJ-Tg (C9orf72)500Lpwr/J; Jax SN 029099) on which we will assess the effect of the AAV10-U7-C9 vector, carrying the most effective C9-AS, to determine its therapeutic potential.

P266

Immunomodulation by gene therapy with adeno-associated vectors for the treatment of multiple sclerosis

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It has been proved that the Th17 and Th1 cell pathway play a crucial role in the immunopathogenesis of multiple sclerosis (MS), in which several key citoquines, like IL-21 or IL-23, are involved in the differentiation and expansion of pro-inflammatory cells and in the inhibition of anti-inflammatory mediators like Tregs or Th2 lymphocytes. The main objective of this study was to carry out immunomodulation to amielorate the clinical symptomatology associated with the Experimental Autoimmune Encephalomyelitis (EAE) in mice, an animal model of MS, by a selective blockade of IL-21 and also through the administration of the novel anti-inflammatory cytokine IL37 using AAV8 as gene vector. We observed that intravenous preventive application of both strategies is capable of producing a clinical improvement of the EAE, in addition to a significant reduction of incident animals. Of note, compared with control animals (AAV8-Null), when the blockade of IL-21 was performed we observed (i) a better spinal cord histological condition with less demyelination, infiltration and axonal damage and (ii) a better immunological profile, with an increase of the anti-inflammatory response during the acute phase, including increased TGFBeta expression and Th2 and Treg response stimulation resulting in a lower activation of compensatory mechanisms during the chronic phase. On the other hand, in animals treated with the processed form of IL37, we observed a decrease in the levels of the main pro-inflammatory cytokines causing pathogenesis (i.e. IL17 and IFNg) as well as a reduction in the capacity of antigenic presentation by dendritic cells.

P267

PROVide: Video based Patient Reported Outcomes for Sanfilippo Syndrome (MPS IIIA): a new and innovative approach to record and measure disease post gene therapy

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Mucopolysaccharidoses (MPS) are a group of lysosomal storage diseases resulting from deficiency of enzymes necessary for glycosaminoglycan breakdown. Mucopolysaccharidosis Type III (MPS III or Sanfilippo syndrome) is differentiated from other MPS subtypes by its' prominent neurobehavioral phenotype. Progression of disease is experienced as a continuum of early developmental delays, behaviour and sleep abnormalities, autistic features, loss of mobility and oral feeding abilities, development of seizures and movement disorder followed by early death. Parents of children with MPS IIIA report that clinic-based assessments do not fully capture the scope of patients' abilities or the real-world disease burden and may miss documenting potential treatment effects. In addition, long term monitoring is a requirement unique to gene therapies under trial, creating further feasibility challenges of sponsoring trials in small rare disease populations to reach approval. This study has developed a home-based method for capturing outcomes for MPS IIIA, as a means to monitor the onset and evolution of disease hallmarks remotely and in a non-invasive way that is suitable for children. Defined video captures were chosen based on identified gaps in clinic-based measures and personalized features identified by caregivers. Up to twenty patients with MPS IIIA, treated on the Lysogene gene therapy P4-SAF-302 trial NCT03612869, are asked to complete qualitative interviews and video captures in their own home and share them via a study-specific smartphone app. Expert clinicians will score each video at the end of the study based on a pre-defined scale.

P268

Single-intrathecal delivery of a new AAV9-mediated gene therapy vector provides long-term safe expression of frataxin and prevents neurodegeneration in a Friedreich Ataxia mouse model

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P269

AAV gene therapy for Parkinson's disease: *in vitro* and *in vivo* effects of AAV-GBA treatment in GBA mutant models

ABSTRACT WITHDRAWN

ABSTRACT WITHDRAWN

P270

RNAi-based gene therapy approach for GNAO1-related neurodevelopmental disorder

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Mutations in GNAO1 were recently linked to a fatal neurodevelopmental disease characterized by infantile epilepsy and movement disorder. GNAO1 encodes Gαo protein that is highly abundant in the brain and involved in signal transmission between neurons. At least some heterozygous mutations in GNAO1 result in toxic gain-of-function and lead to disruption in neuronal signaling. For such conditions, we propose a gene therapy strategy of allele-specific suppression to eliminate mutant GNAO1 while leaving functional protein unaffected. In the present study, we tested applicability of RNAi technology for selective downregulation of GNAO1 missense variant c.607 G>A in the cell culture-based experiments. We screened synthetic siRNAs designed to the mutation site and identified at least one target sequence that efficiently suppressed mutant GNAO1 at RNA and protein levels. Next, using heterozygous assay we demonstrated that candidate RNAi effector discriminates well between wild type and mutant transcripts and downregulates mutant GNAO1 in allele-specific manner. As a step towards adapting RNAi tools for in vivo studies, we constructed shRNA vectors with the selected GNAO1 targeting sequence. Such vectors can be packaged into adeno-associated vectors and efficiently delivered into brain tissues. Potency of GNAO1-specific shRNAs was confirmed in vitro, additional tuning of the hairpin design is needed to further increase allele-selectivity. To complete proof-of-concept studies, we set to verify that shRNA-mediated allele-specific suppression can restore normal Gαo-mediated neuronal signaling in the animal model of GNAO1 disorder. Taken together, our pilot experiments demonstrate the potential of RNAi technology for gene therapy of GNAO1-related neurodevelopmental disorder.

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MRI-guided injections in the brain: a method for gene therapy delivery for NHP and humans

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Selective gene delivery is a powerful approach for treatment of currently incurable diseases such as Huntington disease (HD) which is caused by mutation of huntingtin (HTT) protein. UniQure developed a gene silencing therapy based on a microRNA targeting human HTT which is delivered with the vector AAV5-miHTT directly into affected brain structures by MRI-guided convection-enhanced delivery (CED) delivery. The Renishaw drug delivery system was used for MRI-guided CED to the striatum in cynomolgus macaques. Animals were mounted in a stereotaxic frame, and MRI scans were performed at 3 Tesla for coordinate calculation. After removal of the fiducial arc, a CRW

stereotactic frame was attached to the fixation frame. Using the derived coordinates, animals were surgically implanted with bilateral microstep catheters. The wound was temporarily sutured allowing access to the connecting port. The CRW frame was removed and the animal transferred back to the MR scanner where the infusion lines were connected to the port. Target acquisition was confirmed by a post-implant scan. Infusion pumps were started simultaneously, and MR scans were repeated throughout infusions. 100 μl AAV5-miHTT were injected in each targeted structure. A final MR scan was performed to document vector distribution in the brain. Administration of AAV5-miHTT into the striatum resulted in widespread distribution in striatal and cortical structures and was well tolerated. Follow-up studies demonstrated that the method could be successfully performed with a mobile 1.5 T MRI scanner and confirmed tolerability in a GLP biodistribution and toxicity study. The method is translatable for application in humans.

P273

Long term effect of Prostaglandin E2 exposure on Hematopoietic Stem/Progenitor Cells

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Ex-vivo gene therapy (GT) based on transplantation of autologous genetically-modified hematopoietic stem/progenitor cells (HSPCs) is a promising curative treatment for multiple inherited diseases. Ideal transduction conditions should determine high gene marking in long-term self-renewing stem cells, preserving their homeostasis and clonogenic potential during the in vitro manipulation. Short term exposure to Prostaglandin E2 (PGE2) during lentiviral transduction results in a high Vector Copy Number (VCN) in NSG-repopulating HSPCs, with a controversial effect on the long-term engraftment level. In the context of the clinical development of an ex vivo HSPC-based GT for a Lysosomal Storage Disorder, best transduction results were obtained by transducing mobilized CD34+ cells with the therapeutic lentiviral vector in the presence of PGE2, which increased the VCN $\approx$ three-folds. However, a mild detrimental effect on cell-viability in bulk liquid culture and a significant reduction in clonogenic potential were observed in vitro in multiple CFC-assays. This effect increased with the time of PGE2 exposure, and correlated with the specific increase of a cellular antigen. Inhibition of this factor during HSPC transduction in the presence of PGE2, did not affect the PGE2-mediated transduction enhancement but rescued the HSPC clonogenic potential in vitro. We are currently investigating the effect of PGE2 exposure and of this optimized transduction procedure on long-term NSG-repopulating HSPCs in a competitive repopulation assay. This study, if successful, will identify an effective and clinically relevant transduction protocol, and will provide precious knowledge on the effect of immunomodulatory molecules on long-term repopulating HSPCs.

P274

Transport of therapeutic proteins across the blood-brain barrier using viral gene therapy

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The presence of the blood-brain barrier is a huge challenge for the treatment of neurological diseases. However, the gene therapy strategy using a brain-specific adeno-associated virus (AAV2-BR1) might overcome this obstacle. The AAV2-BR1 has previously been shown to be a promising candidate to transduce brain capillary endothelial cells (BCECs). The present study aims to investigate the possibility of transducing mouse BCECs in vivo for recombinant protein synthesis and secretion of the Niemann-Pick type C2 protein (NPC2) to the brain. Female BALB/cJRj mice were allocated to either a control or a virus group (AAV2-BR1 vector encoding NPC2 coupled to GFP (AAV2-NPC2) or AAV2-BR1 vector encoding luciferase (AAV2-Luc)) and injected intravenously in the tail with either phosphate buffer saline or AAV-BR1, respectively. During the study period, no changes in body weight were recorded between control and AAV2-BR1 injected mice. To evaluate the long-term transgene expression after intravenous injection, the AAV2-Luc injected mice were scanned at different time-points using a bioluminescence scanner. Using immunohistochemistry, GFP immunoreactivity was observed in BCECs throughout the entire brain. GFP immunoreactivity was also observed intraneuronal in several brain regions, including cerebral cortex and cerebellum. Furthermore, it was possible to detect a brain-specific luciferase expression from 14 days post-injection which lasted throughout the study period. Our results show that systemically administered AAV2-BR1 accumulates in BCECs in the entire brain as well as in some neurons in mice. These findings indicate that the gene therapy strategy targeting the BCECs carry the potential to treat genetic diseases affecting the brain.

P275

Gene therapy to the blood-brain barrier with resulting protein secretion as a strategy for treatment of NPC2 disease

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The blood-brain barrier (BBB) denotes a major hurdle in the treatment of disorders affecting the central nervous system (CNS) due to an inability of several therapeutics to cross the BBB. Gene therapy applied to brain capillary endothelial cells (BCECs) represents a novel approach to overcome this hurdle, as genetically modifying BCECs into recombinant protein factories results in protein secretion further into the brain. We know from previous in vitro studies that gene therapy to BCECs leads to a

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bidirectional protein secretion, which is particularly relevant in genetic diseases characterized by a global lack of functional protein like lysosomal storage diseases. We are currently in the process of exploiting this strategy for the treatment of the lysosomal storage disease, Niemann Picks Disease C2 (NPC2), where protein replacement strategies hold therapeutic potential, but are limited by an inability of the NPC2 protein to reach the CNS. In the present study, we investigate the possibility of genetically modifying primary rat brain endothelial cells in an in vitro BBB model, using both non-viral and viral agents, for recombinant protein synthesis and secretion of the NPC2 protein to NPC2 mutant skin fibroblasts, with the aim of correcting the cholesterol accumulation in these cells. We demonstrate NPC2 gene induction and protein secretion in primary BCECs in non-mitotic cultures following gene modification with therapeutic effect on the NPC2 mutant fibroblasts. Moreover, we find a strong association between the secretion of recombinant protein and subsequent therapeutic effect and the gene modification efficiencies.

P276

Extracellular vesicles for therapy of spinocerebellar ataxia type 3

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Extracellular vesicles (EVs) are membrane-contained vesicles produced by cells with the capacity to carry small nucleic acids, particularly miRNAs, a class of small RNAs that regulate gene expression. Interestingly, specific motifs (ExoMotifs) have been shown to interact with ribonucleoproteins promoting enrichment of miRNAs into EVs. Therefore, the aim of this work was to take advantage of ExoMotifs to promote packaging of synthetic miRNA-based silencing sequences into EVs, aiming at their use for therapy of Machado-Joseph-disesase/spinocerebellar ataxia type-3 (MJD/SCA3). MJD/SCA3 is a neurodegenerative disorder caused by abnormal over-repetition of the CAG tract within the ataxin-3 (ATXN3) gene, conferring toxic properties to the corresponding ATXN3 protein. For this purpose we generated silencing sequences targeting mutant ATXN3 (mutATXN3) mRNA and carrying ExoMotifs. Furthermore, neuronal targeting proteins were expressed at EVs surface and their targeting efficiency was evaluated by immunohistochemistry and flow cytometry. A stable packaging cell line was created by using lentiviruses to constitutively express the three elements and the resulting EVs were used to treat neuronal cells encoding mutATXN3. Silencing efficiency was evaluated by a dual luciferase assay, RT-PCR and Western blotting. We found that both endogenous miRNAs as well as the silencing sequences containing the ExoMotifs were enriched into EVs and modifications at EVs' surface increased targeting of primary neurons after 4 hours. Moreover, EVs produced by the packaging cell line were able to significantly decrease mutATXN3 mRNA levels. This study suggests that EVs can be used to deliver artificial silencing sequences to treat MJD/SCA3.

P277

AAV-mediated over-expression of secreted and transmembrane αKlotho isoforms reverse cognitive and molecular aging hallmarks in senescent mice

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Aging of Central Nervous System (CNS) is accompanied by an increase in physiological fragility of neurons, which causes progressive cell dysfunction and death. There are specific conditions that increase the degeneration rate, accelerating the emergence of cognitive deficits. In this context, the senescence-accelerated mouse-prone (SAMP8) represent a good model for aging and neurodegeneration studies as it presents accelerated aging rates when compared with its control strain SAMR1, including neurological degeneration, protein accumulation, epigenetic deregulation, physical weakness, osteoporosis, and shortened life span. αKlotho (KL) has been described as a powerful neuroprotector factor that delays aging consequences. It is mainly expressed in the kidneys and the choroid plexus in CNS and presents two major splicing variants, a transmembrane isoform (m-KL) and a shorter secreted one (s-KL). Interestingly, Klotho expression decreases during non-pathological aging, which causes deregulation of key metabolic pathways and decrease the neuroprotection that this protein confers. Using gene therapy, both isoforms can be constantly over-expressed, allowing the study of their therapeutic potential and the biological basis of Klotho isoforms. During this study, either s-KL or m-KL was over-expressed in SAMP8 and SAMR1 animals by intraventricular administration of AAV9 vectors. After 2 months, a significant improvement in physical condition and cognitive performance of these animals was observed. Histological analysis to detect the neuroprotective effects generated by the treatment, as well as molecular analysis focused on the epigenetic modifications associated with aged CNS have also been performed, showing promising results.

P278

Understanding and challenging the contribution of HSCs to brain myeloid cell turnover

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In the context of metabolic and neurological diseases, microglia-like cells replaced by the progeny of transplanted hematopoietic stem and progenitor cells (HSPCs) can function both as local source of therapeutic drug delivery as well as modulators of the inflammatory environment towards neuroprotection. However, the impact of this approach is affected by the lack of information concerning the brain myeloid cell/microglia reconstitution process that follows HSPCs transplantation. Our previous findings indicate that upon transplantation into myeloablated recipients, HSPCs home into the central nervous system (CNS) shortly after transplant, engraft locally and generate functional microglia like myeloid progeny. To characterize the hematopoietic cells engrafted in the CNS and follow their fate we performed secondary transplantation experiments using, as source of transplantable HSPCs, the bone marrow and brain hematopoietic compartment. Interestingly, not only bone marrow-, but also brain-retrieved hematopoietic cells of primary recipients were able to engraft long-term in hematopoietic tissues and in the brain of secondary recipients, showing multi-lineage differentiation capability. These results were confirmed by integration site analysis-based clonal tracking. Notably these brain-derived transplantable HSPCs were able to generate, both in primary and secondary recipients, a CNS progeny reminiscent of the morphological and functional features of microglia. Overall, our data indicate that cells with microglia progenitor (μP)-like features exist within the bone marrow HSPCs pool and can replace CNS resident μP in defined experimental conditions, retaining their functional features upon long-term engraftment into the brain.

P279

Optimized bicistronic lentiviral vectors to correct β-hexosaminidase deficiency in neural and hematopoietic stem/progenitor cells and progeny

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Tay-Sachs (TSD) and Sandhoff disease (SD) result from mutations in either the HEXA or HEXB genes encoding respectively, the α- or β-subunits of beta-hexosaminidase A (HexA, $\alpha\beta$), the only Hex isozyme that can hydrolyze GM2 ganglioside. GM2 gangliosidoses currently lack a treatment. A major impairment to establishing gene therapy approaches for these diseases is the need to synthesize the α- and β-subunits at high levels and with the correct stoichiometric ratio. Here, we report the generation and in vitro validation of novel bicistronic LVs encoding for both the murine and human codon optimized Hexa and Hexb genes. We show that these LVs drive the safe and coordinate expression of the α- and β-subunits, leading to supranormal levels of β-Hex activity with prevalent formation of a functional HexA in SD murine neurons and glia, bone marrow-derived murine HSPCs, and human SD fibroblasts. The restoration/overexpression of β-Hex leads to the reduction of intracellular GM2 ganglioside storage in transduced and in cross-corrected SD murine neural progeny, demonstrating that the transgenic enzyme is secreted and functional. Importantly, bicistronic LVs safely and efficiently transduce human neurons/glia and CD34+ HSPCs, which are target and effector cells, respectively, in prospective in vivo and ex vivo GT approaches. We anticipate that these bicistronic LVs may overcome the current requirement of two vectors co-delivering the two subunits. Careful assessment of the safety and therapeutic potential of these LVs in a severe SD murine model will pave the way to the clinical development of LV-based gene therapy for GM2 gangliosidosis.

P280

Expression of Igf2 and its binding proteins in the hippocampus during memory formation

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Insulin-like growth factor 2 (Igf2) is the protein hormone in the structure similar to insulin. The biological role of Igf2 has been studied for a long time because it has a powerful mitogenic effect and plays an important role in various physiological processes. In recent years, special attention is paid to its role in nervous system during postnatal period. To study Igf2 role in memory formation we used a model of contextual fear conditioning with consequent real-time PCR analysis of target genes expression. The experiments were carried out on male c57black/6 mice and supported by RSF grant 18-75-10112. Three time points after training were selected for analysis - 1, 4 and 24 hours. Three groups were formed: passive control (mice that were not trained and were in the home cage before sample collecting), an experimental group - animals that were subjected to electric stimulation in training chamber, and active control – without electric stimulation. We reveled a decrease in the expression of Igf2 mRNA in the experimental group at 4 h after training compared with passive control in dentate gyrus but not in CA1 field of hippocampus. In addition, we found an increase of the Igfbp6 mRNA level 4 hours after training in the CA1 field of hippocampus but not in the dentate gyrus. Obtained data indicate that the expression of Igf2-related genes is regulating by different ways in dentate gyrus and CA1 field of hippocampus during memory formation.

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P281

Targeting neurodegeneration using lentiviral gene therapy

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The treatment of neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, Huntington's disease and motor neuron disease, remains challenging due to the lack of effective therapeutic strategies and the side effects linked to systemic drug administration. In most cases, treatments aim at managing the symptoms rather than the cause of the disease itself and require life-long prescription. Lentiviruses are a class of retroviruses that can efficiently integrate large amounts of genetic material into the host genome. At Oxford Biomedica, we have developed vectors based on the lentiviruses for efficient gene transfer to the central and peripheral nervous systems. This platform features include high capacity and broad cell tropism, which are both advantageous for gene therapy aimed at neurodegenerative disorders. We have observed to date that the vectors are generally well-tolerated with no reports of significant safety issues and that they deliver long-term gene expression in both the brain and eye in clinical trials. In addition, our platform is applicable to multiple neurological indications by using different envelope proteins and injection sites to target various central nervous system regions, including striatum and spinal cord. This allows the development of targeted gene therapies for neurodegenerative indications, including Parkinson's disease, amyotrophic lateral sclerosis and spinal cord injury. Herein, we discuss the therapeutic applications of lentiviral vectors and present pre-clinical proof-of-concept data highlighting the potential for the translation to the clinic of the gene therapy for neurodegenerative diseases in the near future.

P282

Processing of IGF-II in rat and mouse brain

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It was shown in several studies that IGF-2 injection as well as the viral delivery of this gene to nervous system can improve memory in animal models of the Alzheimer's disease and aging-related memory impairments (Pascual-Lucas et al., 2014; Steinmetz et al., 2016). However, Igf2 expression in nervous system and its processing in cells are not fully understood. According to previous data mature form of Igf2 has molecular weight 7,5 kDa. We studied the proteins from cortex and hippocampus as well as from neuronal culture of rats and mice by western blot analysis with specific antibodies. This study was supported by RSF grant 18-75-10112. We have shown the presence of about 15 kDa and higher products from neural tissues but not 7,5 kDa while analysis of cell HEK293 cell lysate demonstrated the presence of mature IGF-2. This data is consistent with the results of other groups. We propose that 15 kDa IGF-II corresponds to the protein referred as ''Big IGF-II'' in the article of Qiu at al. (2005) and this protein is product of incomplete processing of pre-pro-IGF-2. For further IGF-II processing analysis lentiviral vector for Igf2 CDS overexpression of as well as two shRNA-containing vectors were constructed. We expect that obtained genetic constructs will help us to clarify the issue about IGF-2 processing and its biological role in the brain.

P283

DNA cytosine methylation contributes to long-term memory maintenance in snails

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According to recent studies, alterations in chromatin structure and DNA methylation can regulate the cellular and molecular mechanisms underlying fear memory consolidation and reconsolidation processes in snails. In the present study, supported partially by Fund 19-75-10067, we investigated whether inhibition of DNA methyltransferase activity affects the contextual fear memory maintenance in snails. We used a contextual fear memory model. For training, animals were placed in the training context and shocked twice per day for 10 days. For testing, we estimated the percentage of maximal tentacle withdrawal in two contexts: on the ball - context in which animals were shocked, and on the flat glass. The DNA methyltransferase inhibitor RG108 was diluted in a sterile saline to the concentration of 2 μM. and 0.1 ml RG108 was injected into the animals. In the first experiments, we found that after training the RG108 treatment without the reminder reduced withdrawal amplitude almost to the non-trained values. Testing session next days demonstrated that memory impaired by RG108 injection was reinstated. Since memory reactivation had a rescue effect on contextual memory, we decided to investigate the joint action of DNMTs inhibitor RG108 and the reminder. Application of RG108 1 hour before the reminder didn't affect the withdrawal response amplitudes. Thus, inhibitor of DNMTs activity impairs the maintenance of contextual fear memory in retrieval-dependent manner – there is no effect when DNMTs inhibition occurs in the presence of memory reactivation. Results support the idea of DNA methylation involvement in maintenance of long-term contextual memory of Helix.

P284

Morphological evaluation of local hypothermia effects after spinal cord injury

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A spinal cord injury (SCI) takes 8% of the total injuries. Pathological processes after SCI are accompanied by a number of complications distal to the place of damage. One of the methods to reduce the area of secondary alteration and prevent complications after injury is applying of local hypothermia. In this study Wistar rats were divided into 3 groups (laminectomy with hypothermia, laminectomy with contusion injury, laminectomy with contusion injury and hypothermia). The damage was caused by a shock rod falling on the open spinal cord at the Th8 level. Animals

were euthanized on 6 and 24 hours after SCI. 8 micron-thick cryosections were stained with hematoxylin and eosin. Mean cross-sectional area of cavities like defects was counted in posterior horns and white matter at the L2 level. No defects were revealed in the group without contusion at the L2 level. The morphological changes were less dramatic in the group with hypothermia. The size of defects was less both in the white matter and in the posterior horns in the group of contusion injury with hypothermia (142,39 ± 68,97 μm2 vs. 891,641 ± 1256,9 μm2 group without hypothermia after 24 hours for white matter, 65,18 ± 135,56 μm2 and 74,39 ± 60,81 μm2 for posterior horns, respectively). Obtained data approve a favorable effect of low temperatures on the posttraumatic period and can serve as supplementary treatment to the gene and cell therapy. The study was carried out with the financial support of the Russian Foundation for Basic Research in the framework of the research project No. 17-04-01746.

P285

Pharmacological protection of the spinal cord in the first hours after injury

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In the first minutes and hours after a spinal cord injury, the administration of the methylprednisolone stabilizes cell membranes, reduces swelling and inflammation of the nervous structures. Starting treatment with methylprednisolone is recommended during the first 6 hours after spinal cord injury. This work was supported by the Russian Foundation for Basic Research under grant №18-315-00267. All stages of study were performed in compliance with ethical and legal norms. The spinal cord injury at the level of 8–9 thoracic vertebrae was applied by the modified method of A. Allen. Methylprednisolone was used as a pharmacological therapy for one hour after the injury. The samples of blood were taken from rat hearts by cardiac puncture in 6 hours after SCI. Caspase-3, Bcl markers were used for analysis of apoptosis. The analytic method western blot with specific marker of autophagy LC3B, p62 was used for identification of autophagosome. The α-tubulin was used as loading control. The apoptosis regulator Bcl-2, Caspase-3 and the autophagy marker LC3 did not appear after injury. Protein p62 accumulated without interacting with LC3, which indicates the incompleteness of autophagy. The presence of procaspases after using methylprednisolone increased to (7.319 ± 0.911, $P < 0.050$) after 6 hours after SCI compared with the group of injured animals (2.075 ± 0.621). It can be assumed that methilpredenesalone played a protective role for cells in response to DNA damage after injury. This suggestions seemed promising and requires further study.

P286

Multiple sclerosis hematopoietic stem cell gene therapy: Promoting remyelination with guidance molecules

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Remyelination failure in chronic multiple sclerosis (MS) lesions is frequently associated with presence of scarce undifferentiated oligodendrocyte progenitor cells (OPCs; the remyelinating cells of the central nervous system (CNS)), which suggests the exhaustion of the local OPC pool followed by inefficient recruitment and differentiation. We have previously demonstrated that the expression of guidance molecule Semaphorin (Sema) 3F increases OPC recruitment to the areas of demyelination and, consequently, myelin repair in a murine model of demyelination, suggesting this molecule as an attractive pharmacological target. However, successful therapeutic targeting to MS lesions is extremely challenging as these are disseminated throughout the CNS.

We hypothesized that gene therapy targeting haematopoietic stem cells (HSCs) may lead to efficient delivery of selected molecules to the areas of CNS demyelination via inflammatory cells (bone marrow-derived monocytes/macrophages). Hence, we used a lentiviral vector encoding Sema 3F and green fluorescence protein (GFP) tag to transduce HSCs in vitro, and then intravenously injected these cells into mice in which myeloablation was achieved using busulfan. Two months following engraftment, focal demyelinating lesions were induced by lysolecithin (LPC) injection in the spinal cord of chimeric mice. Infiltration of GFP-positive (transduced) cells in the lesions was observed as early as 3 days post lesion (dpl), suggesting this therapeutic strategy was relevant for early stimulation of OPC recruitment. Infiltrating cells were predominantly macrophages. Lesions in mice engrafted with Sema3F-GFP- transduced HSCs showed enhanced OPC recruitment compared to those in mice engrafted with control, GFP-transduced HSCs. This increased recruitment was followed by accelerated OPC differentiation into CC1+ mature oligodendrocytes. Our results represent a proof of concept that a combination of gene therapy and HSC transplantation allows to target a given cue "at the right time and at the right place" after demyelination, which might be crucial for efficient stimulation of myelin repair. In addition, this study confirms Sema 3F as a promising candidate for pro-remyelinating strategies in MS.

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CL-participation to advisory boards for Roche, Biogen, Merck-Serono, Genzyme, Vertex, Rewind; scientific collaboration with Vertex and Merck Serono

NC advisory board of Lysogene, BrainVectis

P287

Neuronal differentiation-specific surface antigen expression for tracing *in vivo* implanted stem cells

ABSTRACT WITHDRAWN

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P288

Generation and differentiation of an optogenetic human neural stem cell for Parkinson's disease

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder which involves a selective and progressive loss of dopaminergic neurons and decreasing dopamine levels. Available therapies only transiently ameliorate the symptoms without curing or blocking its progression. Preclinical research and clinical trials in the last decades showed the possible clinical benefits with cell therapy using dopaminergic neuron precursor or neural stem cells. However, the ability to control the biology and function of the transplanted cells is still a breakthrough to achieve. To address this issue a new cell line was designed with the potential to produce dopaminergic neurons than could be functionally controlled and regulated by light. v-myc immortalized human neural stem cells from ventral mesencephalon overexpressing BclXL (Hi-BclXL-hVM1 cell line, Courtois et al, JBC 2010) were genetically modified to express channelrhodopsin-2. Several clones were selected to identify a cell line that expressed the transduced vector retaining neurogenic potential. Immunocytochemistry and western blot analysis revealed that, after differentiation induced with GDNF and cAMP for 2–3 weeks, leads to heterogeneous cell culture with neurons expressing markers of different maturation stages (ß-III-tubulin, MAP-2, synapsin-1) and dopaminergic neurons (TH, Nurr1). Electrophysiology studies confirmed the presence of immature neurons with a resting membrane potential of -75 ± 5mV, moreover, upon blue light stimulation (440–500nm) the depolarization of immature neurons was recorded. In conclusion, the present optogenetic human neural stem cell line constitutes a useful tool for future in vitro and in vivo studies and to help understand the fundamentals of dopaminergic neurogenesis and grafting in PD.

P289

Investigation of microglia cells behavior in spinal cord injury of varying severity

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Microglial cells are known as important mediators of inflammation and immune response in the central nervous system (CNS). M1 and M2 are often considered as two main subsets of microglia in damaged tissue. Phenotypes and functions of microglia in the damaged spinal cord are dynamic and changing depending on the microenvironment during SCI. A possible contribution to the development of microglia responses dichotomy is the degree of nervous tissue damage. In this regard, the purpose of our research, supported by Russian Foundation for Basic Research (to E.R. Akhmetzyanova)(Grant 18-34-00141), was to study the behavior of microglial cells after spinal cord injury of varying severity. After laminectomy, all experimental animals suffered spinal cord injury of varying severity: low - 1.5 m/s, moderate - 2.5 m/s and severe - 4 m/s using the Leica Impactor One for Reproducible Neurotrauma. To study the behavior of microglial cells at different values of spinal cord injury on days 3 and 60 after in vitro damage, we carried out a multiplex analysis of blood serum of injured animals. It has been shown that different conditions can change cytokine profile of spinal cord. Analysis of PCR-RT also brought changes in the expression of genes in microglia cultured under different conditions. Thus, on a model of spinal cord injury varying severity and different duration in vitro, a significant change in the behavior of microglial cells is shown. The results can serve as a basis for the further development of approaches to the stimulation of neuroregenerative processes in nervous tissue.

P290

Isolation of germinal matrix neural stem cells from the cerebrospinal fluid of preterm infants with intraventricular hemorrhage

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Intraventricular hemorrhage is a common cause of morbidity and mortality in premature infants. The rupture of the germinal matrix into the ventricles entails loss of neural stem cells and disturbs the normal cytoarchitecture of the ventricular zone, compromising the organization of the ependymal lining and late neurogliogenesis. Here we demonstrate that neural stem cells can be easily and robustly isolated from the hemorrhagic cerebrospinal fluid obtained during therapeutic neuroendoscopic lavage in severe intraventricular hemorrhage patients. Our analyses demonstrate that these neural stem cells, although similar to fetal ones, display distinctive hallmarks related to their regional and developmental origin in the ganglionic eminences of the ventral forebrain that give rise to cortical interneurons and oligodendrocytes. In addition to regional transcription factors and markers of radial glia, we describe the expression of several membrane proteins, which are maintained

upon purification of the CD133 positive fraction. This novel source of neural stem cells poses no ethical concerns, as the fluid is usually discarded, and could be useful for the development of an autologous therapy for preterm infants, aiming to restore late neurogliogenesis and attenuate neurocognitive deficits. Furthermore, these cells represent a valuable tool for the study of human germinal matrix biology and the final stages of human brain development.

P295

Ocular gene therapy with a synthetically engineered AAV enhances visual function in a pre-clinical model of Leber congenital amarousis

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The long-term follow-up data that has recently emerged from Leber congenital amarousis-2 (LCA2) patients treated with AAV2-RPE65 vectors, highlights the need for high transduction vectors that will permit their administration at significantly low doses to augment visual function. Based on our observation that cellular SUMOylation is inhibitory to AAV transduction, we reasoned that a viral vector engineered to escape this post translational modification may be advantageous. After rational designing and molecular engineering of putative SUMOylation sites in AAV2 capsid, we evaluated its transduction efficiency in ARPE19 cells. A SUMO-site modified AAV2-S, demonstrated a 110% increase in transduction in comparison to wildtype (WT) AAV2 vectors. We further observed that the cellular entry profile was similar between AAV2-S and AAV2-WT vectors. The novel vector was then assessed by in vivo ocular gene transfer with different transgenes (EGFP and RPE65), delivery routes (intravitreal and sub-retinal) and dose of administration (low: 1 to 3x10e8 or high: 7x10e8 vector genomes). AAV2-S-EGFP vectors administered in C57BL6/J mice by either intravitreal and sub-retinal route (n = 8 eyes) demonstrated an enhanced transduction, eight weeks after gene transfer by in vivo fundus imaging. Furthermore, AAV2-S-RPE65 vectors when injected subretinally, at a dose of 7x10e8 vector genomes in rd12 mice (n = 8 eyes), demonstrated a significant visual correction by electroretinography, 16 weeks after gene therapy. These findings were further corroborated by immuno-staining for RPE65 protein in the murine retina. Our pre-clinical data highlights the translational potential of SUMO-site engineered vectors for retinal gene therapy in patients with LCA2.

P296

A mTOR-inhibiting shRNA delivered intravitreally via rAAV tested as a potential anti-angiogenic gene therapeutic in a rat model of oxygen-induced retinopathy

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Currently-used treatments for the wet subtype of age-related macular degeneration (AMD) suffer from limitations including safety concerns regarding the long-term suppression of VEGF and frequent intravitreal injections negatively affecting patient compliance. To address both issues, we explored the potential of a gene therapy targeting mechanistic target of rapamycin (mTOR), which is involved in a number of cellular processes associated with the pathophysiology of the multifactorial AMD. Sprague-Dawley pups were used to generate the oxygen-induced retinopathy model, widely used for angiogenic ocular conditions, and were intravitreally administered with a rAAV expressing a mTOR-inhibiting shRNA (rAAV2-shmTOR-GFP) upon being returned to normoxia. Fluorescein angiography was used to determine the in vivo effects of mTOR inhibition. rAAV2-shmTOR-GFP (4.28 ± 2.86, $p = 0.00103$) treatment significantly reduced the extent to which neovascularisation occurred, as percentage of total retinal area, when compared to model animals that were untreated (20.95 ± 6.85), mock-treated (14.50 ± 2.47), or injected with a control shRNA-containing virus vector (16.64 ± 4.92). Quantifications of retinal avascular area and vessel tortuosity further suggest the therapeutic effectiveness of rAAV2-shmTOR-GFP as a wet AMD treatment, while a TUNEL assay and immunostaining transverse retinal sections for macrophages with anti-F4/80 showed that the virus vector has anti-apoptotic and anti-inflammatory properties, respectively, as well. Therefore, in addition to the procedural convenience that rAAV-delivered therapeutics offer, the ability of mTOR inhibition to address multiple aspects of the condition demonstrates the strong promise of rAAV2-shmTOR-GFP as a gene therapy for wet AMD and potentially other neovascular retinal diseases.

P297

Delivery of CRISPR/Cas9 using AAV-PHP.B in the inner ear leads to allele-specific inactivation of the mutated Tmc1 allele and protects auditory function in Beethoven mice

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Hearing Loss (HL) is the most prevalent sensorineural disorder, affecting 360 million people worldwide. As genetic causes lead to 50% of pre-lingual deafness, gene therapy is considered as a potential therapeutic strategy. Mutations in the TMC1 gene are linked to both autosomal dominant (DFNA36) and recessive (DFNB7/11) forms of non-syndromic HL. DFNA36 is caused by a TMC1 point mutation (p.M418K) and characterized by a post-lingual form of HL, with an onset in the mid-teen years, which leaves a window for therapeutic intervention. The natural disease progression is replicated in the heterozygous Tmc1Bth/+ Beethoven mice, a useful model for the testing of gene therapies. Here, we develop an AAV-based allele-specific gene targeting approach using the CRISPR/Cas9 technology to selectively disrupt and knock-out the Bth-Tmc1 allele. To deliver this system to the neonatal inner ear, we used the AAV-PHP.B vector, which showed effective targeting of both inner and outer hair cells throughout the mouse cochlea (>90% efficacy). This transduction efficacy allowed us to consider a double vector approach, with one vector used to express the spCas9 nuclease whereas a second vector was co-injected to express the gRNA selectively targeting the Bth-Tmc1 allele. The results obtained in the Tmc1Bth/+ mice showed long-lasting improvement of the auditory function, with a significant protection of the auditory brainstem response and the distortion product otoacoustic

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emissions. Furthermore, the treated mice showed a significantly increased startle reflex response. Altogether, these results support the use of AAV-based gene therapy to target genetic defects in hair cells leading to HL.

P298

DNA sequence alterations at an I-SceI target site in murine photoreceptors following AAV mediated gene transfer

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DNA sequence alterations occurring during the repair process after DNA double strand break (DSB) induction are manifold and uncontrolled so far. The purpose of this study was to characterize the DNA sequence alterations following a DSB repair on the X chromosome in photoreceptors in vivo. Two month old mice ((B6J.SV129-Rpgrtm1stie) were used containing an Isce-I target site on the X chromosome. A total of 4 different AAV-vectors (serotypes 5 and 8) were applied that contain the ISce-I endonuclease linked by a T2A linker with the GFP cDNA with or without a template DNA of the target region. Upon euthanasia, eyes were harvested, retinae isolated, and the GFP-positive cell population enriched by FACS. PCR fragments were subjected to T7 surveyor assay and subsequently Sanger sequencing. Analysis of the DNA at the target site revealed DNA-repair activity in all injected eyes. A high frequency of small insertions, deletions, and single nucleotide substitutions (between 10 and 30% of all clones) was observed. Larger DNA sequence modifications were also found, but to a much lesser extent. In retinae injected with an all-in-one vector containing a template in addition to the nuclease, we detected replacement of the I-SceI by a HindIII site (present on the template) with low frequency. In this study, we successfully induced in vivo genome editing in photoreceptors and detected not only NHEJ, but also HDR, albeit at low frequency. These data represent the basis for further studies regarding the occurrence of DNA sequence changes at target sites in retinal neurons.

P299

Long-term efficacy of rhodopsin knockdown and replacement gene therapy in a canine model of autosomal dominant retinitis pigmentosa

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Long-term efficacy of a self-complementary AAV2/5 vector containing an shRNA (shRNA820) under the control of H1 promoter designed to target a homologous region of both human and dog rhodopsin (RHO), and a resistant codon-modified human RHO cDNA (RHO820) under control of a human opsin (HOP) promoter was evaluated in 4 RHO T4R/+ dogs. Testing employed a 1 min light exposure (LE) protocol that causes rapid loss of rod photoreceptors in this model. Following subretinal injection of scAAV2/5-shRNA820-RHO820 (0.15 mL; 5E+11 vg/mL) in the right eye and BSS in the left eye, dogs underwent LE every 12 weeks over the course of 48 weeks. Regular non-invasive retinal imaging by cSLO/OCT and electroretinographic examinations were performed until termination (Wk 50). Eyes were then processed for IHC to examine ONL thickness and structure of rods and cones in treated and untreated areas. Following the 1st LE event, severe ONL thinning was seen 2 weeks later except in the AAV-treated areas, where the ONL was preserved. Similar observations were made after the 2nd, 3rd, and 4th LE events. Full-field ERG assessment showed a positive effect of scAAV2/5-RHO820-shRNA820 on retinal function. Qualitatively and quantitatively, AAV-treated eyes showed better rod- and cone-mediated function than the contralateral vehicle/BSS injected eyes. The functional preservation conferred by scAAV2/5-RHO820-shRNA820 was detectable for up to Wk 50 post-injection. Histological/IHC assessment of AAV-injected eyes confirmed the in vivo OCT findings by showing preserved ONL and retained rod and cone IS and OS structure that was limited to the treated area.

P300

Optogenetics in the clinic: PIONEER, a phase 1/2a, open-label, non-tandomized, dose-escalation study to evaluate safety and tolerability of GS030 in subjects with retinitis pigmentosa

ABSTRACT WITHDRAWN

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P301

Splice modulation therapy for a variety of ABCA4 mutations underlying Stargardt disease

ABSTRACT WITHDRAWN

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P302

Next-generation AAV variants for retinal gene therapy

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Inherited retinal dystrophies (IRD) are, so far, irreversible blindness disorders that affect millions worldwide. They are caused by the absence of individual genes specific to photoreceptors and retinal pigmented epithelium (RPE). This renders IRDs ideal for gene therapy. The most successful applications utilise recombinant adeno-associated virus (AAV) vectors administered via subretinal injection, since natural AAV serotypes require direct contact with their target retinal cells and cannot spread in depth. To overcome this, novel AAV vector variants are being engineered via capsid diversification strategies. Using directed evolution, we have developed two novel capsid variants, herein termed AAV2-GL and AAV2-NN, using AAV2 peptide display libraries carrying random 7-mer insertions flanked by 2–3 linker amino acid (aa) at position 587 of the VP1 protein. With these novel capsids we have achieved fast-onset panretinal expression of a GFP-reporter in mouse photoreceptors after a single intravitreal injection of 2 x 109 total viral genomes. This efficiency was also recorded in retinae of larger animal models, namely canine and non-human primate. Human retinal explants exposed to AAV2-GL/-NN were also successfully transduced with the GFP-reporter detected in all cell layers including photoreceptors. Moreover, both novel capsids showed lower sensitivity for neutralizing antibodies and were easy to produce at high yield. These next-generation AAV capsids expand our current toolbox in retinal gene therapy to battle IRDs.

P303

Towards a clinical trial of gene therapy for Usher syndrome type IB retinitis pigmentosa

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Usher syndrome type IB (USH1B) is a blinding disease caused by biallelic mutations in MYO7A whose coding sequence exceeds the cargo capacity of adeno-associated viral (AAV) which is the preferred vector for retinal gene therapy. Our group has developed dual AAV vectors which upon subretinal administration reconstitute the expression of MYO7A and rescue retinal defects in the Shaker-1 mouse model of USH1B. We received support from the European Union (H2020-SC1 EU Grant, N. 754848 - UshTher) to test the safety and efficacy of this approach in patients with USHIB. Dual AAV8-MYO7A produced under good manufacturing (GMP)-like practices will be tested in Shaker-1 mice and in non-human primates (NHP). During the AAV production process, we found a contaminant vector resulting from recombination between two homologous sequences in the AAV-5'MYO7A vector. This was removed by changing one of the two sequences while maintaining the same MYO7A expression levels in vivo. An initial dose response study in Shaker-1 with research-grade vectors has been performed to define the doses to be used in humans, and this will be confirmed with the GMP-like material. We set at 1 week post-injection the early time-point of the 3-month non-clinical study in NHPs based on an in vivo MYO7A expression onset experiment, and confirmed the tolerability of dual AAV8-MYO7A subretinal administration in NHP in a small pilot study. Overall the results of these studies are crucial for the clinical translation of dual AAV vectors for gene therapy of USH1B retinitis pigmentosa.

P304

The shorter the better? Assessing novel therapeutic strategies for CRB1-associated retinal disease

ABSTRACT WITHDRAWN

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P306

Development of a gene supplementation therapy for PDE6A-linked retinitis pigmentosa

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Retinitis pigmentosa type 43 (RP43) is a inherited retinal disorder caused by mutations in the PDE6A gene. RP43 is characterized by night blindness at an early age, progressive constriction of visual fields, a decrease in visual acuity and, in many instances, progression to legal blindness. We developed rAAV8.PDE6A a recombinant adeno-associated virus (AAV) vector for gene supplementation therapy of RP43. rAAV8.PDE6A expresses full length human PDE6A under control of the human rhodopsin promoter and was packaged with AAV8 capsid. We tested the vector for efficacy in two preclinical models of RP43, the Pde6aD670G mouse and the Pde6a-mutant dog model. In both models rAAV8.PDE6A was administered by subretinal injections and short-term and long-term outcome measures were performed in vivo and ex vivo. Functional and structural rescue was monitored by vision testing, electroretinography (ERG) and spectral domain-optical coherence tomography. rAAV8.PDE6A delivered into the subretinal space led to efficient and stable PDE6A transgene expression and biological activity as determined by immunohistochemistry and/or electroretinography in both animal models. rAAV8.PDE6A resulted in significant rescue of rod function and preservation of cone function as well as retinal structure over the 2-year dog study period. Importantly, the treatment was also efficacious when delivered to late-stage of disease dogs. A GLP safety study in non-human primates confirmed safety of the approach and revealed only limited biodistribution and minimal signs of inflammation. A first in man phase I/II clinical trial focusing on safety and efficacy of rAAV8.PDE6A in patients with RP43 was approved and will be initiated in 2019.

P307

Developing a CRISPR/Cas9 editing approach for the treatment of USH2A-related inherited retinal degeneration

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The most common cause of both Usher syndrome type II (USH2) and nonsyndromic autosomal recessive retinitis pigmentosa (arRP) are mutations in the USH2A gene; these diseases are also known as USH2A-related inherited retinal degeneration (IRD). A single nucleotide deletion in exon 13 (c.2299delG, p.Glu767fs) is the most common mutation in the USH2A gene in the United States. This mutation causes a premature termination codon and a truncated Usherin protein, resulting in loss of protein function. Previous research showed that removal of exon 13 of USH2A results in a functional in-frame protein. Since the size of the USH2A cDNA is too large for AAV-mediated delivery, we are developing an editing approach to treat USH2A-related IRD through CRISPR/Cas9-mediated excision of USH2A exon 13. The optimal guide RNA pair cutting within introns 12 and 13 of USH2A was chosen based on excision rates of exon 13 and on specificity, which was evaluated utilizing three orthogonal methods. Editing with our lead gRNA pair in a cell line that expressed USH2A resulted in up to 60% expression of USH2A ￼delta 13. Moreover, DNA editing and expression of USH2A ￼delta 13 mRNA was observed in human retinal explants, a relevant target tissue, after AAV-mediated delivery of CRISPR/Cas9. These results support the further preclinical development of CRISPR/Cas9 therapies for c.2299delG-associated USH2A-related IRDs.related IRDs.

P308

Set up of a tissue-engineered product consisting of RPE derived from human embryonic stem cells cultured on human amniotic membranes for the treatment of retinal diseases

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Retinitis pigmentosa (RP, a group of rare hereditary disorders) and age-related macular degeneration (AMD) are the main causes of blindness in developed countries. Stem cell-based therapy represents an alternative approach to treat these disorders. The retinal pigment epithelium (RPE) is a monolayer of cuboidal post-mitotic cells, localized between photoreceptors and the choriocapillaris. The structural and functional interaction between the RPE and the

photoreceptors is essential for the maintenance of visual function. RP and AMD could be caused by malfunction or degeneration of this RPE cell layer. Therefore, the goal is to replace RPE cells to achieve a therapeutic benefit. We have developed a tissue-engineered product (TEP) that consists of RPE derived from human embryonic stem cells (hESC) cultured on a denuded human amniotic membrane (hAM). We have optimized and transferred a differentiation protocol to generate RPE cells from clinical grade hESC into a GMP facility. To evaluate the feasibility and safety of the TEP transplantation, the hESC-RPE sheet was implanted in the sub-retinal space in non-human primates and immunocompromised rodents. The localisation-position of TEP remained stable without evidence of degradation in the macula region. Finally, our hESC-RPE sheet will be used for phase I/II clinical trials.

P309

Subretinal injection of rAAV2/8 vector induces dose-dependent retinal alteration and systemic anti-transgene T-cell response similarly in wild type and pathophysiological murine models

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From the first human clinical trials in 2007, recombinant adeno-associated virus (rAAV)-mediated ocular gene therapy has shown successful results. Recently, some clinical trials have reported that gains in visual function declined between 2–6 years after initial treatment. The involvement of antitransgene immune responses may contribute to loss of therapeutic efficacy. This prompted us to evaluate in wild type and ocular pathophysiological murine models whether subretinal rAAV gene transfer leads to an anti-transgene immunization. Here, we characterized the local and systemic immune responses following a co-injection of rAAV2/8 and HY peptides subretinal gene transfer. The transgene expresses the HY male antigen which contains MHC class I and class II-restricted T-cell epitopes, that are immuno-dominant in H-2b female mice. Two μL of endotoxin-free, PBS-formulated rAAV2/8-PGK-GFP-HY with or without HY peptides were injected subretinally in C57Bl/6J or rd10 female mice. At day 14, mice were challenged subcutaneously with the HY peptides adjuvanted in CFA, and the immune responses analyzed at day 21 revealed that: (i) a high dose of rAAV2/8 vectormediated subretinal gene transfer affects the retinal integrity in C57Bl/6J mice, (ii) the injection of rAAV2/8 vector-mediated subretinal gene transfer can trigger a systemic anti-transgene T-cell response in both mice models, and (iii) it seems that retinal degeneration in rd10 mice does not amplify the anti-transgene response. However, a direct T-cell involvement in the retinal alteration is under investigation and will have to be taken into account for biosafety and the long-term efficacy of rAAVmediated ocular gene transfer.

P310

Engineered AAV vector for improved recovery of hearing loss

ABSTRACT WITHDRAWN

P311

Towards the gene therapy for FAM161A associated retinitis pigmentosa in a murine model

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Defects in the ciliary protein FAM161A have been proven to cause autosomal recessive retinitis pigmentosa (RP28). Currently, no effective cure is available. In order to develop a potential gene augmentation therapy, we have constructed adeno associated virus (AAV) vectors to drive photoreceptor-specific expression of wildtype FAM161A cDNA. Following vector administration by subretinal injection, we validated gene expression and retinal functionality in a Fam161a-/- murine model. Human FAM161A proteins could be expressed in mouse retina and localized preferentially to the connecting cilium of photoreceptors. However, mislocalization of FAM161A proteins to the out nuclear layer (ONL) was detected when high dose of AAV vectors were applied. Injection performed early in age (P6) leaded to broader transduced retinal area but also inclined to cause retinal detachment. AAV transduction resulted in mild but persistent improvements in electroretinogram (ERG) up to three months post injection. Further morphological and functional effects are currently under evaluation. Our preliminary results implied an initial therapeutic effect when FAM161A expression is restored in photoreceptors.

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P312

AAV2.7m8 is a powerful viral vector for inner ear gene therapy

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Background: Adeno-associated viruses (AAVs) are commonly used for inner ear gene delivery. While conventional AAVs are capable of transducing inner hair cells (IHC) in the cochlea to varying degrees, outer hair cells (OHC) and supporting cells are transduced less efficiently. In this study, we examined the transduction patterns of two synthetic AAVs (AAV2.7m8 and AAV8BP2) in the neonatal mouse inner ear. Methods: Neonatal (P0-P5) CBA/J mice were used in this study. Synthetic AAV-GFPs were injected into mouse inner ear using the posterior semicircular canal approach. Immunohistochemistry was used to assess the infection efficiency. Auditory function was assessed by auditory brainstem responses (ABR). Results: AAV2.7m8 transduced both IHCs and OHCs with very high efficiency. AAV8BP2 transduced the IHCs with high efficiency, but the transduction efficiency of OHCs was lower. AAV2.7m8 also transduced a subset of supporting cells (inner pillar cells and inner phalangeal cells) with high efficiency. Mice that underwent AAV2.7m8 injections had similar ABR thresholds compared to non-injected controls. Conclusions: AAV2.7m8 transduced both IHCs and OHCs with high efficacy. In addition, it transduced inner pillar cells and inner phalangeal cells with high efficacy. Our results suggest that AAV2.7m8 is an excellent viral vector for inner ear gene delivery.

P313

ABCA4 gene dysfunction in Stargardt retinal disease: charcaterisation of two new rat transgenic models

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The photoreceptor-specific ATP-binding cassette transporter (ABCA4) gene encoding the ABCR protein, serves a crucial role in preventing the build-up of the toxic by-products that result from phototransduction. Localized at the rim of the outer segment (OS) discs, ABCR transports All trans retinal (ATR) that may become trapped in the lumen of the OS disc to the cytosol of the photoreceptor cell where it can be appropriately incorporated into the visual cycle. In the dysfunction of ABCA4 as seen in Stargardts disease (STGD1) dimers of ATR may accumulate in the OS discs forming toxic reactive products that may ultimately lead to retinal cell death. Upwards of 1000 mutations have been identified in the ABCA4 gene and these mutations impact the transport efficiency of the protein and the health of the retina to varying degrees. We have generated and analysed two rat transgenic models of STGD1, one with a knockout of Abca4 and the second carrying the most common mutation found in STGD1 - G1961E. The Abca-/- rat displays a minimal disruption to visual function despite having significant increases in a number of potentially toxic bisretinoid species. The Abca4E/E rat shows no increase in bisretinoid levels however the ATPase and flippase functions of the abca4E/E protein from this model is inhibited in the presence of excess vitamin A. This sheds some light on the compound heterozygosity described for this polymorphism in STGD1. Both models are useful for testing novel therapeutic approaches for STGD1 disease.

P314

Gene therapy to increase and maintain light sensitivity in cones during retinal degeneration

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Rod-cone dystrophy (RCD) is a heterogeneous group of inherited retinal diseases. The majority of RCD mutated genes are expressed in the rod photoreceptors and in the retinal pigment epithelium. Yet, the phenotype is the same and is characterized by the degeneration of rods followed by degeneration of peripheral cones, which leave the patients with tunnel vision in mid stages and blindness in the latest stages of disease. A previous study showed that halorhodopsin, a microbial chloride pump, expressed in mice cones, restored these cells' activity albeit with high light intensities for activation due to the lack of intracellular signal amplification. In order to develop a light sensitive cone reactivation strategy, we first examined the expression of the phototransduction cascade elements in cones during degeneration in RCD mouse models and patients. We found that opsin and arrestin migrate to the cone cell bodies after outer segment loss. We thus hypothesized that cone reactivation based on cone opsin signalling may be feasible which in turn will allow us to recover high sensitivity vision. The ectopic expression of a membranehyperpolarizing target channel activated by G proteins recruited by cone opsin in degenerating cones improved visual function in two RCD mouse models. In RCD patients, we found the same phenotype as in the mouse models ensuring a possible clinical translation. This new approach has the potential to restore, for the first time, high acuity and color vision requiring only low light intensities.

P315

Ex vivo efficacy study to evaluate neuroprotection in a retinal organotypic culture system: A gene therapeutic strategy to deliver growth factors to the retina to treat avascular age-related macular degeneration

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The pathogenesis of avascular age-related macular degeneration (aAMD) appears to include a reduction in neuroprotective factors, oxidative stress and inflammatory reactions. We have hypothesized that, to prevent retinal cell degeneration in aAMD, it is essential to restore the neuroprotective environment in the retina, which can be achieved by the subretinal transplantation of autologous iris pigment epithelial (IPE) cells that have been genetically modified to overexpress the neuroprotective factors pigment epithelium-derived factor (PEDF) and granulocyte macrophage-colony stimulating factor (GM-CSF) using the non-viral Sleeping Beauty transposon system. The neuroprotective effect of recombinant

PEDF and GM-CSF was examined in organ cultures of retinas exposed to H2O2. Rat retinas isolated immediately after euthanasia cultured in Ames's medium were treated with 500 ng/ml rPEDF or GM-CSF for 3 days. On day 3, retinas were exposed to 350 μM H2O2 for 3 h. Retinal morphology was examined in H&E-stained sections at days 0, 4, 8 and 12. Retinas, pre-treated with PEDF and GM-CSF showed increased photoreceptor preservation, decreased inflammation as evidenced by immunohistochemical analysis of rhodopsin and GFAP, and increased levels of the anti-oxidant tri-peptide glutathione vs. untreated controls (n = 3 rats) indicating reduced oxidative stress. These data substantiate the hypothesis that PEDF and GM-CSF protect the retina by reducing oxidative stress and inflammation and validate the hypothesis that transplantation of IPE cells expressing increased levels of PEDF and GM-CSF would be beneficial to aAMD patients.

P316

OXB-203, a lentiviral vector expressing aflibercept as a single dose, long-term treatment for wet age-related macular degeneration

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Wet age-related macular degeneration (wet AMD) is a leading cause of blindness in older people, caused by abnormal blood vessel leakage leading to progressive degeneration of the macula. Vascular endothelial growth factor (VEGF) over-expression has a key role in the development of wet AMD. A number of anti-VEGF therapeutic strategies have been developed and are the current standard of care for wet AMD. However, these treatments are only effective for 1–2 months and require frequent injections causing a significant burden and are associated side effects. Clinical data from our OXB-201 programme (EIAV-endostatin and angiostatin) program (Campochiaro et al, 2017) demonstrated stable long term expression of the transgenes providing proof-of-principle for a single-injection gene therapy approach. An Equine Infectious Anaemia Virus (EIAV) lentiviral vector (OXB-203) was constructed encoding the anti-VEGF protein aflibercept. Anti-VEGF ELISAs and binding analysis of EIAV-aflibercept transduced cell supernatants demonstrated comparable binding characteristics to that of recombinant aflibercept. In vitro angiogenesis assays demonstrated that both cell proliferation and tubule formation were significantly inhibited with vector derived aflibercept similar to that of recombinant aflibercept. Preclinical studies using a rat choroidal neovascularization model following a subretinal administration of EIAV-aflibercept vector are ongoing.

P317

Establishment of an in vivo protocol for non-viral gene delivery to ocular tissue

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In vivo gene delivery to the eye using viral vectors is well established; however, the drawbacks of viral gene delivery have motivated the search for non-viral gene delivery. Ex vivo gene delivery to retinal pigment epithelial (RPE) cells using the non-viral Sleeping Beauty (SB) transposon mediated by electroporation is very efficient; however, in vivo non-viral gene delivery has only been reported successful in rodent eyes. In this study, we investigated gene delivery using the SB system and electroporation to transfect RPE cells in explanted whole pig eyes. Gene delivery was accomplished by injecting subretinally plasmids encoding the green-fluorescent Venus protein gene and electroporated using external paddle or needle electrodes. RPE cells were harvested from the electroporated area, cultured for 21 days and analyzed by fluorescence microscopy. Control cells were isolated from the opposite side of the same eye. Electrode type and positioning, DNA injection volume and injection site, voltage, length, interval and numbers of electrical pulses were investigated. Using paddle electrodes on opposite sides, 20 μL of DNA (250ng/μL) injected subretinally and 150V/cm resulted in the highest transfection of RPE cells. No fluorescence was observed in cells isolated from the control area. The methods developed here are critical to establish an in vivo protocol for the delivery of genes to ocular tissue without the use of viral vectors, which have the potential for acute immune responses, insertional mutagenesis and dissemination in tissues other than the organ and cells of interest.

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Non-virally transfected primary human pigment epithelium cells overexpressing the oxidative stress reduction factors PEDF and GM-CSF to treat retinal neurodegeneration

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Disturbed balance of neuroprotective factors (e.g. PEDF, GM-CSF), oxidative stress, and inflammation are significant events that are assumed to cause retinal degeneration in avascular age-related macular degeneration (aAMD). We hypothesize that transplantation of transfected pigment epithelium (PE) cells that overexpress PEDF and GM-CSF would prevent retinal RPE and neural cell degeneration by reducing oxidative cellular stress. The antioxidant potential of these factors was determined in the human RPE cell line (ARPE-19) and in human primary RPE cells (n = 6 donors), transfected with the genes for PEDF and GM-CSF. The genes were delivered to the cells by electroporation using the Sleeping Beauty Transposase System; gene expression and protein secretion were determined by RT-qPCR, WB, and ELISA at 1–8 weeks post-transfection. Transfected and non-transfected cells pre-exposed to the recombinant proteins, were treated with H2O2 for 24 h and the level of glutathione, an endogenous antioxidant, and phosphorylated Akt (pAkt), implicated in survival and growth pathways, were determined. Transfected cells showed a significant increase in gene expression and protein secretion (ANOVA, $p < 0.05$). Cells transfected and pre-treated with PEDF and GM-CSF, showed higher glutathione levels after H2O2 treatment than non-transfected/untreated controls (ANOVA, $p < 0.05$). A significant higher level of pAkt was detected in cells that overexpress GM-CSF indicating that this protein enhances the survival pathway. The

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results suggest that overexpression of PEDF and/or GM-CSF will reduce oxidative stress in retinal cells and may offers a promising approach to reconstitute the neuroprotective milieu to prevent cell degeneration in aAMD.

P319

A novel modified deep intravitreal injection improves Adeno-associated virus (AAV)-mediated retinal transduction

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Intravitreal (IVT) route is a choice when targeting the inner retina. However, the transduction efficiency of AAV vectors administered by IVT injection is usually low and may be affected by several factors. To improve the transduction efficiency, we developed a novel illuminated long-needle attached injection system and injected AAV2-CMV-eGFP (2.11 x 1011 vg/0.1ml) just in front of the retina in the right eye of New Zealand White Rabbit (n = 7). With the conventional needle (30 Gauge, 13mm), AAV2-CMV-eGFP was also injected to the same number of rabbits. To compare transduction efficiency and side effects between modified injection and conventional group, ophthalmological examinations, including slit-lamp biomicroscopy, and fundus photography, were serially performed for one month. Pro-inflammatory cytokines in the aqueous humor were assessed at the baseline and during 1 month. Chorioretinal tissues were used for immunohistochemistry. In the ophthalmological examinations, no significant inflammatory signs were detected in both groups, except transient, mild hyperemia. In the whole mount and the vertically sectioned tissues of the test group, significantly increased GFP expression was detected at the ganglion cell and the inner nuclear layers at 1month ($p < 0.01$). However, glial activation detected with GFAP/Iba-1 antibody were no differences between the two. Differences of Interleukin-6 and -8 expressions in aqueous humor were not observed either. These results suggest that modified deep intravitreal injection in front of the retina would be safe and efficiently transduce AAV2 vectors into the retina of large animals and considered as a potential method for use in clinical trials.

P320

New gene therapy modalities targeting oxidative stress and microglial activation are needed for effective treatment of retinitis pigmentosa

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Retinitis pigmentosa is a retinal degenerative disease, which results from genetic mutations disrupting photoreceptor function and structure. As rod cell death mechanism and degeneration process are mainly governed by the type of mutation, cone cells don't even start to die until the majority of the rod cells are lost. Reduced oxygen consumption due to the absence of rod cells results in an increase in oxygen levels of the outer retinal layer. Excessive oxygen, then, activates NADPH oxidase leading to the generation of superoxide radicals in cytoplasm through mitochondrial electron transport chain. Progressive oxidative stress eventually causes photoreceptor dysfunction and loss. Activated microglial cells by dead photoreceptors migrate from retinal plexiform layer to subretinal space, and further exacerbate photoreceptor degeneration by virtue of releasing proinflammatory cytokines. Consequently, correcting genetic defect by gene replacement therapy does not necessarily cure or prevent photoreceptor degeneration as revealed by recent clinical trials. Thus, inhibition of oxidative stress and microglial activation might be necessary to halt the degenerative process. To give such an example, anti-inflammatory Vasoactive Intestinal Peptide (VIP) gene delivery can be tested as a strategy to prevent retinal cell degeneration. To check if lentiviral vectors are suitable for the therapeutic gene delivery into retinal pigment epithelial cells, ARPE-19 cells were infected with a 3rd generation of lentivirus vectors encoding red fluorescein protein (LentiRFP). Our initial results suggested that lentiviral vectors can effectively be used to deliver therapeutic genes into retinal cells. TUBİTAK-218S543

P325

AVR-RD-01 lentiviral gene therapy reduces Gb3 substrate in endothelial cells of renal peritubular capillaries, in a previously untreated classic Fabry male patient

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Fabry disease (FD) is caused by GLA gene mutations leading to deficiency in alpha-galactosidase A (AGA) activity and accumulation of substrates and metabolites, including globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3). Symptoms include renal and cardiac insufficiency, and stroke. Despite Enzyme Replacement Therapy (ERT) standard of care, progressive complications develop leading to significant morbidity and early mortality. Initial results on 5 previously ERT-treated patients in a Phase 1 trial of AVR-RD-01, an investigational ex vivo autologous lentiviral gene therapy, showed increases in plasma and leucocyte AGA activity and decreases in substrate (Gb3) and metabolite (lyso-Gb3) in plasma. Here we report the kidney biopsy result for the first patient completing a Phase 2 open-label study investigating the efficacy and safety of AVR-RD-01 in treatment-naïve classic FD males. Reduction in Gb3 inclusions per renal peritubular capillary (PTC) were quantitatively assessed using the BLISS methodology. At 48 weeks, Gb3 inclusions were reduced from an average of 3.55 to 0.47 per PTC corresponding to 87% reduction versus baseline (BL). Leucocyte and plasma AGA activity increased, associated with declines in plasma and urine Gb3 and lyso-Gb3, including an 87% reduction in plasma lyso-Gb3 at 48 weeks versus BL. Treatment-emergent adverse events were as expected with conditioning, underlying FD and pre-existing conditions, with no serious adverse events related to AVR-RD-01 drug product. Low-titer anti-AGA antibodies were transiently detected at week 24. Substantial kidney biopsy Gb3 reduction was observed in the first Phase 2 patient at 48 weeks following AVR-RD-01 investigational gene therapy, with no unexpected safety events identified.

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P326

Development of a universal extraction protocol for multiple AAV-based gene therapy shedding assays

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Gene therapy is evolving rapidly for the treatment of genetic disorders, and for many patients the success of these therapeutics is their only hope for a cure. During gene therapy, a healthy copy of the gene causing the disease is delivered to the patient via a viral vector. Many regulatory bodies (EMA, FDA, etc) require monitoring of the shed virus as part of the gene therapy clinical trial. Detection of the virus in bodily fluids might be critical to understand environmental consequences or potential long-term effects that may lead to an increase of neutralizing antibodies (NAb) against adeno-associated virus in society. We have developed new protocols for monitoring viral shed DNA from many different bodily fluids including semen, saliva, urine, whole blood, PBMC, plasma, and stool. Here we present data on how we optimized the extraction from a diverse cohort of shedding compartments to establish a consistent and reproducible shedding assay. In parallel, we developed a rAAV infectivity assay for establishing sensitivity performance and show comparative data of the two assays for different rAAV vectors.

P330

Concurrent muscle and liver transduction imposes immune tolerance for muscle rAAV gene transfer

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Reaching long term immune tolerance to therapeutic transgenes represents an important goal for gene therapists, particularly for the treatment of monogenic muscular pathologies, such as Duchenne muscular dystrophy. Indeed, when the transgene contains sequence elements divergent from endogenous ones, a collection of lymphocytes that have not been purged during their development is available to initiate rejection of transduced cells. The ensuing recruitment and activation of transgene-specific lymphocytes generates adverse humoral and cellular responses, which are prominent in tissues subjected to vector transduction and inflammation. To overcome this obstacle, we harnessed the tolerogenic properties of the liver and explored how dual muscle-liver expression of a foreign transgene allows muscle transgene engraftment. We found that concurrent rAAV transduction of muscle and liver promotes a state of transgene-specific tolerance, resulting in the absence of CD8+ T cell responses combined with lower humoral response to the transgene. This tolerance is achievable in muscle weeks after liver transduction and is equally able to override preexisting immunity to the transgene. Regarding the mechanism, we found that dual muscle-liver transduction converts preexisting polyclonal, transgene-specific CD8+ T cells into typically exhausted T cells with high programmed cell death 1 expression and low IFN-γ production. Our results complemented with muscle confocal imaging demonstrate that transduction of muscle tissue can be protected from immune attack by liver-mediated control of humoral and cytotoxic T cell responses. Importantly, this liver-based tolerance induction process applies even in the presence of preexisting immunity to the transgene of interest.

P331

Transgene immunity following AAV-mediated gene transfer to the liver is associated to persisting viral genomes and T cell exhaustion

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The liver is a singular organ where immunity can be biased toward ineffective response and even immune tolerance. When T cell immunity is unable to completely eradicate an antigen in the liver, it can result in chronic viral inflammation and even tissue injury and fibrosis that could resolve over time. In the context of recombinant Adeno-Associated Virus (rAAV)-based gene transfer, host immunity balance against the transgene product depends on multiple factors. We performed a long term follow up of 12 non-human primates from three different protocols in which all the individuals received a rAAV8 vector carrying GFP transgene. We analyzed viral genome copies, GFP-directed immunity, GFP hepatic expression as well as liver histology. Despite the detection of an acute short-term cytotoxic immunity leading to the loss of transgene expression in the liver, we were still able to detect persisting viral genomes until 1 year post-injection. This long term unexpected observation was associated to in situ liver inflammation in the majority of animals. Interestingly, long term inflammatory fibrogenesis was systematically correlated to T cell-associated hepatic immune regulation with the detection of PD1-positive CD8 T cells in liver infiltrates. In conclusion, our study shows for the first time that anti-transgene immunity following hepatic rAAV-mediated gene transfer can lead to a non-conventional immune response in the macaque liver.

P332

GLP-compliant long-term evaluation of toxicity and tumorigenicity following haematopoietic stem cell gene therapy in a mouse model of chronic granulomatous disease

ABSTRACT WITHDRAWN

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P333

Sensitivity of different AAV serotypes to pre-existing NAbs

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While it is clear that pre-existing neutralizing antibodies exclude many potential patients from gene therapy treatment with AAV-based vectors, current exclusion levels are very rigorous (usually titres of >1:5). Testing a large cohort of patient samples and using the same experimental conditions, the approximate percentage of eligible subjects was tested for AAV8 and AAVAnc80. 300 serum samples of individuals of different geographical and ethnic origin, age and equal gender distribution were tested. Approximately 37% and 40% for of the individuals were seronegative for AAVAnc80 and AAV8, respectively. Subsequently, the sensitivity of the Anc80 and 8 serotypes to NAbs was tested in mice passively immunized with human sera of different serotype-specific titres. AAVAnc80 was found to be more sensitive than AAV8, i.e. much lower existing NAb titres could partially or fully inhibit transduction. The high sensitivity of AAVAnc80 to NAbs was further confirmed in NHPs. Immunoadsorption was required for an efficient transduction of NHP liver, even in in animals with low NAbs. Transcription levels and expression of the reporter gene were in line with the amount of vector genome present in liver. Based on the findings, we can argue that the real impact of a NAb positive signal obtained using an in vitro assay should be further tested in a more relevant biological assay and the seroprevalence values need to be reconsidered accordingly.

P334

Antigen-encoding bone marrow deletes mature antigen-specific B cells in recipients and inhibits antigen-specific antibody production

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Detrimental antibody responses arising from dysregulated B-cell responses underlie many diseases. Existing treatments use non-specific immunosuppression, and induction of antigen-specific tolerance remains elusive. Immunotherapies typically manipulate the T-cell component of pathogenic immune responses but few directly target B cells. Hematopoietic stem cell (HSC)-mediated gene therapy is endowed with features that engender great promise for antigen-specific immunotherapy. Particularly, the potential to deliver antigen in a form directly tolerogenic to B cells, but this approach must be used in a way that preserves bystander immunity. Gene-engineered bone marrow encoding ubiquitous ovalbumin (OVA) expression was transferred after low-dose (300cGy) immune-preserving irradiation. Homeostasis of pre-existing OVA-specific B cells and those arising after BM transfer was monitored using flow cytometry and responsiveness to immunisation was tested. OVA-specific B cells were purged from the pre-exiting mature B-cell population in recipients following transfer of OVA-encoding BM as well as from newly-developed B cells that arose after BM transfer. OVA-specific antibody production was largely prevented after OVA-encoding BM transfer and this was consequent to inhibition of B-cell activation, development of germinal centres and plasmablast differentiation. Low levels of gene-engineered bone marrow chimerism ($\sim$20%) were sufficient to limit antigen-specific antibody production. These data show that antigen-specific B cells within an established B cell repertoire are susceptible to de novo tolerance induction and this can be mediated by transfer of gene-engineered bone marrow. This study provides an important proof-of-principle that HSC-mediated gene therapy has the capacity to modulate a mature developed B-cell repertoire under conditions where bystander immunity is preserved.

P335

Neutralizing anti-AAV antibody impact on vector transduction following intravitreal administration of AAV in non-human primates

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Pre-existing immunity remains a challenge for many adeno-associated virus (AAV) gene therapies due to the high prevalence of AAV in the general population. Ocular gene therapies appear less affected by neutralizing antibodies (Nabs) possibly due to the partial immune privilege status of the eye, but some degree of vector neutralization is thought to take place when AAV is injected intravitreally (IVT). The current study was designed to answer questions around its potential significance. Macaca fascicularis with low (<4), intermediate (277 ± 51) or high (11599 ± 4748) systemic anti-AAV2tYF NAb titers received IVT

injections of rAAV2tYF-CBA-hGFP (1.0E+11 vg) into their right eyes. Ocular exams and fluorescent fundus imaging were performed at weeks 1, 4, 6, 8 and 12. Serum, together with vitreous humor samples from the left (untreated) eyes were collected at study weeks 0, 6 and 12. In addition, vitreous humor samples were collected from the right (treated) eyes and aqueous humor samples from both eyes at termination (week 12). Anti-AAV2tYF NAb titers were measured for all collected samples. No severe ocular inflammatory events occurred, and there was no difference in the extent of inflammation between groups. At termination, neither serum NAb titers nor injected eye NAb or uninjected eye NAb titers were significantly different between groups. The typical foveal ring pattern of retinal ganglion cell GFP expression following IVT injection was detected at variable intensities in all groups, indicating that high pre-existing systemic NAb titers per se are not sufficient to block retinal AAV transduction.

P336

Prevalence and affinity/avidity assessment of pre-existing NABs against AAV2, 5 and 8 analyzed in the serum of 300 healthy donors

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Low levels of anti-AAV2 or anti-AAV8 neutralizing antibodies (NABs) have been related to a decrease or even total impairment of AAV liver transduction after systemic delivery in both non-human primates (NHPs) or humans. Currently patients with detectable anti-AAV NABs titers, as low as 5, are commonly excluded from AAV gene therapy trials. We have reported that AAV5-neutralizing antibodies did not impair the efficacy of in vivo transduction of AAV5-based vector up to a measured titer of 340 in humans and 1030 in NHPs. Those results suggest that differences in the neutralization ability of antibodies might exist between AAV serotypes. The aim of the present study was to assess the binding characteristics of the pre-existing anti-AAV NABs to AAV antigen for AAV2, AAV5 and AAV8 serotypes. The results obtained from 300 healthy donor serum samples demonstrate that the avidity of pre-existing AAV-specific IgG antibodies in healthy human population is significantly different between AAV serotypes. Pre-existing anti-AAV2 or anti-AAV8 NABs form stronger antibody-antigen complexes with AAV2 and AAV8 antigens than pre-existing anti-AAV5 NABs with the AAV5 antigen. Overall, these data suggest why pre-existing anti-AAV5 NABs measured in vitro do not interfere with the AAV5-based in vivo transduction as much as pre-existing anti-AAV NABs against other serotypes do. Furthermore, no significant differences between gender or ethnic origin on the prevalence or avidity of pre-existing anti-AAV antibodies was observed in the analyzed healthy human population.

P337

A retrospective literature review of the safety outcomes of clinical studies of haemotopoietic stem cell gene therapy using lentiviral vectors in non-oncologic diseases

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Haemotopoietic stem cell (HSC) gene addition therapy using third-generation lentiviral vectors (LVVs) is being evaluated as a treatment option for various diseases. This literature review describes the safety of LVVs for *ex vivo* HSC gene therapy as reported in clinical studies published between January 2005 and October 2017. A total of 125 patients received LVV-mediated HSC gene therapy across 17 studies encompassing 8 genetic diseases and 13 LVVs. Diseases included haemoglobinopathies (36% of patients), immunodeficiencies (32%), leukodystrophies (24%) and Fanconi anemia (8%). Age, reported for 112 patients, ranged from 0.4–42 years. Cell dose ranged from 0.5-25x10^6cells/kg. Drug product vector copy number ranged from 0.17–6.3 copies/diploid genome. Follow-up, reported for 107 patients, ranged from 1–96 months; 20 patients had ≥3 years of follow-up, with longest follow-up in a patient with beta-thalassemia. There were no reports of replication competent lentivirus in the 16 studies that evaluated this outcome. In 13 studies, integration site analysis (ISA) was performed to assess clonal diversity; ≥1 ISA result was reported for 94 patients. While a few subjects experienced transient predominance of a single clone, only 1 instance of a relatively predominant clone over an extended period of time was reported. No studies reported evidence of insertional oncogenesis or LVV-derived HIV infection. In summary, at the time of the analyses, there were no reports of serious vector-related safety events following LVV-mediated HSC gene therapy in non-oncology related diseases. Longer follow-up is warranted to better assess the long-term safety of third-generation LVV gene therapy.

P338

Tolerogenic ImmTOR™ nanoparticles enhance vector transduction, mRNA synthesis and transgene expression after initial and repeated administrations of AAV-based gene therapy vectors

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We have previously shown that tolerogenic ImmTOR™ nanoparticles encapsulating rapamycin block adaptive immune responses against the AAV capsid, thereby enabling repeat administration of AAV vectors. Here we further demonstrate that ImmTOR™ also enhances transgene expression after the first dose of AAV vector in naïve mice. This beneficial effect of ImmTOR™ is independent of its effects on adaptive immunity, it is seen in beta 2-microglobulin- and Rag2-deficient mice and cannot be achieved in vivo by free rapamycin. Admixing ImmTOR™ and AAV is required for enhanced transgene expression after the first dose but not for inhibition of the antibody response to AAV. ImmTOR™ affects multiple aspects of AAV biology at first dose, including trafficking, inhibition of acute inflammation, and autophagy. The physical association of ImmTOR™ and AAV facilitates AAV uptake by liver cells and results in increased vector copy numbers and transgene mRNA expression. The combination of ImmTOR™ and AAV also inhibits NF-κB activation in the liver of transgenic NF-κB reporter mice. Finally, ImmTOR™ appears to enhance autophagy and is synergistic with other autophagy-inducing interventions. This multi-pronged mechanism of ImmTOR™ action makes it an attractive candidate to enhance systemic gene therapeutic applications. The first dose benefit of adding ImmTOR™ to AAV gene therapy is immediate, dose-dependent and not mouse

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strain-specific. It can also overcome low levels of pre-existing antibodies to AAV. The rapid and enhanced transgene expression may enable faster onset of therapeutic effects achieved at lower AAV doses and coupled with the inhibition of antibodies against AAV to enable vector redosing.

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Treatment with valoctocogene roxaparvovec in a Ph1/2 study (BMN 270-201) elicits cross-reactive antibodies against divergent AAV capsids that exhibit different kinetic profile than anti-drug antibodies

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Adeno-associated virus (AAV)-mediated gene therapy offers great promise to the hemophilia A population by providing substantial and sustained FVIII activity levels resulting in a clinically relevant reduction of bleeding episodes. Valoctocogene roxaparvovec (BMN 270) is a recombinant AAV5-hFVIII-SQ vector, currently in development for the treatment of hemophilia A. To evaluate the cross reactivity of treatment-induced antibodies BioMarin has validated identical electrochemiluminescent (ECLA)-based methods to detect antibodies against AAV2, 5, 6, 8, and rh10 serotypes in a central laboratory and with comparable limits of detection. Antibody levels were determined at baseline and up to 3 years of post-BMN 270 treatment across multiple dose levels, 6x10e12 vg/kg through 6x10e13 vg/kg, in a Ph1/2 study. All patients demonstrated considerable increases in antibody titers against the AAV5 capsid at 8 weeks post-dosing. The kinetic profile in BMN 270-treated patients illustrates that AAV5 titers continued to increase during long-term assessment and generally were greater than 1e6 in all treated patients. Cross-reactive antibodies against non-drug related capsids (AAV2, 6, 8, and rh10) increased to a lesser extent (1e2 to 1e4 titer range) within the first few months post-treatment and gradually declined to levels similar to those observed in untreated, naturally exposed subjects with pre-existing immunity against such AAV serotypes. Together, these data demonstrate that persistence of treatment-induced antibodies varies in specificity to AAV serotypes over time as immune responses evolve following gene therapy dosing.

P340

Potential innate immune responses to adeno-associated virus mediated gene therapy

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Adeno-associated virus (AAV) vectors have been established as gold standard in terms of safety and efficacy in retinal gene therapy. However, previous studies revealed that AAV could induce innate and adaptive immune responses potentially compromising the therapeutic success. Here we aim to evaluate the pattern recognition pathways which are activated in an innate immune response to AAV serotype 8 using human differentiated THP-1 cells as a model for macrophages. THP-1 cells were differentiated into macrophages by incubation with 100nM phorbol 12-myristate 13-acetate (PMA) and subsequently incubated with AAV8-CMV-eGFP at different multiplicities of infection (MOI; 1:10^4, 1:10^5, 1:5x10^5 and 1:10^6). Bacterial lipopolysaccharide (LPS) served as positive control. Supernatant was collected at various time points after stimulation and analysed for the presence of inflammatory cytokines and chemokines (IL-1β, TNF-α, IL-8, MIP-1α, MIP-1β) and type I IFNs (IFN-β) release using ELISA. We found that AAV8-CMV-eGFP induced the release of TNF-α, IL-1β, IL-8, MIP-1α and MIP-1β but did not stimulate the production of IFN-β. The time when this release peaked varied between the different cytokines/chemokines. For all cytokines/chemokines the response was dose-dependent: highest at the higher MOIs (1:5x10^5 and 1:10^6) and low or absent with the lower MOIs (1:10^4 and 1:10^5). All cytokines were detected after stimulation with LPS. We conclude that AAV2/8 can trigger a dose-dependent inflammatory innate immune response in a model for human macrophages. In a next step, this model will allow us to dissect the dynamics of relevant pattern recognition pathways involved in the innate immune response to AAV.

P341

Induction of humoral and cellular immune responses in mice immunized with a DNA vaccine for Zika virus

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Zika virus (ZIKV) is a single-stranded, positive sense RNA virus within the Flavivirus genus, primarily transmitted through the bite of Aedes mosquito species. The World Health Organization (WHO) declared ZIKV a Global Emergence of Public Health Concern because of massive rise in teratogenic outcomes and neurological complications such as Guillain Barre Syndrome and microcephaly. There are no vaccines and antiviral therapies available against this pathogen. In this study, we generate and evaluate the in vivo efficacy of novel plasmid DNA vaccine targeting the pre-membrane (prM) and envelope (E) proteins of ZIKV. Following initial in vitro development and evaluation studies of the plasmid DNA vaccine construct, mice were immunized with this PrME DNA vaccines via the intramuscular or intradermal route followed by electroporation. All vaccinated mice induced antigen-specific cellular immunity and antibody response, which provided neutralizing immunity. In addition, immunization with DNA vaccine generated a balanced IgG response. Taken together our data illustrate that DNA vaccine can induce strong immune responses against ZIKV infection, suggesting that this strategy may have value as a promising vaccine against this emerging pathogen.

P342

The antitumor activity of a DNA vaccine encoding glioma-associated antigens is enhanced by surgical resection in a GL261 glioblastoma orthotopic model

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DNA vaccination against cancer has become a promising strategy for inducing a long-lasting and specific immune response. However, the activity of DNA vaccines is limited when used as single therapy. To enhance their activity, DNA vaccines can be combined with other therapies that can drive the vaccine-induced immune response into the tumor microenvironment. This work evaluates the potential synergic effect of a DNA vaccine encoding glioma-associated antigens (GAAs) and tumor resection and their ability to reduce the onset of glioblastoma recurrences, in an orthotopic model. Hence, a DNA vaccine encoding the GAAs TRP2 and gp100, expressed by GL261 glioblastoma cells, has been designed and delivered by electroporation in the tibialis muscle of mice 16, 23 and 29 days after the GL261 orthotopic inoculation. A significant increase in the survival of vaccinated mice that underwent surgical resection compared to the controls (vaccine or resection) was observed. Immunological analysis by flow cytometry and ELISpot show a significant decrease of the infiltrated immunosuppressive cells 13 days after the priming dose of the vaccine (at day 29 post-tumor inoculation) and the presence of antigen-specific and immunologically active T cells in the brain when the two therapies are combined. Hence, the combination between the glioblastoma tumor resection and DNA vaccine immunotherapy can increase mice survival in a clinically-relevant preclinical model, opening the door to a new standard of care for GBM patients.

P343

Robust solutions for navigating challenges associated with development and validation of cell-based neutralising antibody assays

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Pre-existing immunity to adeno-associated virus (AAV) or dose related immune response can limit effective gene transfer and neutralising antibodies (NAb) pose a safety and efficacy liability. Therefore, an effective and robust strategy to measure pre-existing or generated NAbs are an important component of AAV-based gene therapy programs. We developed a GLP compliant AAV2 cell-based NAb assay with commercial reagents to support cynomolgus NHP studies and tested assay robustness to production lot changes. The NAb method measured luminescence signal emitted from HEK-293 cells transduced with an AAV2-Luciferase control virus after preincubation with a commercial NAb. The assay was developed to characterize screening and confirmatory cut points, accuracy and precision, sensitivity, stability, specificity and selectivity. Assay feasibility was established, then 110 individual serum lots were pre-screened. We identified 60% seropositivity and true naïve lots were pooled. The method was validated and Cut Point statistics resulted in a Normalization Factor of 6093 Relative Light Units (RLU) and sensitivity of 714 ng/mL. A second production lot of AAV2-Luc was bridged into the assay and failed to meet acceptance criteria. Thus, the assay was re-optimised and partially validated to re-establish Cut Points and required assessments. This resulted in a more sensitive assay (234 ng/mL) with a Normalization Factor of 34010 RLU. Our results demonstrate substantial AAV2 seroprevalence in a sample NHP population. These data highlight that NAb analysis is an essential component of AAV-based programs and should be implemented early with sufficient critical reagents to help ensure safety and efficacy of the therapeutic.

P344

Metabolic insight into Cyclosporine-mediated improvement of HSPC engraftment in the context of *ex vivo* gene therapies

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We have previously demonstrated that both Cyclosporines A (CsA) and H (CsH) increase Lentiviral Vector (LV) transduction efficiency in SCID-repopulating human hematopoietic stem and progenitor cells (HSPC). CsA also improves HSPC engraftment in vivo by preserving the more primitive stem and multipotent progenitors in culture. Interestingly, our preliminary results suggest that also CsH favours HSPC engraftment as higher levels of CD45+ cells were retrieved from the peripheral blood and bone marrow of NSG mice transplanted with human mobilized peripheral blood (mPB)-derived CD34+ cells pre-exposed to CsH. To further investigate the impact of CsH on HSPC, we performed a Mass Spectrometry (MS) analysis in primary human HSPC exposed or not to the drug. Out of 3136 proteins identified, CsH significantly downregulated 100 and upregulated 140 proteins (T-test FDR <0.05). Interestingly, pathway enrichment analysis revealed that the proteins upregulated by CsH are mainly involved in metabolic processes such as TCA cycle, mitochondrial activity, fatty acid and glucose metabolism. Oxygen consumption assays in HSPC exposed to CsH compared to untreated controls are on-going to understand whether CsH could impact mitochondrial respiration. MS analysis to detect the metabolic mechanisms specifically affected by CsH treatment will also be performed. Finally, gain- and loss-of function experiments targeting the identified pathways, in the presence and absence of CsH, will confirm the CsH-mediated regulation of HSPC metabolism. Altogether these experiments will contribute to elucidating the mechanisms governing HSPC metabolic programs and help improving their ex vivo expansion, manipulation and transplantation in the context of gene and cell therapies.

P345

Innate immune response of the retinal pigment epithelium to gene therapy vectors

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The low immunogenicity of adeno-associated virus (AAV) vectors was considered ideal for ocular gene therapy. Recent pre-clinical animal experiments, however, have found that transduction of the retinal pigment epithelium (RPE) by these vectors can result in toxicity. Further, clinical trials using AAV vectors for ocular gene therapy have independently reported

varying degrees of ocular inflammation. We hypothesize that RPE cells are capable of mounting an inflammatory response to gene therapy vectors. We have cultured RPE cells derived from induced pluripotent stem cells (iPSC-RPE) to evaluate the in vitro innate immune response of RPE cells to AAV vectors. Three iPSC-RPE cell lines were studied: a commercially-available iPSC-RPE line, a line derived from a healthy patient, and a line derived from a choroideremia (CHM) patient. iPSC-RPE cells were grown on permeable supports for no less than 3 weeks prior to transduction with AAV vectors encoding GFP or CHM, with or without a non-coding DNA sequence that suppresses the TLR9-mediated innate immune response. Cell morphology, expression of RPE markers BEST1 and PMEL17, and expression of tight junction markers ZO-1 and CLDN19 were evaluated by immunofluorescence. Inflammatory cytokine concentrations in the apical and basal compartments were evaluated by ELISA analysis. Transduction of the iPSC-RPE, regardless of transgene, stimulated secretion of IL-6, IL-8, and MCP-1 on both the apical and basal sides of the RPE. In the future, alternate vector constructs may be required to mitigate the innate immune response of the RPE to viral vectors to enable safer and more efficacious gene therapy.

P346

Design of dna vaccine for prevention of hemorrhagic fever with renal syndrome

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Background: Viral hemorrhagic fevers represent a group of diseases caused by RNA (-) viruses belonging to various families. This group of diseases is known to be widespread worldwide with mortality rate up to 40% dependent on viral strain. Climate change and the global economy contribute to the emergence of more dangerous strains of the virus and the spread of hemorrhagic fever. Therefore, development of the new approaches for therapy and preventive treatment is critical. At the present time, there is no vaccine developed to protect against Puumala virus (PUUV), circulating in the Republic of Tatarstan.

Methods: All procedures with animals were approved by the Kazan Federal University Animal Care and Use Committee (Permit Number: 5 dated 27 May 2014) and study was supported by President's Grant MK 2393-2019.4. Experiments were carried out in accordance with international bioethical standards. Plasmid vectors encoding various combinations of PUUV glycoprotein and/or nucleocapsid protein and reporter genes were used for intramuscular animal immunization. Immune response, morphological changes in tissues and viral protein expression in parenchymal organs were analyzed at 7, 14 and 21 days after vaccination. Antibody titer was determined by ELISA. Production of IFNγ by cytotoxic lymphocytes was evaluated using ELISPOT (Murine IFNγ ELISPOT Kit, USA).

Results: Immunized mice had significantly high titer of IgG antibodies to hantaviral proteins 14 days after vaccination. Activation of cytotoxic lymphocytes was detected 14 and 21 days after immunization. These data suggest that DNA coding for PUUV nucleocapsid protein and glycoproteins could be effective against hantavirus infection.

P347

Novel AAV capsids show increased evasion to wild-type AAV9 neutralizing antibodies

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The use of adeno-associated virus (AAV) vectors is emerging as an effective method to deliver corrected genes to patients. A recurring issue in AAV clinical trials is the prevalence of pre-existing immunity to the AAV vector being utilized to deliver the gene of interest. It has been shown that low levels of AAV neutralizing antibodies (nAb) can drastically reduce or eliminate the effectiveness of the gene transduction. Novel AAV vectors with tropisms similar to wild-type AAVs have been developed as a potential way to evade this immune response. A novel AAV capsid library was developed with tropisms comparable to AAV9 but different immune recognition, both pre- and post-exposure. A luciferase-based nAb assay was developed to evaluate the prevalence of AAV9 nAbs compared to those of our novel capsids AAV204 and AAV214. Initially, these capsids were tested against commercially-obtained human donor serum to evaluate their sero-positivity in the general population. In the tested population, the prevalence of sero-positivity for AAV9 was higher than that of both AAV204 and AAV214. NAb levels were also assessed after dosing non-human primates with either AAV9 or AAV204 to evaluate development of cross-reactive neutralizing antibodies. Importantly, animals administered AAV9 developed few or no cross reactive antibodies to either AAV204 or AAV214. Primates injected with AAV204 displayed limited development of antibodies that cross-reacted with AAV9. Both AAV204 and AAV214 exhibit characteristics of lower general population prevalence and less cross-reactivity to AAV9, allowing them to evade the immune response in patients seropositive for AAV9.

P348

The inflammatory state of Hepatic stellate cells following infection by coagulation factors-shielded Adenovirus

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The inflammatory effect of Adenovirus is a challenging issue and coagulation factors seem to be determinative. Herein, the effect of Adenovirus shielding by VII and FX coagulation factors on the inflammation of stellate cells was investigated. Ad36) and Ad5-GFP were separately loaded with VII and FX. The Ad5-GFP was employed to assess the transduction efficiency. The Ad36, Ad36-FVII and Ad36-FX were subjected to Zetasizer to determine size and charge parameters. They were added to stellate cells then expression of PKR and inflammatory cytokines including IL-1β and TNF-α were measured by qPCR and ELISA. The activation status of LX-2 was evaluated by measuring TIMP-1, Collagen, and TGF-β expression. The shielding by coagulation factors enhanced the transduction rate of Ad5-GFP to 60% and 75%, respectively. The PKR expression analysis showed a

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significant up-regulation following treatment with all Ad36 forms especially lonely virus (P = 0.0152). The IL-1β and TNF-α cytokine analysis demonstrated that Ad36-FVII elicited the most inflammatory response (P = 0.05). The result of fibrosis-related genes expression also showed the enhancing effect of FVII as increased the TGF-β and collage-I expression compared to Ad36-FX. The findings suggested that coagulation factors particularly FX increase the transduction efficiency of Adenovector. However, Ad36 loaded with FVII induce more inflammation as well as activation of stellate cells than FX shielded virus.

P349

Prevalence of NAb against AAV8 in micromini pigs

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Utility of adeno-associated virus (AAV) vectors has been extensively tested in vivo in various animal species, including humans. Few reports, however, have examined the utility of pigs in gene therapy. Pigs are potentially useful in preclinical studies because of their anatomical and physiological similarity to humans. Here, we tested the prevalence of NAb against AAV8 capsid in micromini pigs. Blood samples collected from 48 pigs in the same facility were analyzed in our assay system. As a result, the overall NAb positivity was 62.5%. The proportion of animals with NAb titers of (14×), (28×), (56×), and (112×or higher) was 27.1%, 16.7%, 8.3%, and 10.4%, respectively. The lowest positive-NAb subjects formed the largest group within the NAb positive animals. Along with the NAb, the amount of anti-AAV8 antibody in pig sera was quantitated by ELISA and its relationship with NAb titer was analyzed. No relationships between these two parameters were demonstrated, showing that overall anti-capsid antibodies by ELISA cannot predict the titer of NAb. These results imply that micromini pigs may be suitable to test the role of low titer NAb in preclinical studies especially for liver-targeted AAV gene therapy.

P350

Epitope display on adenovirus capsid as a potent vaccination strategy to elicit cytotoxic cellular responses

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Vectors derived from human serotype 5 adenovirus (Ad) were investigated as vaccine delivery vehicles. However their efficacy is confronted with pre-existing anti-Ad immunity that limits gene transfer and the subsequent expression of the antigen. Therefore, there is a need to investigate new vaccination approaches. Epitope display relying on genetic insertion of peptides into Ad capsid proteins constitutes an alternative method to induce immune responses against a heterologous protein. Although the capacity of Ad displaying heterologous epitopes to induce potent humoral immune responses was previously demonstrated for different epitopes, their potential to induce CD8+ cellular immune responses has never been investigated. The present study reports for the first time the capacity of the epitope display strategy to trigger CD8+ cellular immune responses. First, we demonstrated that immunodominant ovalbumin-derived Ova257–264 T-cell epitope inserted into the hexon or the fiber protein of Ad capsid was efficiently processed by antigen-presenting cells leading to activation of ovalbumin-specific T cells. After administration to mice, Ad displaying Ova257–264 epitope into the hexon protein led to an increase of antigen-specific CD8+ T cells compared to Ad displaying the same epitope into the fiber protein. In addition, this increase in CD8+ T cell number was associated with a stronger capacity of these T cells to produce IFNgamma upon antigen challenge. Altogether these results provides the foundations for the use of epitope display on Ad capsid as a strategy to elicit cytotoxic cellular responses against relevant antigens.

P351

Detection of Macro-throtropin in patients with Hashimotos Thyroiditis and subclinical hypothyroidism

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The level of thyroid stimulating hormone (TSH) is one of the diagnostic markers of thyroid function. In subclinical hypothyroidism, the concentration of TSH in the blood serum increases, while the level of thyroid hormones stay the same. The reason for this observation is the macroTSH phenomenon, in which the macro isoforms of TSH (a complex of TSH with IgG) are present in the blood. Blood serum collected from 50 patients (30 patients with subclinical hypothyroidism, 10 - with manifest hypothyroidism, 10 - control group) was screened for the presence of macroTSH by polyethylene glycol (PEG)-precipitation and analyzed using gel filtration chromatography. As a result of the screening, 56–98% of 50–100% serum TSH was identified as true macroTSH. In patients with subclinical hypothyroidism with the TSH level of more than 10 μIU/ml, a trend towards an increase in the level of macroTSH has been shown. It is known that elevated levels of antibodies to thyroperoxidase (TPO) can lead to the generation of macroTSH. Thus, we identified that the amount of macroTSH complex in patients with subclinical hypothyroidism, in whom the level of antibodies to TPO is >500 U/L, is significantly higher compared with patients with manifest hypothyroidism. Patients with the TSH level of more than 10 μIU/ml are candidates for screening for the presence of the macroTSH complex. This research was supported by the subsidy of the Russian Government to support the Program of the competitive growth of Kazan Federal University.

P352

Analysis of autophagy and LC3-associated phagocytosis in T-lymphocytes of patients with severe asthma

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A lot of diseases are characterized by a dysfunction of apoptosis and immune activity, although one process is distinguished as a link between the appearance of autoantigens and impaired immune tolerance. Autophagy is a process of intracellular degradation, which is aimed at maintaining the cell homeostasis by delivering the cytoplasmic components to lysosomes. According to the studies, the greatest interest in the context of human diseases represents the non-canonical form of autophagy – LC3-associated phagocytosis (or LAP). For years, it has been assumed that asthma and autoimmune diseases have little in common, however, both of them are characterized by involving the immune system components, and in case of asthma, the autoimmune component. Thus, we performed a comprehensive ultrastructural analysis of apoptosis, autophagy and LAP in patients with severe bronchial asthma. By using the transmission electron microscopy, we found that the majority of T-lymphocytes in the control group have a morphology that is common for the early and late stages of apoptosis. Autophagosomes were not detected, however, a significant number of single-membrane phagosomes associated with the LAP pathway were found. In patients with severe asthma, in addition to the signs of the early stages of apoptosis, increased content of autophagosomes and a small number of single-membrane phagosomes were detected. As a result, a weak manifestation of LC3-associated phagocytosis, and the inhibition of apoptosis in the final stages may cause the appearance of autoantigens and the development of an autoimmune response. Study was funded by RFBR according to the research project №18-34-00739.

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P353

Evaluation of T- and B-cell receptor diversity using different immune repertoire sequencing methods

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Diversity of T-cell (TCR) and B-cell receptor (BCR) repertoires, generated by somatic recombination of variable (V), diversity (D) and joining (J) gene segments, constitutes a key aspect for immune system functionality. As malignancies, infections or immunological disorders may result in reduced diversity, the analysis of TCR/BCR repertoires enables investigation of disease progression and/or immunotherapeutic efficacy. We performed side-by-side comparison of a PCR- (RACE-PCR) and a target enrichment sequencing (TES)-based approach to analyse TCR and BCR diversity. Method's performance was evaluated on mono-/oligoclonal controls and healthy donor samples regarding sensitivity, accuracy and quantitative readout. Both techniques performed in replicates yielded comparable results of >30,000 clonotypes for TCR and BCR when normalised to sequencing depths, with a sensitivity threshold of 0.01%. The V and J segment usage profiles obtained, as well as the predominant clonotypes identified, were analogous for both methods. Either approach revealed the deepest insights into clonal repertoires with 500ng-1000ng RNA template, although reliable results on dominant clonotypes were achieved down to 30ng RNA input. TES yielded a more representative TCR/BCR repertoire overview at comparable input, as it allowed the simultaneous capture of all TCR and BCR chains, respectively. Therefore, TES is suitable for analysis of limited sample material and, in presence of a dominant clonotype, for identifying the paired chains. In fact, TES was performed on three ADA-SCID patient samples of a clinical lentiviral gene therapy trial (NCT01380990) for improvement of immune reconstitution and we retrieved information about TCR diversity even at low sequencing depths (up to 31,418 reads/sample).

P354

A redundant control approach based on a bicistronic suicide gene construct enhances the safety and efficacy of the system

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Inducible suicide genes are designed to provoke cellular death upon activation. This property is especially useful in therapeutical applications in which exogenous, ex-vivo modified cells are introduced into patients. If the transplanted cells are provided with inducible suicide constructs, the activation of the transgenes could eliminate harmful effects arising from their malignant transformation. The Herpes Simplex Virus (HSV) deoxythymidine kinase (TK) gene and the iCaspase 9 inducible construct are the two most used of such cassettes. Although both exhibited promising results in a considerable number of cell types, none has shown complete elimination of the targeted cell. In order to improve those results and to provide a level of redundancy to the system, we have devised a bicistronic construct in which HSV-TK and iCAspase9 are joined by a 2A element in a same coding unit. The construct has been tested in vitro in human colon carcinoma HCT116, human placenta choriocarcinoma JAR, human prostate adenocarcinoma PC3 and human iPS cell lines, and in vivo upon subcutaneous injection of the cells into nude mice. In all cases, activation of the two suicide genes produces significantly better results that each one of them alone. We have also observed that serial activation of the two genes improves upon the efficiency of simultaneous activation, but it is dependent on the order in which the genes are set into action.

P355

Scalable allogeneic cell therapies derived by reprogramming inducibly-immortalised adult stem cells

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1: ReNeuron

Induced Pluripotent Stem Cells (iPSCs) derived by somatic cell reprogramming have great potential in cell therapy. However their clinical application remains challenging due to their teratoma potential, the inefficiency of differentiation protocols and the fact that target cells are typically progenitors unstable in vitro. CTX is a neural stem cell line in clinical trials for stroke. It is transgene-immortalised, with cell cycle progression

controllable with 4-hydroxytamoxifen (4-OHT). We have reprogrammed CTX cells and find that CTX-iPSCs display many features characteristic of hPSCs. After reprogramming, cell morphology changes from a neuronal phenotype with extended processes to densely-packed undifferentiated cells with prominent nucleoli. They express alkaline phosphatase, the transcription factor OCT4 and the embryonic antigens TRA-1-60 and SSEA-4, but do not express the early differentiation marker SSEA-1. In vitro differentiation to endoderm, mesoderm and ectoderm confirms that they are truly pluripotent, unlike many reprogrammed derivatives of immortalised cells. Differentiation of CTX-iPSCs can produce medically-relevant cells such as mesenchymal stem cells. CTX-iPSC-MSCs conform to ISCT criteria such as plastic adherence and marker expression but in the presence of 4-OHT they can be expanded indefinitely (> 20 passages) at a high rate without apparent change in phenotype. CTX-iPSCs represent a useful clinical resource. They may be differentiated like normal iPSCs, but the immortalising transgene allows scalable production of differentiated derivatives as allogeneic off-the-shelf treatments. Furthermore, as CTX-iPSCs are derived from a cell line which has already passed clinical safety trials, their application to new indications is likely to be accelerated.

P356

Scalable GMP-compliant cultivation and hematopoietic differentiation of human iPSC enables the production of macrophages for immunotherapies

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Macrophage-based therapy has been shown to be effective for a variety of diseases affecting different tissues including lung, brain, liver or bone. To derive sufficient quantities of macrophages, we propose the use of human induced pluripotent stem cell (hiPSC) technology. In order to translate macrophage-based therapies into clinical practice, we established a xeno-free, fully-defined hiPSC-based production platform suitable to derive scalable quantities of macrophage populations in stirred tank bioreactors. To achieve this aim, hiPSCs were expanded as a monolayer in fully defined and GMP-compatible Essential 8 media, followed by cultivation of hiPSCs as pluripotent aggregates in suspension culture using 150 ml fully-equipped stirred tank bioreactors. Flow cytometry analysis showed more than 90% positive cells for pluripotency marker Tra1-60. After 7 days of mesoderm priming using BMP4, VEGF and SCF, aggregates were cultured in fully-defined X-VIVO15 media supplemented with hematopoietic differentiation cytokines for macrophage production. We observed that successful generation of macrophages was dependent on formation of a CD144+CD34+CD43-CD73- cell population during mesoderm priming. Efficient production of hiPSC-macrophages started from day 12 of differentiation onwards and continued for more than six weeks. Generated hiPSC-macrophages represented classical morphology, homogenous surface marker profile of CD45+CD11b+ CD14+CD163+ and phagocytic capacity of bacterial bioparticles. Moreover, hiPSC-macrophages were able to eradicate viable Staphylococcus aureus (S. aureus) including methicillin-resistant S. aureus in-vitro in comparable efficiency to peripheral blood monocyte derived macrophages. To summarize, we here introduce a GMP-compatible platform to derive macrophages from hiPSCs, which can be applied for various cell based therapies including S. aureus mediated infections.

P357

In vivo progressive degeneration of Huntington's disease patient iPSC-derived neurons reveals human-specific pathological phenotypes

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Research on neurodegenerative disorders, such as Huntington's disease (HD), has been considerably hampered by the limited access to patients' brain tissue and the absence of relevant physiological models with human neurons, accounting for the little success of clinical trials. HD patient-derived induced pluripotent stem cells (HD iPSCs) have been used to examine human pathology in vitro, but this approach has the disadvantage of depriving cells of their natural environment, which is critical for neuronal development and aging. New in vivo chimeric models using HD iPSC-derived progenitor cells transplanted into newborn mice could avoid these shortcomings, thanks to the physiologically relevant environment of the developing mouse brain. Therefore, to characterize progressive human neurodegeneration in vivo we transplanted HD iPSC-derived forebrain progenitors into the striatum of neonatal wild-type mice. Most grafted cells differentiated into striatal neurons that sent axonal projections and established synaptic connections within the host basal ganglia circuitry. HD human neurons progressively developed mutant huntingtin (mHTT) oligomers and aggregates, which primarily targeted mitochondria, endoplasmic reticulum and nuclear membrane to cause structural abnormalities. Selective death of human medium spiny neurons and striatal degeneration altered mouse behavior, suggesting disease propagation to non-mutated host cells through extracellular vesicle-mediated transfer of soluble mHTT. Our findings cast new light on human neurodegeneration, unveiling cell and non-cell autonomous mechanisms that drive HD progression in patients. This work has been supported by Ministerio de Ciencia, Innovación y Universidades; the ISCIII; and the Generalitat of Catalunya, Spain; and CHDI foundation, USA.

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P358

Genome-wide analyses of regulatory regions and transcripts in hiPSC-derived neural stem/progenitor cells to define their safety and efficacy in cell therapy approaches for neurological disorders

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Human fetal-derived neural stem/progenitor cells (hfNSCs) display a favorable safety profile in allogeneic experimental transplantation settings for several neurodegenerative diseases, but require long-lasting immunosuppression of patients. Human induced pluripotent stem cells (hiPSC)-derived neural stem/progenitor cells (hiPS-NSCs) are promising alternative cell sources in view of autologous cell transplantation approaches. We gave proof-of-principle of safety and efficacy of hiPS-NSCs in ex vivo gene therapy protocols for metachromatic leukodystrophy (MLD), a fatal demyelinating disease caused by genetic mutations of the arylsulfatase A (ARSA) lysosomal enzyme. The optimization of hiPS-NSC production (purity, homogeneity) and a better assessment of their safety profile are mandatory in view of prospective clinical application. To this end, we envisage that defining transcriptional and epigenetic mechanisms underlying hiPSC to neural commitment well complement phenotypic and functional studies. Computational integration of RNA-seq and ChIP-seq data revealed a strong downregulation of pathways regulating pluripotency, cell cycle, and cancer-related processes with the concomitant appearance of a distinct ''neural signature'' in hiPS-NSCs. Interestingly, a dramatic change in the usage of cell-specific enhancers and super-enhancers during hiPSC neural commitment plays a major role in the generation and maintenance of hiPS-NSCs. Differences in the transcriptomic and epigenetic profiles between hiPS-NSCs and hfNSCs can be ascribed to culture conditions, regionalization pattern, and differentiation potential, with no major signs of abnormal differentiation and activation/misregulation of cancer-related pathways attributable to a pluripotent ''memory''. The definition of a consistent and comprehensive ''NSC signature'' might aid strategies for increasing safety and efficiency of hiPS-NSC-based cell therapy approaches.

P359

Single cell developmental trajectories reveal early phenotypes in Duchenne muscular dystrophy prior to skeletal muscle commitment

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Directed differentiations of induced pluripotent stem cells (iPSCs) have become a trusted model to study lineage commitment, as well as the initiation and progression of genetic diseases. For neuromuscular disorders in particular, they provide a powerful tool to decipher the early pathological events happening during muscle development, which are not precisely characterized from studies in animal models. Duchenne muscular dystrophy (DMD) is often considered an affection of children and young adults, with an incidence of approximately 1/3,300 male births. Symptoms appear after 2–4 years of age, but the precise moment at which disease phenotypes arise – even asymptomatically – is still unknown and prevents early therapeutic interventions. We generated skeletal muscle myotubes using iPSCs from a DMD patient and a healthy control, and compared their transcriptome dynamics by single-cell combinatorial indexing RNA-sequencing (sciRNA-seq) on more than 2,000 individual iPS cells sampled at multiple differentiation time points. Overall, we confirmed the expression of well-known regulators of myogenesis in defined clusters of cells expressing brachyury, Tbx6, Meox1, Pax3, Pax7, or Myog. However, reconstruction of the developmental trajectories at the single cell resolution revealed that early after mesoderm induction, a population of DMD cells shifted to an alternative path illustrated by a distinct branch in which myogenic regulators are strongly downregulated. Mining of previously published data showed that a significant fraction of the genes deregulated in this alternative branch is involved in somitogenesis, particularly in the formation of cell junctions and extracellular matrix, advocating for a modified muscle development program in DMD.

P360

Modeling Parkinson's disease using human midbrain-specific organoids

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Induced pluripotent stem (iPS) cells derived from somatic cells of patients represent an innovative tool for in vitro modeling of diseases, including neurodegenerative disease such as Parkinson disease (PD). We have previously generated a collection of iPSC lines representing both sporadic PD and familial PD patients, and identified distinct PD-related neurodegeneration phenotypes arising, upon long-term culture, in DAn differentiated from these PD-iPSC. Here, we demonstrate that three-dimensional (3D) differentiation of midbrain floor plate neural progenitor cells leads to organoids that resemble key features of the human midbrain. Indeed, ventral midbrain organoids (vmO) efficiently express crucial markers of dopaminergic fate such as FOXA2, LMX1A, EN1, OTX2 at their progenitor state as well as TH, MAP2 and FOXA2 at their mature state. Midbrain-specific organoids derived from PD patients carrying the LRRK2 G2019S mutation recapitulate disease-relevant phenotypes, such as α–synuclein accumulation and impaired autophagy (CMA). Interestingly, by using a co-culture system in which midbrain-specific organoids derived from PD patients carrying the LRRK2-G2019S mutation were exposed to SNCA-flag tagged astrocyte, we found that α–synuclein can spread from PD astrocyte to vmO neurons and induce endogenous α–synuclein accumulation. Thus, we have efficiently developed a robust method to generate 3D human midbrain organoids containing dopaminergic neurons and provided first evidence, in a physiological model, of synuclein

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''prionoid'' behavior. These studies may impact significantly on our understanding of the mechanisms that promote the spread of α–synuclein in vivo.

P361

Modelling chronic cervical spinal cord injury in aged rats for cell therapy studies

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Currently, there are increasing numbers of elder people suffering spinal cord injury (SCI) who could be candidates for spinal cord surgery; however, there are few available preclinical data in aged individuals. The objective of this study is to analyse how aged individuals can endure all the procedures required for a preclinical SCI model - extensive behavioural testing, surgical procedures, post-operative complications, intra-spinal cell transplantation and immunosuppression-, and to examine the potential effectiveness of human iPSC-derived Neural Progenitor Cells (hiPSC-NPCs). Behavioural tests were performed in rats before and after cervical hemi-contusion. The Fourth Generation Ohio State University Injury Device was used for inducing SCI. Four weeks after the lesion, hiPSC-NPCs injections were carried out in immunosuppressed animals receiving daily cyclosporine injections. Locomotor behaviour abilities and mortality were analysed. Survival and phenotype of transplanted human NPCs was assessed histologically four weeks after injection. As rats aged, their success at completing behavioural tests decreased. In addition, high mortality rates during behavioural training time (41.2%) and after cervical injury (63.2%) and transplantation (50%) procedures were observed. The animals did not show locomotor recovery one month after hiPSC-NPCs injection. Nevertheless, the injected cells survived and remained at and around the grafted site and did not cause tumours. Elderly rats are highly vulnerable to experimental interventions; thus, in order to study the potential efficacy of cell-based therapies in age-related chronic myelopathy, large groups of study must be set initially.

P362

Mitochondrial dysfunction in iPSC-derived Parkinson's disease astrocytes

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Parkinson's disease (PD) is associated with the degeneration of ventral midbrain dopaminergic (vmDA) neurons and the accumulation of toxic α-synuclein. Leucine-rich repeat kinase 2 (LRRK2) mutations are the most common genetic cause of Parkinson disease (PD). We recently demonstrated a non-cell autonomous contribution, in particular of astrocytes, during PD pathogenesis using iPSC-derived astrocytes and neurons from familial mutant LRRK2 G2019S PD patients and healthy individuals. Considering the established role of LRRK2 in regulating mitochondrial function and oxidative stress, here we used our PD patient-specific iPSC-based model to explore the relative contribution of astrocyte mitochondrial dysfunction to the neurodegeneration phenotype of patient-derived DAn. We found that the G2019S mutation causes severe mitochondrial fragmentation and accumulation in the perinuclear area, respiration impairment and ATP deficits only in astrocytes derived from LRRK2 PD patients. In addition, mitochondrial biogenesis and clearance were also impaired in LRRK2 iPSC-derived astrocytes leading to an increased production of reactive oxygen species (ROS). Reconstitution of normal levels of LRRK2 in PD-derived astrocytes normalized mitochondria morphology and function. Intriguingly, selective induction of mitophagy by Urolithin A effectively rescued these deficits. Future studies will evaluate the beneficial effects of treated astrocytes on dopaminergic neurons to hopefully aid in the discovery of new therapies.

P363

Patient-derived pancreatic tumor organoids identify therapeutic response to oncolytic adenovirus

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Pancreatic organoids are a well-established model to study PDAC carcinogenesis and to predict clinical responses to chemotherapy. Oncolytic virotherapy is envisioned as a novel treatment modality for pancreatic cancer and candidate viruses are being tested in clinical trials. In this work we explored the feasibility to use the organoid model as a platform to screen for oncolytic adenovirus response. We generated organoids from healthy pancreas and PDAC patients' tissues and propagated them in culture. This system was used to assess infectivity and spreading of different replication-competent adenoviruses. Oncolytic adenoviruses (OA) displayed good selectivity, by replicating in tumor organoids and not in healthy pancreas organoids. Furthermore, responses of individual patient's organoids to a set of OA showed clear differences in terms of cytotoxicity and synergism with standard chemotherapy. Adenoviral cytotoxicity in patient derived-organoids (PDO) predicted the antitumor efficacy in subcutaneous xenografts. Moreover, response to OA in organoids from orthotopic tumors and metastasis in nude mice mirrored original PDOs sensitivity. Our data suggest that patient-derived pancreatic tumor organoids can be a predictive tool to screen for sensitivity to oncolytic adenoviruses. Thus, we propose tumor organoids as a suitable and easy-handling platform to identify more personalized viral therapies alone or in combination.

P364

Identification of *in vitro* differentiated striatal progenitor cell sub-types for cell therapy treatment of Huntington's disease

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Cell therapy is a promising strategy to treat Huntington's disease (HD) where striatal medium spiny neuron (MSN) degeneration occurs. This approach requires human pluripotent stem cell (hPSC) in vitro differentiation protocols that efficiently generate the striatal progenitors required for transplantation. Previously we have successfully transplanted hPSC-derived neural progenitors into mouse striatum with a subset of transplanted cells differentiating into MSNs. However, various neural progenitor sub-types were present within the transplanted population and our goal is to transplant pure striatal progenitor populations. To achieve this we performed single-cell RNAseq on hPSC lines differentiated in vitro to the transplantation timepoint to establish the identity of the neural progenitor sub-types present at this stage and to identify specific cell surface markers of those sub-types. Data analysis has identified two main populations, neural precursor cells (NPCs) and neuroblasts (NBs), and a third transient intermediate population differentiating from NPCs to NBs. Promising specific cell surface markers for each population have also been identified. Further analysis has revealed that distinct cell sub-types are present within the NPC and NB populations. Work is ongoing to establish their identity and to define specific cell surface markers of each sub-type. We anticipate that this approach will permit the purification of specific striatal progenitor sub-types from in vitro differentiated cultures to produce a homogeneous population for transplantation, which in turn will improve HD cell therapy efficacy. This work has been supported by Ministerio de Ciencia, Innovación y Universidades; the ISCIII; and the Generalitat of Catalunya, Spain; and CHDI Foundation, USA.

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P365

Patient iPSC-derived macrophages to study interferon gamma related pathologies

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Interferon gamma (IFNγ) is a central mediator in cellular immunity and is essential for protection against a multitude of pathogens, including viral and mycobacterial species. One way IFNγ mediates these effects is by being the main activator of macrophages (MΦ). Defects affecting the IFNγ-immunity can lead to severe immunodeficiencies and a heightened susceptibility to different pathogens. One of these conditions is Mendelian Susceptibility to Mycobacterial Disease (MSMD) which is characterized by recurrent severe infections by otherwise only weakly virulent mycobacteria. MSMD is caused by mutations in the IL12-IFNγ-loop leading to an impaired activation of MΦ. Here we show a platform based on patient-derived iPSC-MΦ to recapitulate defects in the IFNγ-signaling pathway and study related pathologies. Material from patients suffering from MSMD or connected immunodeficiencies due to mutations in the IFNGR1/2 or STAT1 genes was reprogrammed into iPSCs and differentiated into MΦ. Cells showed typical MΦ morphology and IFNγ-independent functionality e.g. phagocytosis or GM-CSF clearance. In the IFNγ-dependent characteristics there were however marked differences. MΦ with complete or partial deficiency in the IFNγ-signaling pathway failed to fully upregulate associated surface markers e.g. HLA-DR and CD64 or induce IFNγ-dependent genes like IRF1 and CXCL10. Complete or partial impairment could also be observed at the level of STAT1 phosphorylation and reactive oxygen production. Furthermore, MΦ also showed defects when challenged with Bacillus-Calmette-Guérin as a common mycobacterial species. In summary, we provide an iPSC-based platform to study the diverse effects of IFNγ-signaling and defects of this pathway in the form of immunodeficiencies like MSMD.

P366

Using an iPS cell-based model to study neuroinflammation in Parkinson's disease

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Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by motor and non-motor symptoms. For decades, research on PD has focused on the two pathological hallmarks of the disease: the death of dopaminergic neurons (DAn) in the substantia nigra (SNc), and aggregation of

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abnormal protein alpha-synuclein. However, an extensive and consistent neuroinflammation component has been also described in PD, including an increased microglial activation, reactive astrocytes and lymphocyte infiltration in post-mortem PD brains as well as elevated levels of inflammatory cytokines in cerebrospinal fluid and serum of PD patients. To investigate the role of astrocyte function and neuroinflammation on the progression PD pathology, here we generated induced pluripotent stem cell (iPSC)-derived astrocytes from familial mutant LRRK2 G2019S PD patients and age/gender-matched controls (Ctrl). PD astrocytes displayed alterations in autophagy and mitochondrial dynamics, and a progressive accumulation of α-synuclein, when compared with Ctrl astrocytes. Analysis of transcriptome from iPSC-derived astrocytes indicates that the PD-derived astrocytes have a distinct expression signature compared to Ctrl. Additionally, PD-derived astrocytes were found to be robustly reactive, since they expressed high levels of GFAP along with an extreme retracted morphology, suggesting that they could also sustain inflammation by producing pro-inflammatory cytokines themselves. Follow-up analysis will identify the impaired pathways in PD glial cells that can potentially be targeted for future therapies focusing on inflammation pathways. These results represent an important step for modeling a complex disease such as PD with an inflammatory component, allowing for the investigation of the role of diseased astrocytes in neuronal function.

P367

Innovative 3D model for the establishment of primary paediatric low-grade glioma cultures: new platform for the preclinical study of immunotherapeutic approaches

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Solid tumours are characterized by a high structural complexity that is difficult to reproduce with common bidimensional (2D) systems. We developed an innovative fibrin-based hydrogel 3D model to establish primary low-grade glioma (LGG) cultures, otherwise difficult to maintain owing to the activation of senescence pathways. To date, 37 samples (19 astrocytomas and 18 gangliogliomas) were cultured in both 2D and 3D platforms, with an average culture duration of 177 ± 13 days. Cell lines identity was verified by short tandem repeats (STRs); immunohistochemical and immunofluorescence characterization (H&E, Ki67, tumour and differentiation markers such as GFAP, olig2 and Synaptophysin) revealed phenotype, cellular organization and a proliferative rate closer to those observed in the onset sample, as compared to 2D. Moreover, stabilized 3D cultures show a complex structural architecture. The analysis of cell senescence using ß-galactosidase assay revealed a lower senescence in the 3D cultures (3D:12,28% ± 4,3% of the cells vs 2D:50,69% ± 18,46%; p = 0,008). Finally, the evaluation of responses to radiotherapy, chemotherapy and to the innovative oncolytic adenovirus immunotherapeutic approach by MTS assay, demonstrates that cells in 2D culture are more sensitive to treatment than in 3D, suggesting an overestimation of the efficacy of such treatment by the 2D-setting, therefore reducing the predictive power. These data suggest that the 3D platform is able to recreate LGG structures more representative of the real sample and provides a better model to predict response to treatments, offering an innovative model for biological and therapeutic studies.

P368

Comparison of mobilized peripheral blood with cord blood CD34+ hematopoietic stem cells as source for artificial thymic organoid derived Natural Killer cells

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Natural Killer (NK) cells are promising tools for the development of anticancer therapies due to their high cytotoxic activity. The possibility to generate NK cells from hematopoietic progenitors is restricted by availability of umbilical cord blood (CB). We here show a method of generating high numbers of cytotoxic NK cells from CB hematopoietic stem cells (HSC) and mobilized peripheral blood (PB) by artificial thymic organoid (ATO). The MS5 cell line was transduced with human Delta-like protein 1 (DLL1) and human CD34+ HSC were isolated from mobilized PB and CB via magnetic beads. MS5-hDLL1 cells were aggregated with HSCs per ATO by centrifugation and cultured on a cell culture insert in serum-free media supplemented with Interleukin 7 (IL7), Interleukin 15 (IL15) and Fms-related tyrosine kinase 3 ligand (Flt3L). The functionality of generated NK cells was determined by Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) assay. In both PB and CB derived ATOs CD56+CD3- NK cells were observed, whereas a mature CD16+ population occurred after 2 weeks in CB derived cells compared to 5 weeks in PB derived cells. The procedure generated 1*106 NK cells out of 7500 CB derived HSC. ADCC assay for CB derived NK cells showed up to 70% killed target cells. The previously established in vitro T cell differentiation system (ATO) modified by supplementing IL15 to the culture media showed the potential for the generation of functional NK cells out of CB derived HSCs. Mobilized PB HSCs are able to give rise to NK cells but in low numbers.

P369

Establishment of a 3D co-culture model for the study of tumour-stroma interactions in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) has a dismal prognosis than can be partly attributed to its dense, active stroma. Cancer-associated fibroblasts (CAFs) represent the most abundant cell type of the pancreatic stromal compartment and they maintain a crosstalk with neoplastic cells that modulate cancer progression and therapeutic response. Recent identification of different CAF subpopulations with promoting or restricting tumour growth activities highlights a complex scenario of interactions within the tumour. Thus, model systems that recapitulate tumour microenvironment are envisioned as interesting tools to study tumour-stroma interactions and evaluate therapeutic responses. Here, we present a 3D coculture model of human PDAC derived organoids with CAFs grown in serum-reduced medium. We observed organoid shape diversity based on the spatial distribution of CAFs, with a morphologic change in organoids growing in close contact to CAFs. The cocultures showed increased tumour epithelia organoid viability as compared to organoids in monoculture, demonstrating a cooperative interaction between cancer cells and CAFs. Furthermore, signs of CAF plasticity were evidenced when CAFs were grown in organoids conditioned-media, as shown by decreased αSMA expression and increase in IL-6 content. These findings suggest that the 3D organoids-CAF co-cultures represent a model of neoplastic-stromal cells interactions and hold promise to study the role of key molecules in this interplay facilitating the testing of therapeutic drugs.

P370

Elongated human 3D neural tissue for developmental and regenerative studies: NEUROTUBES

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Current methods to build 3D neural tissue result in spherical structures of small size, lacking a developmental axis, and difficult to be prospectively patterned by the application of diffusible signals. Building efficient, valid and robust in vitro models for brain circuitry and disease studies is an important goal to understand, treat and cure them. The long-term goal is to provide diffusible signals (growth and survival factors, morphogens) to 3D neural tissues that would confer new functional properties. Therefore, we have generated a new neural 3D tissue with an axis, so called ''Neurotubes'': elongated 3D tissues resulting from the aggregation and differentiation/maturation of human Neural Stem Cells. The mini-wells for the generation of neurotubes have been manufactured in PDMS. A protocol for neurotubes generations has been optimized and established, using both human Neural Stem Cells (line hNS1, forebrain) and human Embryonic Stem Cells (line WA09, H9). The length of the neurotubes is enough (3–4mm long) for the application of localized signals. The first spatial patterning test has been conducted with hNS1 cells, using a GSK3 inhibitor and activator of the Wnt signaling pathway. Histological studies revealed the presence of non-differentiated cells (Nestin+, Sox2+), neurons (β-III-tubulin+ and MAP2+), with only occasional apoptotic cells (Activated Caspase3+). Besides, on going gene expression studies and proteomics are being conducted, and in the mid-term-future, studies of electrical stimulation, cell death and extracellular matrix will be done. This type of tissue has never been neither achieved nor reported earlier and could represent a useful advancement in neuroscience.

P371

IPS-PANIA project to develop a HLA matched bank of iPSC in Spain

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Induced Pluripotent Stem Cells (iPSC) derived cell therapies are part of an interesting new portfolio in the word of regenerative medicine. One of the approaches to make affordable iPSC derived therapies is the use of allogenic HLA matched donors to generate the cell lines. To address this, IPS-PANIA is a project lead by the CMRB-BST (Blood bank Barcelona) consortium to derive a collection a of seven clinical grade iPSC lines with homozygous HLA haplotypes that could cover a significant percentage of the Spanish population. Haematopoietic progenitors isolated from stored frozen cord blood samples from homozygous donors will be reprogrammed and expanded in clinical grade conditions to provide suitable starting material for cell therapy medicinal products and future clinical trials.

P372

Prediction of sgRNA specificity for CRISPR/Cas9 genome editing by measuring DNA damage response in induced pluripotent stem cells

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CRISPR/Cas9 recently emerged as straightforward method for genome editing and its potential for the treatment of genetic human diseases is currently under investigation. Although CRISPR/Cas9 is supposedly very precise, its specificity is not error-free. Indeed, edited cells could present Cas9 mediated cleavage in undesired regions (off-targets), which can lead to an oncogenic potential of the modification in patients. Since off-target genome editing highly depends on the specificity of a sgRNA, extensive off-target analysis of a potential therapeutic sgRNA is necessary. Here we characterized the DNA damage response in human iPSCs upon CRISPR/Cas9 genome editing, in order to find a correlation between DNA damage response and the specificity of a sgRNA. iPSCs were chosen as model since they are particularly sensitive to DNA damage. To this aim, iPSCs expressing an inducible Cas9 protein were transduced with lentiviruses carrying specific and unspecific sgRNAs that generate 0 to over 1000 double strand breaks in genes that are not essential for cell survival. We demonstrated a correlation between number of double strand breaks and increased expression of p21 and FAS, thus being able to distinguish the DNA damage response of a non-targeting control, single-cutter as well as multi-cutter sgRNA. Our final goal is to identify a DNA damage response signature to generate a tool able to predict the specificity of a potential therapeutic sgRNA.

P373

Targeting the AAVS1 locus for genetic correction of p47-CGD in iPSC revealed differential transgene silencing of myeloid-specific promoters

ABSTRACT WITHDRAWN

P374

Therapeutic effects and homing of mesenchymal stem cell and neural progenitor cells after intra-arterial transplantation into rats with experimental ischemic stroke

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The aim of this study was to compare curative effects and homing of mesenchymal stem cells and neural progenitor cells derived from different sources and transplanted intra-arterial into rats with experimental stroke. 24h after transient occlusion of the right middle cerebral artery, Wistar rats were transplanted through the ipsilateral internal carotid artery with 1 ml saline or 5x10^5 cells in 1 ml. The following cell types were tested: mesenchymal stem cells from human placenta (pMSCs) or dental pulp (dMSCs), and neural progenitor cells differentiated from induced pluripotent cells (iNPCs) or directly reprogrammed from human bone marrow MSCs without genetic manipulations (drMSCs). Therapeutic effects, infarct volume and cell distribution and homing within the brain were studied for 14d after cell transplantation using 7T-MRI, behavioral tests and immunohistochemistry. Transplantation of all the studied cell types provided significantly better recovery from stroke compared to the control group. However, only iNPCs and drNPCs accelerated reduction of stroke volume. Immediately after the intra-arterial injection, all types of transplanted cells were found in the hemisphere of administration mainly at the periphery of the infarction zone and in the brain stem. Cells were visualized inside small vessels in tight contact with the vascular wall. pMSCs remained in the brain tissue up to 3–4d that is longer compared to the other cell types (1–3d). Our results indicate that all transplanted cells, despite some differences, exert their therapeutic action through paracrine trigger mechanisms. This work was supported by the Ministry of Education and Science of the RF (project №14.604.21.0184RFMEFI60417X0184).

P375

Bioreactor grown iPSc derived hepatocyte-like cells for testing liver directed gene therapy

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Background: Liver-targeted AAV mediated gene therapy is an effective treatment for Haemophilia in adults despite low affinity of some vectors for human compared with murine hepatocytes. In early childhood during rapid liver growth high rate of hepatocellular proliferation is accompanied by loss of episomal AAV genomes. Thus, the effect of AAV gene therapy is expected to be short-lived. Therefore, it is important to test the affinity of vectors in human cells. Human mice are appropriate models to study human hepatocyte vector transduction, however this complex model may not be available to all scientists.

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Aims: We propose to use human iPSc-derived bioreactor cultured hepatocyte like cells (hi-Heps) that can be maintained in long-term culture conditions as an in-vitro model to test suitability of vectors.

Methods and Results: Standard hi-Hep differentiation protocol was used. After initial stages of differentiation human iPS cells were transferred into a custom-built bioreactor seeded on decellularised mouse liver scaffold and cultured for up to 35 days. The hepatocyte functionality was assessed by immunohistochemistry: expression of liver specific proteins e.g. albumin, afp, hnf4a; urea, glycogen, bile acid production assays. The assays confirmed improving maturity of hi-Heps over time. Different titres of Lentiviral and AAV vectors were injected into the bioreactor grown hi-Heps and demonstrated vector transduction and transgene expression.

Summary and Conclusions: In this study we were able to demonstrate that long-term bioreactor cultured hi-Heps can be used as an in-vitro model for studying the efficacy of potential therapeutic gene therapy vectors.

P376

Modeling haematopoietic niche in osteopetrosis using patient-derived induced pluripotent stem cells

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Malignant infantile osteopetrosis is an autosomal-recessive-disorder of bone-metabolism leading to defective-bone-resorbtion. The only available-treatment is allogeneic-hematopoietic stem cell-transplantation. The objective of this study was to model hematopoietic-niche in osteopetrosis. Induced pluripotent stem cell-(hiPSC)-lines were generated from peripheral blood mononuclear cells of three patients carrying TCIRG1-mutation using SeV (Sendai-viral-vector). IPSC-lines were differentiated first into hematopoietic stem cells-(iHSCs), and then into myeloid-progenitors, and osteoclasts using a step-wise protocol. Immunophenotype and colony-forming-capacity (CFU) of IPS-derived-hematopoietic-stem/progenitor cells-(iHSCs) were evaluated.iHSCs-derived-osteoclasts were characterized by scanning-electron-microscope (SEM), flow-cytometry (CD14, CD16, CD18, CD45, andCD51/61), IFstaining and expression of osteoclast-specific-molecular-markers. Different coculture conditions with bone-marrow-derived-hMSCs and iHSCs were set up to study the interaction between osteopetrotic-iHSCs and healthy-and/or osteopetrotic-MSCs as an in vitro hematopoietic-niche-model. After coculture, expression of the genes (Ang, Sdf-1, Jag-1, N-Cad, Kit-L, Opn) related to HSC-kinetics were analyzed, and migration-assays were done. Over-95%of iPSC-lines were positive for pluripotency-markers (OCT4, SSEA-4, SOX2, TRA-1-60), and expressed pluripotency-associated-genes. All-lines were differentiated succesfully into-iHSCs, myeloid-progenitors and osteoclasts. Osteopetrotic-iHSCs had three-fold increased ability to form CFU-M compared to the controls, while BFU-E-forming-ability was observed only in donor-iHSCs. Osteopetrotic-iPSCs-derived osteoclasts were positive for Cathepsin-K, Rhodamine, and TRAP, and exhibited osteoclast-spesific surface markers, but showed significantly reduced expression of Cathepsin-K, Calcitonin-R, and NFATC1. There was a significant-difference between the podosome-size of patient-and control-osteoclasts. Following coculture with healthy-control-iHSCs, the expression of Jagged-1, Ang-1, Kit-L, and Sdf-1 required for maintenance and homing of HSC in the niche, increased and Opn, negative-regulator of HSC pool, increased in osteopetrotic-MSCs. Overexpression of N-cadherin, probably related to its negative-role in MSC-osteoblastic-differentiation, observed in osteopetrotic-MSCs and decreased after coculture. The migration-potential of osteopetrotic-iHCSs were found impaired compared to control-iHSCs. In conclusion, our results indicates that dysfunctional-osteoclasts in osteopetrosis lead to defective-HSC niche formation resulting from altered MSC-compartment, and abnormal HSC-homing besides not providing space for hematopoiesis.

P377

AAV gene therapy for Parkinson's disease: Effects of AAV-GBA treatment in iPSC-derived neurons from Parkinson's disease patients carrying GBA mutations

ABSTRACT WITHDRAWN

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P378

Investigating neural differentiation capacity in Alzheimer's disease iPSC-derived neural stem cells

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Neurodegeneration in Alzheimer's disease (AD) is associated with impaired intraneuronal clearance and dysregulated hippocampal neurogenesis. Neural stem cells (NSC) maintain adult neurogenesis and depletion of the NSC niche has been associated with age-related cognitive decline and dementia. We hypothesise that factors associated with age-related cognitive decline such as inflammation and defective anti-oxidant response modulate homeostatic NSC activity in AD resulting in depletion of the stem cell niche and exacerbation of disease. NSC from patients with familial AD (PSEN1 A246E and PSEN1 M146L) and healthy controls were reprogrammed to induced pluripotent stem cells (iPSC) to provide an inexhaustible resource of patient-derived cells for this study. Patient and control iPSC lines were confirmed as Tra-181+/OCT4+ by immunocytochemistry, amplified, cryopreserved and then differentiated back to neural rosette forming SOX2+/Nestin+ NSCs. AD patient and control NSCs were cultured in suspension as neurospheres under defined culture conditions for 11 and 25 days and assessed for relative expression of markers of progressive neural maturation (SOX2, Nestin, N-Cadherin, NeuN, MAP2 and βIII-tubulin). Preliminary data indicates a depletion of neural stem cell markers in NSCs containing the PSEN1 A246E mutation when compared to controls, which may impair generation of mature neurons and exacerbate the disease phenotype.

P379

Generation of genetically modified osteoclasts from induced pluripotent stem cells derived from an infantile malignant osteopetrosis patient with a TCIRG1 mutation

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Infantile malignant osteopetrosis (IMO) is an autosomal recessive disorder characterized by non-functional osteoclasts and a fatal outcome early in childhood. About 50% of patients have mutations in the TCIRG1 gene, encoding for an osteoclast proton pump subunit. To develop a disease model for TCIRG1-deficient IMO, we generated iPSCs from a 2-year-old female IMO patient who carries the homozygous c.11279G>A (IVS18 + 1) mutation in TCIRG1. Fibroblasts were isolated from a skin biopsy and reprogrammed using integration-free Sendai viral vectors. CTRL-iPSCs were generated from healthy fibroblasts. Pluripotency of the cell lines was confirmed by their expression of typical ES cell markers at iPSC stage and by their capacity to differentiate into cells of all three germ layers. IMO-iPSCs were transduced with a lentiviral vector expressing TCIRG1 and containing the CBX3-UCOE element. EBs were generated from the iPSCs, transferred to tissue culture plates after 4 days of culture, and differentiated in X-VIVO media supplemented with M-CSF and IL-3. Non-adherent cells were harvested from week 3 onward and were differentiated into osteoclasts on bovine bone slices in the presence of M-CSF and RANKL. TRAP activity in the media of all osteoclast cultures was comparable. CTX-I release into the media of IMO-iPSC-TCIRG1-derived osteoclasts was in average 5-fold higher than that of IMO-iPSC-derived osteoclasts and 35% that of CTRL-iPSC-derived osteoclasts. This indicates that non-resorbing osteoclasts were generated from an IMO patient-derived hiPS cell line and that the phenotype was partially rescued, providing a valuable resource for pathobiology studies and therapy development for this form of severe osteopetrosis.

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Human induced pluripotent stem cells-derived cardiomyocytes efficiently engraft and improve murine heart function after myocardial infarction

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INTRODUCTION: We evaluated whether the therapeutic potential of human PSC-derived cardiomyocytes (hiPSC-CMs) in the murine model of MI is linked with their permanent engraftment.

METHODS: HiPSC obtained by reprogramming of blood leukocytes were transduced with lentiviral vectors harboring luciferase (luc) or luc and heme oxygenase-1 (HMOX1). HiPSC were differentiated to cardiomyocytes and 5 x 105 cells were administered into the hearts of NOD-SCID mice immediately after MI induction. Control mice were subjected to sham operation or underwent MI and were injected with saline or with luciferase-overexpressing human adipose stromal cells (ADSC). VEVO2100 ultrasonography was performed on day 7, 14, 28 and 42 whereas the presence of cells was monitored using IVIS Spectrum upon administration of luciferin and finally analyzed in heart sections.

RESULTS: IVIS demonstrated strong luciferase activity throughout the whole experiment in hearts injected with hiPSC-CMs-HMOX1, while it was low in hiPSC-CMs-luc animals. Left ventricular ejection fraction (LVEF) significantly deteriorated to about 20–25% after MI and remained low in animals injected with saline or ADSC. LVEF in mice injected with iPSC-CMs-Luc or hiPSC-CMs-Hmox1 were significantly deteriorated up to 28 days but then it almost doubled at 42 days in all animals receiving iPSC-CMs independently of their modification. Importantly, the presence of hiPSC-CMs-Luc or hiPSC-CMs-Hmox1 was confirmed in murine heart sections by detection with specific anti-human-Ku80 antibody.

CONCLUSION: These results strongly indicate that administration of hiPSC-CMs, unlike ADSC, restores murine heart function after MI. Delayed improvement together with cells' engraftment indicate for the direct contribution of hiPSC-CMs to heart function.

P381

Stem cell derived brain organoids, a promising model to study Adeno associated viruses for CNS gene therapy

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P382

A human iPSC-based model of globoid cell leukodystrophy uncovers mutation- and cell-type specific neurodevelopmental defects

ABSTRACT WITHDRAWN

ABSTRACT WITHDRAWN

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Interrogation of the molecular role of p62 in iPS reprogramming and maintenance of pluripotency

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The reprogramming of adult cells to induced pluripotent stem cells (iPSC) has huge potential for disease modelling, drug screening and regenerative medicine. The inefficiency of the reprogramming process from somatic cell to pluripotency using established methodologies is a major roadblock to realising the full potential of iPSC in cell therapy. The sequestosome protein p62 is a highly conserved scaffolding protein with roles in nutrient sensing, autophagy, inflammation and disease. The canonical role of p62 is to aggregate ubiquitinated proteins to form the sequestosome, a precursor to the autophagasome but it has six known functional domains with a range of binding partners including Nrf2 and LC3 as well as ubiquitinated proteinsinvolved in the anti-oxidant response and inflammation. There is a well-established role for autophagy in reprogramming, and a growing body of evidence for the role of p62 in maintaining stemness in cancer stem cells. As yet, the role of p62 in establishing or maintaining pluripotency in iPSC has not been elucidated. Utilising p62 null patient fibroblasts, and lentiviral knockdown and mutant p62 over-expression vectors I aim to establish the contribution p62 makes on iPSC reprogramming and the maintenance of the pluripotent state. My data so far suggests that p62 null patient fibroblasts reprogram with the same efficiency as healthy controls, but the resultant iPS cells are inextricably primed to spontaneous differentiation. Future experiments, including RNA sequencing will aim to elucidate the mechanism behind these findings.

P384

Improvement of pluripotent stem cell-derived skeletal muscle myotube maturation through the combination of physical and chemical factors

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Genetic disease modelling with pluripotent stem cells (PSCs) requires the optimization of differentiation protocols to recreate the tissue context in which phenotypes manifest as closely as possible. For skeletal muscle in particular, this was initially achieved by overexpressing master regulators of myogenesis such as MYOD1 or PAX7, directly at the pluripotent state,

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thereby missing the early stages of paraxial mesoderm development. More recently, several protocols based on defined culture media have been proposed to engage PSCs into the paraxial mesoderm lineage, generate myogenic progenitors and ultimately plurinucleated cells expressing muscle markers. However, the morphology of the resulting myotubes markedly differ from their in vivo equivalent. Despite the observation of typical striation patterns and sporadic spontaneous contractions, the myotubes are thin and spindly, with centrally located nuclei. Here, we propose to combine growth factors, small molecules and biophysical cues such as nanopatterned culture substrates or electromechanical stimulations, to mimic the in vivo microenvironment and improve the maturation of PSC-derived myotubes. In particular, we identified inhibitors of the TGF-beta I receptor as powerful maturation enhancers if added during terminal differentiation. We are using this proof of principle to optimize a battery of ''maturation metrics'' (e.g. fluorescent stainings, gene expression profiles, electrophysiological measures), that will help us compare the structure and function of PSC-derived myotubes submitted to different culture conditions in a multiparametric manner. Going forward, we hypothesize that more mature myotubes will help recapitulate the phenotypes seen in neuromuscular disorders more faithfully, and facilitate the identification of relevant therapeutic targets.

P385

Pall Xpansion® Bioreactor supports progenitor cell growth to >1 million cells/cm2 and proper cell differentiation

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1: Pall Life Sciences

With the recent increase in early phase cell therapy clinical trials, there is a need for a manufacturing platform which can be implemented in research labs and easily scaled up to expedite process development studies, pre-clinical testing, and large-scale expansion of adherent human cells. Although various platforms such as traditional flatware and stirred tank bioreactors with microcarriers exist and have been well characterized, traditional flatware often does not allow for tight control of the cellular environment and is not scalable. Pall's Xpansion single-use bioreactor offers a tightly-controlled, scalable manufacturing platform for cell therapy applications, allowing for expedited process development, pre-clinical testing and large scale, high-density expansion of progenitor cells while maintaining their differentiation capacity. We have previously demonstrated efficient expansion of both epithelial cells (Vero, HEK293) and human mesenchymal stem/stromal (hMSC) in the Xpansion single-use bioreactor. Here we extend these findings by successfully expanding a proprietary progenitor cell to extremely high cell densities (> 1 million cells/cm2). The first stage of this process begins with a pluripotent stem cell line, which is expanded and differentiated into proprietary lineage-specific progenitor cells. We then demonstrate growth and differentiation of this progenitor cell in the Xpansion bioreactor using a 14-day and 21-day protocol. In summary, both protocols resulted in 1) cell expansion to >1 million cells/cm2 and equivalent cell densities to the traditional flatware process control; 2) equivalent differentiation to a more mature cell fate as indicated by expression of key cell surface markers; and 3) self-assembly into characteristic three-dimensional structures.

P386

Development of CRISPR/Cas9 gene editing double-KI and brain organoid differentiation system for the study of brain development and disease

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CRISPR/Cas9 gene Editing and Organoids are revolutionizing the fields of developmental and stem cell biology as they have enabled precise and efficient gene editing combined with the in vitro generation of complex structures resembling whole organs. When stem cells are grown in a 3D environment, they recapitulate the mechanisms of organogenesis seen in vivo. These complex structures provide a unique opportunity to model human organ development in a system remarkably similar to development in vivo. Human brain development, which is the most sophisticated organ of our bodies, it is currently properly modeled in vitro by the use of brain organoids. To this end, with the aim of developing an screening system for neurodevelopmental assays, we have developed a double KI human ES cell line for Sox2 targeted with Cherry and Tuj1 targeted with GFP. Further to that, we have implemented an efficient brain organoid differentiation system for modeling brain organogenesis, and we have optimized imaging for proper visualization of the double KI-fluorescent brain organoids. With this technology we aim to introduce neurodevelopmental perturbations in the in vitro culture system and to quantify them.

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Generation of human microglia-like cells from PD patient-specific iPS cells

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Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc) and accumulation of cytoplasmic protein inclusions known as Lewy bodies and Lewy neurites. In addition, microglial activation and increase in astroglia and lymphocyte infiltration occur in PD. Thanks to the development of induced pluripotent stem cells (iPSCs) technology, we have been able to previously model neuronal cell death and α-synuclein accumulation in DAn and more recently have demonstrated neurodegenerative phenotype in iPSC-derived neurons due to non-cell autonomous effect of PD astrocytes. To mimic neuron-glia interactions in the PD brain, here we generated iPSC- derived human microglia (hMG) cells from two familial mutant LRRK2-PD patients and two age/gender-matched controls. hMG cells expressed key microglia-specific markers, were functionally phagocytic and respond to inflammatory stimuli. Interestingly, LRRK2-PD hMG cells expressed at basal conditions similar pro-inflammatory mediators but less anti-inflammatory genes. Not only that, upon stimulation,

LRRK2-PD hMG cells express less pro-inflammatory genes compared to the control hMG, suggesting a disbalance of the microglial pro-healing and pro-killing properties within our LRRK2-PD hMG cells. The neuron-glial crosstalk will be evaluated in the 2D and 3D co-culture system, to depict the exact contribution of hMG cells to the neural maintenance in vitro. So far, our results represent an important step for investigating the role of diseased microglia in PD and may serve as a valid human cellular model to identify compounds that can improve microglial homeostatic clearance functions and reduce chronically activated microglia.

P388

Cytoskeleton proteins and its epigenetic regulation in the endometriotic lesions

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Endometriosis is a widespread gynecological pathology that can lead to a loss of fertility in women of reproductive age. Despite the long history of studying the cause and pathogenesis of the disease, it remains not completely clear, which makes most treatment approaches palliative. In this work, we decided to evaluate the content of the main cytoskeletal proteins and proteins that epigenetically regulate the expression of various genes in intrasurgery endometriotic lesions biopsies of women with endometriosis compared with the control group. Subjects were informed of the purpose of the study and all of them provided written consent to participate. The study was complied with the guidelines of the Declaration of Helsinki. The results indicate that the content of the main structural proteins (beta- and gamma-actin, beta-tubulin) does not change, however, the content of actin-binding protein alpha-actinin-1 decreases by 24% ($p < 0.05$) in the group of patients with endometriosis compared to control. At the same time, the content of its structural homolog alpha-actinin-4 does not change. This pattern of distribution of alpha-actinin isoforms is usually associated with an increase in the migratory ability of cells. At the same time, the content of hydroxylase TET3, which ensures active demethylation of the genome, is reduced by 16% ($p < 0.05$) in the group of endometriosis compared with the control against the background of the constant content of methylase DNMT3a, acetylase HAT1 and deacetylase HDAC1. The latter may lead to a change in the regulation of genome methylation status.

P389

The acetylase and deacetylase content in mice ovaries, testes, heart and lung tissues under modeling weightlessness

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Human exploration of deep space is difficult, including because of a number of medical problems, the solution of which requires an understanding of the mechanisms of interaction between the cell and the gravitational field. It is well known that, under space flight conditions and under modeling its effects on the Earth, there is a change in the expression of various genes, for example encoding cytoskeletal proteins, in different types of tissues. However, the reasons for this change are unclear. In this study, we simulated the effects of weightlessness on Earth for mice by long-term hind limbs antiorthostatic suspension. The content of acetylase HAT1 and deacetylase HDAC1 in the tissues of the ovaries, testes, heart and lungs as well as the expression of the genes encoding them, was evaluated. The results show that after suspension, the content of HAT1 in the mice ovaries did not change, while the content of HDAC1 decreased by 17% ($p < 0.05$) relative to the control level. The content of HAT1 and HDAC1 did not change in the mice testes after 30 days of modeling microgravity, but the content of HDAC1 mRNA decreased by 46% ($p < 0.05$). At the same time, in the heart and lungs of mice, the acetylase content also did not change, but the HDAC1 deacetylase content increased by 32% and 26% ($p < 0.05$), respectively, as well as mRNA – by 74% and 38% ($p < 0.05$), respectively. The opposite changes let us suggest the mechanism of formation an adaptive pattern of gene expression.

P390

Platelet production from murine induced pluripotent stem cells by the inducible overexpression of supporting factors

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Platelets are small anuclear blood cells that circulate in the blood in a resting state but can be activated by external cues. Platelets then secrete numerous factors from their granules, a feature potentially employable to deliver therapeutic proteins. Platelets can be produced from induced pluripotent stem cells (iPSC) as unlimited source; however, recovered numbers are low. To optimize megakaryocyte (MK) and platelet output from murine iPSC, we investigated overexpression of the transcription factors Gata-1, Nfe2 and Pbx1, or the small GTPases RhoA and Cdc42. To avoid off-target effects, we generated iPSC from mice carrying the transactivator in the Rosa26locus and expressed the factors from Tet-inducible gammaretroviral vectors. Differentiation of iPSC was initiated by embryoid body (EB) formation and hematopoietic commitment supported by SCF and IL-3. After EB dissociation, CD41-positive cells were co-cultivated on OP-9 feeder cells with Thpo and SCF. Overexpression of Gata-1 and hyperactive Cdc42 increased MK output 7.5-fold and 6-fold, respectively, but had no effect on final differentiation and platelet release. Nfe2, in contrast, supported platelet production per MK (100/MK). Cytomorphological and electron microscopic analyses identified the typical MK morphology with enlarged cells, multilobulated nuclei, alpha and dense granules and the internal membrane system. Also platelets showed typical morphology although their size was larger than for in vivo counterparts. In vitro platelets from Gata-1-overexpressing cultures were able to spread on fibrinogen or collagen-related peptide indicating that they were functional in that aspect. Our strategy identified factors increasing in vitro MK/platelet production and can be used for further optimizations.

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Extracellular vesicles with enhanced immunosuppression capacity secreted by genetically improved mesenchymal stem cells derived from dental pulp

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Despite the rise of MSC-based therapeutic strategies during the last years, clinical trials performed heretofore delivered inconsistent results. In recent years, it has been described that extracellular vesicles (EV) secreted by MSCs are able to recapitulate tissue regeneration and immunesuppressive properties of MSC, although, the effective dose is quite high, complicating any translational study in humans. In this piece of work, we generated a non-senescing MSC line with increased immunosuppressive potential using gen editing tools. In addition, we developed a specific cytokine based preconditioning culture medium for EV isolation that improves immunomodulatory capacity of the vesicles secreted by our cell line. Our results indicate that MSCs derived EVs are incorporated by monocytes, T, B and NK cells. Once incorporated, EVs derived from improved MSCs are able to reduce CD3/CD28 activated T cell proliferation more effectively than none improved EVs. At the same time, our EVs impair the activation of LPS stimulated monocytes. Furthermore, we observed that improved EVs reduced the swelling of mouse ear, declined immune cell infiltration and improved tissue integrity in DTH mouse model. Taking all together, our data indicates that we have generated a long standing EVs source which secretes not only more suppressive EVs but more EVs, so it will facilitate obtaining a more standard and cost-effective therapeutic product. Thus, our EVs can provide a new and safe tool for treating autoimmune diseases and other inflammatory diseases. Co-funded by FEDER ''Una manera de hacer Europa'', RD6/0011/0004, PI16/00107, ACIF/2018/254.

P392

Retinitis pigmentosa disease model from iPSC of a patient with PRPF31 c.165G > A mutation

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Mechanism of disease associated to mutations in PRPF31 and other splicing factors causing retinal degeneration is under debate. Human-derived cellular models are useful tools to study these diseases and to search for therapeutic targets. In the present study, a retinitis pigmentosa patient and a heathy family member were the donors of the starting biological material after informed consent. From a peripheral blood sample, DNA and mononuclear cells were obtained. Genomic DNA analysis identified heterozygous PRPF31 c.165G > A change as the causative mutation for autosomal dominant retinitis pigmentosa. Yamanaka factors transduction via Sendai virus was used to obtain the iPS cell lines from the primary monocytes of both the patient and healthy control. A complete characterization of the iPS cell lines was performed before starting the preparation of retinal pigmented epithelium (RPE) cellular models. In the recent literature RPE is considered as the primary retinal cell type affected by mutations in PRPF31. After differentiation and maturation, RPE model was authenticated checking for pigmentation, cell morphology, gene and protein expression of RPE markers and physiological features of transepithelial resistance and phagocytosis of photoreceptor outer segments. Protein quantification and sub-cellular distribution showed not only the expected decrease in PRPF31 protein, but also an abnormal localization of the splicing factor. RPE presented a more pronounced phenotype compared to iPS cells, correlating to the specific retinal disease associated to PRPF31 mutations even when it is an ubiquitously expressed gene.

P393

Enteroendocrine cell-derived hormone A is involved in the intestinal homeostasis by directing the differentiation of the mouse intestinal organoid

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As the largest endocrine system in the body, enteroendocrine cells (EECs) produce the highest level of hormones and bioactive molecules despite that they comprise only 1% of the intestinal epithelium. In this study, we cultured intestinal epithelial organoids and focused on EEC-derived endogenous signals to evaluate their impact on the intestinal homeostasis. It was noted that one of EEC-secreted hormone A was impeded the normal generation of intestinal organoids; upon treatment of A, organoid growth was retarded and the typical budding pattern was almost disappeared, resulting in the round to oval shaped-organoids. The epithelial lining was intact and budding ability was restored after A withdrawal, suggesting that A did not induce epithelial cell death. We found that the morphology of A-treated organoid was similar to that of IWP-2(Wnt inhibitor)-, DAPT (Notch inhibitor)-and U0126(MEK inhibitor)-treated organoid. Also, rebudding process after A removal was accelerated with Chir99021 and Epiregulin, which activates Wnt and EGF pathway, respectively. Then we performed qPCR to screen the ISC and differentiated intestinal cell markers. Importantly, both active ISC and proliferation markers are down-regulated, while secretory lineage markers such as Neurogenin3, defensin-a and Gob5 were increased upon A treatment. Immunohistochemistry analysis also revealed that lineage-specific differentiation was induced after A treatment. These data suggest the novel endogenous impact of EEC-derived hormones on ISC maintenance, differentiation and intestinal homeostasis.

P394

Production and characterization of two human induced pluripotent stem cell lines from patient with krabbe disease

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Krabbe disease (globoid cell leukodystrophy, GLD) is a rare lysosomal storage disorder affecting the central and peripheral nervous systems, It is caused by an inherited deficiency of galactocerebrosidase (GALC). The main pathological changes observed, including the presence of globoid cells and decreased myelin, appear to result from the toxic nature of psychosine and accumulation of galactosylceramide that cannot be degraded due to the GALC deficiency. Current treatment for globoid cell leukodystrophy is limited to bone marrow or umbilical cord blood transplantation. However, these therapies are not curative and simply slow the progression of the disease. We generated two human iPSC lines from primary dermal fibroblasts, one derived from GLD patient carring two mutations (c.461C >A, c.1244G >A), the other comes from the patient's father with one mutation (c.461C>A). Two cell lines, Krabbe C and Krabbe D, were characterized for expression of pluripotency marker genes (Oct4, Nanog, SOX2, TRA-1-60) and differentiated into all three germ layers using embryoid body (EB) formation. The two hiPSC lines were genetically stable and retained the GALC mutations of each donor. This clones represent a useful tool to study the pathomechanisms underlying the GALC deficiency and a new model for the treatment of GLD.

P395

Regenerative effects of solid neural tissue grafts located in gelatin hydrogel conduit for treatment of peripheral nerve injury

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Damage of the peripheral nerves is one of the most widespread and severe types of injury. The cell therapy and nerve guide conduits construction are promising modern approaches to the treatment of peripheral nerve injury. Here, we investigate the regenerative effects and development patterns of the mouse solid fetal neocortex and spinal cord grafts located in gelatin hydrogel conduit. The frontal neocortex tissue and spinal cord tissue were obtained from the fetuses harvested from transgenic EGFP mice. The solid grafts were injected into the hydrogel conduits which were connected the nerve stumps after cut. Eight weeks after surgery we revealed that only fetal neocortex solid graft cells had survived after implantation. Immunohistochemical study showed that some of the transplanted cells expressed neural marker NeuN, but the most differentiated in glial lineage, which was confirmed with immunostaining for GFAP and S100β. Estimation of the motor function recovery by the walking-track analysis showed that 8 weeks after surgery the neocortex solid graft group differed significantly from the control. We revealed that the hydrogel conduit is suitable for nerve re-growth and that the fetal neocortex grafted cells can survive, differentiate, and stimulate functional recovery after the nerve injury. The study was supported by the IDB RAS Government basic research program and RAS Presidium Program ''Innovations in biomedicine'', project '' Development of a new biomedical technology for the treatment of peripheral nerve injury''.

P396

Neuroprotective effects of adipose-derived stromal vascular fraction stem cells on acute traumatic spine cord injuries in rats

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Spinal cord injuries remain a real public health issue. However, despite more than half a century of intensive research and numerous clinical trials, the different strategies for repairing the spinal cord remain still inconclusive, even if the diagnosis and the patient care have improved considerably. When Japan and China are embarking on cell therapy trials for chronic lesions, there is currently no treatment that would allow spinal cord injured people to preserve damaged tissue avoiding the secondary expansion of the lesion and recovering of their sensorimotor functions. We thought the stromal vascular fraction (SVF) from fat could be neuroprotective in the acute phase of spinal cord injuries. SVF is extractable in just 2 h and contains mesenchymal and hematopoietic stem cells known for their regenerative and anti-inflammatory properties. Our strategy based on an autologous injection of the SVF within 4 hours after spinal cord injuries. To check our hypothesis, we conducted a preclinical study in adult male rats. Contusions performed at thoracic level using an impactor. From a fat sample of 11.7 +/- 3.5 cc it is possible to extract 8.9 +/- 2.1 million cells of the SVF with 92.2 +/- 1.6% of viability and 77.7 +/- 0.04% of yield. A million cells then injected around injuries. The following 3 months devoted to kinematic analyses of movement, evaluation of sensorimotor recoveries using different behavioral tests (BBB test, Ladder rung walking test, CatWalk…), electrophysiology (evoked potentials, sensorimotor reflexes…), biochemistry (Elisa…), immunohistology (neurofilament labeling…). We present for the first time the results.

P397

Exploiting targeted epigenetic editing to increase the yield, homogeneity and purity of human iPSC-derived oligodendrocyte cell populations

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The generation of human induced pluripotent stem cells (hiPSCs)-derived oligodendrocyte (OL) cell population at yield, purity and homogeneity required for advanced biochemical and molecular studies, and for in vivo testing is still a challenge. Several differentiation protocols have been proposed over the years to enhance OL differentiation from hiPSCs, including those relying on the transient and/or stable overexpression of oligodendroglial Transcription Factors (TFs), which are not without risks of gene misregulation and/or genotoxicity. Our project aims to exploit novel epigenetic editing technologies to timely and physiologically modulate the expression of OL related genes in hiPSC-derived Neural Stem Cells (hiPS-NSCs).

We have selected genes known to drive or suppress OL commitment and, based on the acetylation and DNA methylation profile of their regulatory regions, we have identified target sites for epigenetic modulators (epieffectors). The epieffectors, built on catalytically dead Cas9 (dCas9) or TALEN, are fused to enzymatic or scaffolding domains able to activate or repress gene transcription, based on previous results (Amabile et al., Cell 2016). The best setting for gene activation/repression will be selected to treat iPSC-NSCs during their differentiation into neuronal/glia progeny (Frati et al., Cell Death Dis. 2018). We expect an enhancement in OL yield, purity, and maturation in the epieffector-treated iPSC-NSC-derived cultures. These experiments will allow to transiently and physiologically modulate the endogenous OL regulatory network in hiPSC-NSCs, with the aim of obtaining high-yield, homogeneous and pure OL populations to be used for basic and pre-clinical research.

P398

Resistance in human foreskin fibroblasts against lipofectamine-aided transfection of CoMiP plasmids

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Novel codon-optimized mini-intronic plasmids (CoMiPs) were recently reported for use in iPSC generation, as non-integrating gene transfer vectors for efficient, cost-effective, and xeno-free induction tools of pluripotency. We have tested CoMiPs that carry codon-optimized (Co) sequences of the reprogramming Yamanaka factors, along with short hairpin RNA against p53 (4-in-1 CoMiPs), as a part of the procedure to generate iPSCs from BJ human foreskin fibroblasts. BJ fibroblast and 293T cell cultures grown in 24-well plates were transfected with tdT-encoding 4-in1 CoMiPs or RFP reporter plasmids. Transgene luminescence was monitored at 18 and 48 hours to evaluate the efficiency of transfections. According to our results, lipofectamine-aided CoMiP transfection did not work in BJ fibroblasts but efficiently worked in 293T cells, which were also efficiently transfected with RFP plasmids. The resistance of the BJ fibroblasts to lipofectamine-aided CoMiP plasmid transfection in our study is not likely to be related to the passage number (10–11) of the cell lines used. Use of a different transfection reagent, or different means of transfection such as electroporation may be among alternative approaches to be tested to provide efficient transfection of CoMiPs into BJ fibroblasts (TUBITAK grant no: 218S617).

P399

Study of the effect of electrical stimulation in brain development using human brain organoids

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Human neural tissue is hardly accessible and therefore difficult to study. However, we need a way to study the brain in order to understand better how it is formed and how different diseases develop (such as Huntington's or Parkinson's disease). It has been shown that brain organoids can be generated from human pluripotent stem cells (PSC) (by Lancaster's group and many others). This is a very promising tool to study the brain in vitro using human cells. In our lab we managed to obtain brain organoids from embryonic stem cells (ESCs) and induced PSCs. Nevertheless, there is still a need to improve the quality and reproducibility of these organoids. Electrical stimulation is known to have an important role in certain development processes. In this study we use an in-house developed system to stimulate the cerebral organoids at different time points during development (first during the generation of the neural stem cells and later on during the maturation face). Then, the effects are analyzed at both protein and structure level using immunohistochemistry techniques. Understanding the effects of electrical stimulation can lead us to find ways to improve brain organoid generation. This way we are studying ways to reduce variability within organoid batches and increase the amount of neural-like tissue generated in the organoids.

P400

Decoding human cardiac progenitor cells regenerative potential using advanced proteomic tools

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Upon Acute Myocardial Infarction (AMI) and inherent Ischemia/Reperfusion (I/R) injury, endogenous cardiac progenitor cells (CPCs) are activated, contributing to myocardial repair through an auto/paracrine crosstalk between CPCs and cardiomyocytes (CMs) in stress. Transplantation of CPCs is being tested in clinical trials, and although improvements have been reported, the mechanisms of action of these cells are still mostly unknown. Our work combines the development of I/R in vitro human cell models with advanced mass spectrometry proteomic tools to further characterize hCPC and unveil associated regenerative mechanisms. hCPCs employed in the clinical trial CARE-MI (NCT02439398) were used. Different strategies were explored to recapitulate I/R, including: use of human adult/mature cells, 3D culture and bioreactor technology. Firstly, we developed a transwell co-culture I/R model, with hCPCs and human induced pluripotent stem cell derived CMs (hiPSC-CMs). Following this work aiming at further improving the relevance of the I/R in vitro setup, 3D hiPSC-CM cultures and bioreactors were combined, allowing the control/monitoring of critical environmental parameters. Important features of I/R injury were successfully captured, including hiPSC-CM death, cell ultrastructure disruption, as well as increased release of inflammatory cytokines. hCPCs response to I/R was probed using whole proteome analysis (including quantitative SWATH-MS), allowing to propose new pathways in the hCPCs-mediated regenerative process along I/R injury. Our data shows that our AMI-setup upregulates hCPC proteins associated with migratory, proliferation and stress response-related pathways. Moreover, our results reinforce the idea that paracrine-mediated mechanisms are central for hCPC activation, with the enrichment of several paracrine signaling pathways.

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P401

Reconstruction of neuronal network by transplantation of human skin-derived iPS cells after brain stroke

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Brain repair after damage represent a major challenge for current clinical and basic research. Stem cell-based approaches hold much promise, among other strategies, as potential novel treatments to restore brain function after stroke. However, there are still unanswered questions regarding the mechanism underlying the functional integration of grafted cells into the neuronal network of the injured brain. First, we showed that transplantation of human skin-derived neuronal precursors is a good strategy to improve functional recovery following cortical stroke in a rat model (Tornero, D. Brain, 2013). Grafted cells give rise to mature neurons that re-build the damaged tissue and send fibers to several host structures. We also showed that human iPS-derived cells transplanted into the stroke damaged cortex, beyond cell survival and differentiation, can integrate into the host network and remained so even at long time-points (Tornero, D. Brain, 2017). Immuno-electron microscopy (iEM) revealed synaptic contacts between host neurons and the neurons generated from grafted cells; and monosynaptic tracing of afferent connections with modified rabies virus showed a pattern that resembles that of the local endogenous neurons lost during stroke damage. More recently, we have observed synaptic contacts from grafted cells to host neurons located in specific locations of the brain, regenerating neuronal networks lost during stroke. Reconstruction of functional synaptic connections between host neurons and transplanted cells is of crucial importance in order to accomplish true cell replacement, which would lead to long-term recovery after stroke.

P402

Large scale manufacturing of regulatory macrophages (Mreg) in wave-type of bioreactor for clinical use

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Regulatory macrophages (Mreg) are activation-induced derivatives of peripheral blood monocytes. Due to their claimed immunoregulatory, anti-inflammatory, and angiogenic properties, Mregs have been considered as potential allogeneic cell therapy product to treat clinical conditions, e.g., non-healing diabetic foot ulcers. Earlier, Mregs have been manufactured either in tissue culture flasks or in gas-permeable differentiation bags that are not feasible when aimed to produce large scale clinical batches. Here we introduce a fully closed, automated Mreg process, ready to be transferred to the clinical production. We isolate monocytes from leukapheresis product by using CliniMACS Prodigy® and differentiate them to Mregs in a wave-type of bioreactor Xuri™. Mregs produced by our method are >95% viable and exhibit special immunophenotype with higher expression of CD163, CD206, CD209, Syndecan3, CD11c, and CD16 when compared to other types of macrophages (i.e., Mregs cultured in differentiation bags, M1, and M2a). Functionally, our Mregs inhibit T cell proliferation and owe the most potent phagocytic capacity when compared to afore-mentioned macrophages. In an in vitro human fibroblast scratch wound model, Mregs do not accelerate closing of the wound. This may be an indication of their regulatory function on fibroblasts that are the major actors in wound closure, scar formation, and skin remodelling. Our preliminary results show that when injected around the wounds of Balb/cAnNRj-Fox1nu/nu mice, Mregs persist in the wound vicinity, without major migration, at least for 7 days. This is the first fully automated, large scale and clinical grade process available for allogeneic therapeutic regulatory macrophages.

P405

Pulmonary macrophage transplantation-based therapy for alpha-1 antitrypsin deficiency

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Alpha-1-antitrypsin (AAT) is an acute phase antiprotease, deficiency of which may cause childhood liver failure as well as early-onset emphysema, the latter as a result of the uninhibited neutrophil elastase activity. In mice in vivo lentiviral transduction of alveolar macrophages (AMs) has been described to yield protective AAT level (2,7μM) in lung epithelial lining fluid (ELF) and ameliorate emphysema development. Here, we investigate the pulmonary transplantation of macrophages (PMT) overexpressing AAT (AAT-MΦ) as novel therapy for AAT-associated emphysema. For this purpose, human AAT-cDNA in combination with a GFP marker gene was expressed in a murine AM cell line as well as human and murine macrophages differentiated in vitro from hematopoietic stem/progenitor cells employing a 3rd generation SIN-lentiviral vector and different promoters. AAT-MΦ showed highest AAT secretion into culture supernatants using the constitutive CAG and Cbx3-EF1α promoters and secreted AAT was able to bind elastase in vitro. AAT-MΦ showed normal morphology, surface phenotype (CD11b, F4/80) and functionality (phagocytosis, MHCII upregulation, GM-CSF uptake). To study the potential of AAT-MΦ to engraft and secrete AAT in vivo, murine AAT-MΦ were intrapulmonary transplanted into Csf2rb-/- mice. Two months after PMT, donor-derived cells were detected by FACS in bronchoalveolar lavage fluid (BALF) and homogenized lung tissue. These cells preserved GFP expression and gained a CD11c+/Siglec-F+ AM-specific phenotype. Moreover, human AAT was detected in the BALF of transplanted animals reaching 0,12μM in ELF. Currently, we evaluate various strategies such as changes of promoter elements or signalling peptides to further increase AAT secretion from our AAT-MΦ.

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P407

Novel cardioactive factors selected *in vivo* from an AAV library encoding the secret

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Biological therapies for patients with myocardial infarction and heart failure are urgently needed. To search for novel cardioprotectors, we developed FunSel, a procedure for the unbiased in vivo functional selection of active biologics. FunSel is based on the in vivo delivery of a library of 1200 different AAV vectors coding for the murine secretome. Pools of 50 AAV vectors from this library were injected into mice hearts after infarction to identify factors conferring protection against myocardial cell death; three weeks later, viral genomes were recovered, and the frequency of each vector was determined by NGS. After two subsequent rounds of selection, we identified three novel proteins (Chrdl1, Fam3c and Fam3b) that markedly promoted cardiac function and alleviated left ventricle remodeling after either direct AAV9-mediated intracardiac delivery or recombinant protein administration. In particular, Chrdl1 promoted cell survival and enhanced cardiomyocyte autophagy by inhibiting extracellular BMP4 activity and attenuated cardiac fibrosis by blunting fibroblast response to TGF-β. The three identified proteins represent exciting new leads for the development of effective cardioprotectors.

P408

From heart impairment characterization to pathway investigation in a Titinopathy model

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Dilated cardiomyopathy is the most common form of cardiac damage associated with progressive muscular dystrophies. Two mouse models of muscular dystrophies with heart damage are used in this study, with both of them presenting left ventricular chamber enlargement and interstitial fibrosis, characteristics of dilated cardiomyopathy. The first one is a dystrophin-deficient Mdx mouse strain backcrossed on a DBA2/J background, used as a Duchenne muscular dystrophy (DMD) mouse model. The other one is a model carrying a deletion into the penultimate exon of the titin gene. Titin plays a crucial role in sarcomeric integrity and muscle stiffness, especially in heart functions. The aim of the study is to characterize these models, and then to identify abnormal molecular cascades implicated in the cardiac impairment of the two DCM models in order to evaluate the potential therapeutic effects by targeting them pharmacologically or genetically. RNAseq analyses of dysregulated RNA of the heart on both mice models at different ages highlight common pathways that have been validated at RNA and protein level. Modulations of the expression of these pathological pathways are under evaluation in the mouse models.

P409

Muscle expression of a secreted LDLR/Tfchimeric proteinameliorates lipid profile inLDLR-deficient mice

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Familial hypercholesterolemia is an inherited disorder mainly due to mutations in the LDL receptor gene characterized by premature onset of cardiovascular disease due to high plasma LDL cholesterol concentrations. Homozygous FH patients do not always respond to conventional therapies and have often a poor prognosis. Therefore, more effective therapeutic strategies, such as gene therapy, are still of main interest. We developed a safe and effective gene therapy strategy based on liver expression of a secreted chimeric protein composed of the extracellular portion of the LDLR linked to a transferrin dimer using a helper-dependent adenoviral (HD-Ad) vector. This chimeric protein binds LDL and removes them from the bloodstream through the transferrin receptor (TfR). Intravenous administration of this HD-Ad vector ameliorated the lipid profile and a reduced aortic atherosclerosisin LDLR-deficient mice. To improve this strategyfor a possible clinical application, we generated a HD-Ad vector for a muscle-restricted expression of themLDLR/mTF chimeric protein using a muscle-specific promoter and intramuscular administration of the vector. We observed expression of the chimeric protein after infection of myoblast C2C12 cells with our vector and an amelioration of lipid profile after intramuscular administration in LDLR-deficient mice. In summary, we developed an innovative strategy for FH therapy based on the expression of a secreted chimeric protein after intramuscular administration of an HD-Ad vector. This approach reduces risks due to systemic administration of viral vectors and is applicable to other genetic diseases; collection of additional efficacy and safety data will further define its applicability in clinical settings.

P410

Development of constructs for cardiac conduction system targeted gene therapy

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Heart failure is characterised by generalised dysfunction of the cardiac conduction system (CCS). Arrhythmogenic ion channel remodelling has been documented throughout the failing CCS. In particular, aberrant expression of HCN4, which carries the pacemaker funny current (If), occurs in the dysfunctional sinus node in various pathophysiological states including heart failure. This pathological remodelling has been linked to dysregulated expression of specific microRNAs (miRs). We therefore aim to develop a method of modulating gene or miR expression in the failing CCS specifically. In this study, we analysed four promoter constructs based on the regulatory sequence of two major genes expressed in CCS- KCNE1

and HCN4. AAV constructs were generated in which transcription of a GFP reporter gene was regulated by the KCNE1 promoter, a K+ channel subunit expressed throughout the CCS, or the HCN4 promoter, to target the sinus node. Two variants of each promoter were tested; 0.8-kb and 1.2-kb HCN4, and 0.8-kb and 1.6-kb truncations of the KCNE1 promoter that had been previously characterised. GFP expression from KCNE1 and HCN4 promoters was low or negligible in primary neonatal ventricular rat cardiomyocytes, where their physiological gene expression is also low. Significantly higher GFP expression was observed in the sinus node-like Shox2 cell line, suggesting tissue specific promoter activity. Both KCNE1 variants yielded stronger transgene expression compared to HCN4 promoters, among which the 0.8-kb variant performed best. Further experiments in vivo will assess the efficacy of AAV9 packaged promoter constructs in driving transgene expression in the CCS after systemic administration in mice.

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P411

Decellularized extracellular matrix in modelling lung fibrosis development *in vitro*

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As stated progression of lung fibrosis depends on extracellular matrix (ECM) properties such as composition, stiffness, containing of growth factors. Although single proteins such as collagen are widely used in vitro to establish the role of ECM in fibrosis progression it is not sufficient as in vivo ECM environment includes tens of components. We invented the way to model extracellular microenvironment using the matrix produced by lung fibroblasts and than decellularized. Two primary fibroblast cultures were derived from healthy C57 mice or mice with bleomycin-induced lung fibrosis. Fibroblasts were cultivated in high density and than cells were eliminated by CHAPS and DNAse treatment. Obtained decellularized ECM (dECM) was a complex of extracellular proteins with stored structure as were established by scanning electron microscopy and immunohictochemistry. dECM promoted cell survival and proliferation and did not increase the monocytes to macrophages activation. We suppose dECM from lung fibroblasts is a relevant model to study the processes occur during progression of lung fibrosis in vitro such as myofibroblast differentiation or endothelial-to-mesenchymal transition. This study was supported by Russian Fond of Fundamental Research #19-015-00437 ╲ 19.

P412

Proteomic analysis reveals angiogenic and immunomodulatory function for placental stromal cell-derived extracellular vesicles

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Allogeneic regenerative cell therapy has shown surprising results despite the lack of engraftment of the transplanted cells. Their efficacy was so far considered to be mostly due to secreted trophic factors, but extracellular vesicles (EVs) can also contribute to their mode of action. Here we provide evidence that EVs derived from therapeutic placental-expanded (PLX) stromal cells are potent inducers of angiogenesis and modulate immune cell proliferation in a dose dependent manner. Crude EV preparations were enriched >100-fold from large volume of PLX-conditioned media via tangential flow filtration (TFF) as determined by tunable resistive pulse sensing (TRPS). Additional TFF purification was devised to separate purified EVs from cell-secreted soluble factors. EV identity was confirmed by western blot, calcein-based flow cytometry and electron microscopy. Surface marker profiling of tetraspanin-positive EVs identified expression of cell- and matrix-interacting adhesion molecules. Tandem mass tag proteomics comparing PLX-EVs to PLX-derived soluble factors revealed 1116 differentially expressed proteins mainly involved in angiogenesis, cell movement and immune system signaling. At functional level PLX-EVs and cells but not the corresponding soluble factors inhibited T cell mitogenesis. PLX-EVs and soluble factors displayed dose-dependent proangiogenic potential by enhancing tube-like structure formation in vitro. Our findings indicate an alternative mode of PLX action based on EV-mediated proangiogenic function and immune response modulation that may help explaining clinical efficacy beyond presence of the transplanted allogeneic cells.

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In vivo delivery of CRISPR/Cas9 to the liver using lipid nanoparticles enables gene knockout in multiple species

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A wide range of diseases currently exist where the reduction or inhibition of a protein produced in the liver may represent a treatment option. Current therapeutic modalities generally utilize repeated dosing to accomplish this. Here, we describe the delivery of CRISPR/Cas9 components to the liver of mice, rats and non-human primates (NHP), using lipid nanoparticles to achieve long-term reduction of a target gene's expression with a one-time intravenous administration. This modular system can be used to target virtually any gene in the liver by changing the targeting sequence of the guide RNA. We utilize a high-throughput screening system based on next-generation sequencing for rapidly identifying highly potent guide RNAs with no detectable off-target

editing. In both mouse and NHP, substantial liver editing has been achieved in the TTR, SERPINA1 and Albumin genes. Editing in the liver using this system leads to a high rate of frame-shift indels, causing a dose-proportional reduction in mRNA via nonsense-mediated decay and an associated reduction in protein levels. With this system we have achieved over 95% reduction in TTR protein in mice, rats and NHPs from a single administration. These levels of TTR protein reduction are stable for at least one year in mouse and 10 months in NHP. Delivery utilizing this system is well-tolerated, with no significant safety observations at therapeutic dose levels. This new therapeutic modality has the potential to treat a range of diseases including transthyretin amyloidosis, alpha-1 antitrypsin deficiency, primary hyperoxaluria and for uses in gene insertion.

P416

Modulation of F8 promoter activity in response to Ets-1 and Ets-2 transcription factors for hemophilia A gene therapy application

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Since liver sinusoidal endothelial cells (LSECs) are the major source of FVIII and are involved in tolerance induction, we were interested in dissecting the transcriptional control promoting FVIII expression in this cell-type. By performing an in-silico analysis of F8 promoter (pF8) we disclosed the presence of several transcriptional factor binding sites (TFBS) recognized by Ets-1 and Ets-2 and distributed with an overlapped pattern in the pF8. Luciferase assays performed by co-transfecting pF8 with Ets-1, Ets-2 or combined, demonstrated a 3-fold up-regulation of promoter activity in the presence Ets-1 and 5-folds in the presence of both, while Ets2 alone showed no effect. Generation of Ets1/2-DNA binding domain mutants (DBD) and their combined expression with the WT counterpart, abolished promoter up-regulation only when the Ets-1 DBD was removed. Indicating that pF8 up-regulation occurs through Ets-1 and Ets-2 protein-protein interaction. To elucidate the regulatory role of these proteins we generated several pF8 variants with multiple or single Ets-BS deletions. One of these minimal promoters containing all the Ets-TFBS required for the basal activity and for the response to Ets-1/2 stimulation was characterised in vivo. Tail vein injection of C57/Bl6 mice with LVs expressing GFP under the control of the shortened promoter, confirmed longterm transgene expression in LSECs. Moreover, LV-delivery of FVIII in B6-129 HA mice, promoted long-term therapeutic levels of FVIII without inhibitors formation. These data strongly support the potential application of these short pF8 variant in gene therapy, extending its suitability also for other vector systems, such as AAV.

P417

Differentiation of human liver-derived stem cells into hepatic lineage for therapeutic liver reconstitution

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In patients with end-stage liver disease, liver transplantation is the only available curative approach. But due to organ shortages and long latencies, stem cell therapy is being proposed as a promising tool to extend the latency. We here present modified two-step methods for differentiating adult stem cells into the hepatic lineage, especially using human liver-derived stem cells (LD-SCs) as an optimal candidate for cell therapy. Liver tissues for obtaining LD-SCs were acquired by needle biopsy from healthy donors. LD-SCs presented mesenchymal stem cell markers (CD90 and CD105) by flow cytometry. Fadusil and Decitabine (5-aza-2'-Deoxycytidine) were supplemented with Nicotinamide, FGF, and HGF to initiate two-step hepatocyte differentiation. The protocol produced a robust bipotent progenitor cells (BPCs) in both LD-SCs and umbilical cord matrix-derived stem cells (UCM-SCs) in 7 days and could differentiate both cells into hepatocyte-like cells (HLCs) in 14 days. When comparing each BPCs and HLCs differentiated from each UCM-SC and LD-SC, LD-BPCs showed most advanced mitochondrial functions. During in vivo cell transplantation, both UCM-SC and LD-SC migrated into the liver within 6 hours in hepatic ischaemia-reperfusion injury mice model. All stem cells and differentiated BPCs and HLCs settled in the liver and differentiated into new functional cells after 14 days in immune-deficient mice with TAA-induced liver injury. Particularly BPCs comprised hepatocytes and biliary cells together resulting in fair hepatic microenvironments. Those results cumulatively support that LD-BPCs obtained by our protocol is a promising source for cell therapy with unique regenerative potential in liver reconstruction for severe liver diseases.

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Liquid-Biopsy-Integration-Site-Sequencing (LiBIS-Seq) allows the detection of genetically modified cells residing in solid tissues and the earlier detection of premalignant clones triggered by insertional mutagenesis

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Clonal tracking techniques enable monitoring of the fate of thousands cells in tissues of gene therapy (GT)-patients and permit assessment of the safety and efficacy of these procedures. However, the impossibility to study cells residing in solid organs (e.g liver/brain) without performing invasive biopsies, reduces the diagnostic power of clonal tracking analyses as safety readout, and provides only a superficial snapshot of the clonal repertoire of the engrafted transduced cells. To overcome these issues, we developed Liquid-Biopsy-Integration-Site-Sequencing (LiBIS-Seq), a PCR technique designed for high-throughput retrieval of vector integration sites (IS) from cell-free blood plasma DNA (BP-cfDNA) released from dead cells. We employed LiBIS-Seq to analyze cfDNA from BP samples harvested at different time points from 7 Metachromatic leukodystrophy patients treated by lentiviral (LV)-based hematopoietic stem cell GT, and retrieved >10,900 IS. The genomic distributions and clonal abundances of cfDNA-derived IS showed a polyclonal reconstitution and a positive safety profile as described. LiBIS-seq was also applied to BP-cfDNA collected from animal models treated by LV-based liver-directed GT and harvested at different time points post-

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injection. From this analyses, we retrieved >11000 IS and observed that many of those IS shared across time points. Moreover, LiBIS-Seq enabled earlier detection of malignant T-cell lymphoblastic leukemia clones triggered by LMO2 insertions in γ-retroviral-based clinical trials for X-linked-SCID and Wiskott-Aldrich syndrome. In conclusion, LiBIS-Seq allows for longitudinal monitoring of genetically modified cells residing in potentially any organ and reveals aberrantly expanding clones earlier than canonical IS retrieval techniques based on cellular DNA.

P419

Targeting of hepatocyte subpopulation contributing to liver post-natal growth is crucial for maintenance of transgene expression in liver-directed gene therapy

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Liver-directed gene therapy with adeno-associated viral (AAV) vectors delivering a clotting factor transgene into hepatocytes has shown successful results in adults with hemophilia. However, because of their non-integrating nature, AAV are diluted upon cell division during liver growth, thus challenging their use in paediatric patients. In contrast, lentiviral vectors (LV) integrate into the target cell genome and are maintained as cells divide. We developed LV that provide stable and therapeutic levels of coagulation factor IX (FIX) in adult mice, dogs and non-human primates, after intravenous (i.v.) delivery. We then set out to evaluate the fate of LV-modified liver cells during growth by administering increasing doses of LV to newborn mice. Exploiting 3D imaging of cleared livers and bioluminescence, we show that transduced hepatocytes are maintained over time and proliferate locally. Unexpectedly, we observed an initial, promoter-independent, decrease in transgene expression followed by stable maintenance, indicating some extent of tissue remodelling. We then administered LV-FIX to 2-week old mice and showed 3-fold higher FIX output compared to newborn, suggesting targeting of a different hepatocyte subpopulation contributing to liver growth at that time. Molecular studies are underway to investigate the nature and the clonal composition of transduced hepatocytes at the different ages. In addition, we show in vivo transduction of cholangiocytes able to generate LV-positive liver organoids in vitro. Our work will provide a rationale for application of LV-mediated liver gene therapy to paediatric patients and may shed light on the role of different cell populations involved in post-natal liver growth.

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LV.InsulinB9-23/anti-CD3 inhibits recurrence of autoimmunity in NOD mice after allogeneic pancreatic islets transplant allowing T1D resolution

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Type 1 diabetes (T1D) is an autoimmune disease resulting in the destruction of pancreatic beta-cells by auto-reactive T cells, and induction of antigen (Ag)-specific tolerance represents a therapeutic option. We demonstrated that administration of Lentiviral vector, enabling expression of insulin B9-23 in hepatocytes (LV.InsB), arrests beta-cell destruction in pre-diabetic NOD mice, by generating InsB9-23-specific FoxP3+ T regulatory cells (Tregs). Moreover, LV.InsB in combination with a suboptimal dose 1X5μg of anti-CD3 mAb (combined therapy, CT) reverts diabetes and prevents recurrence of autoimmunity following islets transplantation in $\sim$50% of NOD mice. Here, we investigated whether optimization of the CT can increase efficacy in arresting recurrence of autoimmunity and possibly suppress allo-response to transplanted islets. We optimized CT protocol by transplanting islets when CT-driven tolerogenic program was induced. Additionally, to obtain stabilization of disease progression till the time of islet transplantation, anti-CD3 dose was elevated to 1X25μg (CT25). Results indicate that 100% of diabetic NOD mice treated with CT25 and transplanted with Balb-c islets (at glycemia <500mg/dL) remained normoglycemic for 100 days, displaying unresponsiveness to InsB9-23 stimulation and increased % of Tregs infiltrating islets and pancreatic LN. Moreover, cured mice showed a reduced insulitis compatible with normoglycemia but no persistence of the transplanted islets. Results confirmed the previously described mode of action of LV.InsB: induction of Ag-specific Tregs that, accumulating in PLN, control Ag-specific effector T cells. Moreover, optimized CT25 represents a curative treatment for T1D when associated with allogeneic islets transplantation to restore activity of the endogenous beta-cell mass

P421

Structural characterization of AAV5-FVIII-SQ vector DNA in human blood by Real-Time and Droplet Digital™ PCR

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Recombinant AAV-based gene therapies deliver DNA to a wide range of cells. While AAV5 has a predominant liver tropism, vector DNA can be detected in different blood fractions after administration of BMN 270 (valoctocogene roxaparvovec), an investigational AAV5-FVIII-SQ therapy for hemophilia A. DNA from unfractionated whole blood, and blood fractionated into plasma, peripheral blood mononuclear cells (PBMCs), and red blood cells (RBCs), was analyzed by qPCR, a Droplet Digital PCR (ddPCR) assay to determine ITR fusion events, and a panel of drop phase ddPCR assays to analyze vector fragmentation and full-length assembly. Most vector rapidly disappeared from the blood in all subjects with less than 1% of the original administered BMN 270 DNA detected in blood by Day 2. The kinetics of DNA clearance were very similar in all subjects, with three phases corresponding to the lifespan of granuloctyes, RBCs, and PBMCs. Linear and ITR-fused episomal structures were formed in PBMCs, and persisted beyond 24 weeks, consistent with preferential clearance of non-ITR fused DNA. Transgene

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DNA in blood continued to decrease from 6 months to 2 years, consistent with slow turnover of long-lived PBMCs containing stable episomes. These data demonstrate differential micro-distribution of a rAAV5 vector in the different compartments of blood, differential processing of the DNA in each compartment, and illustrate the value of using blood subsets to non-invasively monitor molecular processes in a surrogate sample.

P422

Characterization of MHC class II-restricted T-cell receptors for T-cell therapy of HBV infection

ABSTRACT WITHDRAWN

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P423

Durability of factor IX expression in mice treated neonatally with a nuclease-free, promoterless, AAV-based gene therapy, GeneRide™

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Hemophilia B is a hereditary bleeding disorder caused by loss-of-function mutations in the gene encoding factor IX. Current standard care consists of chronic replacement therapy by intravenous infusion of recombinant or plasma-derived factor IX. Several clinical trials have begun using canonical AAV gene therapy in adult hemophilia B patients with the potential to provide long-lasting production of factor IX from hepatocytes following a single injection. While this is an exciting prospect for adult patients, pediatric patients will not benefit from this approach due to progressive loss of therapeutic levels of Factor IX as hepatocytes divide and the liver grows. To overcome this limitation, we have developed GeneRide, a novel AAV-based, promoterless, nuclease-free, genome editing technology that leverages the natural process of homologous recombination to insert a functional copy of the factor IX gene in the genome site-specifically. The factor IX transgene is precisely inserted into the albumin locus, ''hitching a ride'' on the highly active endogenous promoter of albumin and resulting in high levels of expression, selectively in hepatocytes. This promoterless transgene approach addresses the risks associated with random integration in the genome of active promoters used in canonical AAV therapies. By being nuclease-free, GeneRide also avoids the potential for offsite mutagenesis that has been observed with other gene editing approaches using nucleases. Here we present data comparing the durability of Factor IX expression in mice treated with either a canonical gene therapy vector or GeneRide delivering factor IX.

P424

Improved in vivo efficacy of clinical-grade cryopreserved human hepatocytes in mice with acute liver failure

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Clinical hepatocyte transplantation short-term efficacy has been demonstrated; however, some major limitations, mainly due to shortage of organs, the lack of quality of isolated cells and low cell engraftment after transplantation, should be solved for increasing its efficacy in clinical applications. Cellular stress during isolation causes an unpredictable loss of attachment ability of the cells, which can be aggravated by cryopreservation and thawing. In this work, we focused on the use of a GMP solution in comparison with the standard cryopreservation medium, the University of Wisconsin medium, for the purpose of improving the functional quality of cells and their ability to engraft in vivo, with the idea of establishing a biobank of cryopreserved human hepatocytes available for their clinical application. To this end, we evaluated not only cell viability but also specific hepatic functions indicators of the functional performance of the cells such as attachment efficiency, ureogenic capability, phase-I and II enzymes activities and the expression of specific adhesion molecules in vitro. Additionally, we also assessed and compared the in vivo efficacy of human hepatocytes cryopreserved in different media in an animal model of acute liver failure. Overall, the results indicate that the new tested GMP solution maintain better hepatic functions, and, most importantly, showed better results in vivo which could imply an increase in long-term efficacy when used in patients.

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P425

Design, investigation and delivery of advanced trans-splicing RNA for suicide gene therapy

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Suicide gene therapy involves the delivery of cell suicide transgene into target cells inducing cell death. Spliceosome-mediated RNA trans-splicing represents a technology that allows endogenous labelling of target pre-mRNAs with an artificial trans-splicing RNA (tsRNA) triggering the formation of chimeric RNAs and proteins. We combined these two technologies and developed a RNA trans-splicing-based Herpes simplex virus thymidine kinase (HSVtk)/ganciclovir (GCV) suicide gene therapy approach that can be programmed to target any pre-mRNA disease biomarker. This therapeutic approach is not limited to cancer treatment and is also being explored for targeting of cells that are irreversibly transduced by integrating viruses. Rationally designed tsRNAs greatly improved on-target activity and on-target specificity. These suicide vectors triggered selective death of liver cancer cells or HPV-16 transduced cells. Our latest generation of multi-targeting constructs comprising recognition domains for up to 5 different hepatocellular carcinoma (HCC) pre-mRNA biomarkers exhibited the highest cell death activity even at 330-fold reduced GCV concentrations of 0.3 μM. Currently we explore non-viral dumbbell-shaped DNA minimal vectors for delivery. These are tested in patient-derived xenograft (PDX) 3D cell culture models and will be tested in both immune-deficient and humanized mouse models. For targeted delivery in vivo, HCC-targeting vectors will be furnished with tri-antennary N-acetylgalactosamine (GalNAc3) residues. We further study dumbbell delivery following topical application onto the skin of domestic pigs. Our observations suggest RNA trans-splicing represents a promising approach to suicide gene therapy targeting viral infection, cancer or other diseases characterized by the expression of one or multiple disease-specific pre-mRNA biomarkers.

P426

Biodistribution and pharmacokinetics of AAVrh.10-A1AT mediated gene therapy in humanized liver mice as a predictor of A1AT human expression levels following intravenous delivery

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Alpha-1 antitrypsin (A1AT) deficiency (A1AD) is an autosomal co-dominant disease, resulting in impaired neutrophil elastase regulation and a high risk of pulmonary emphysema. A1AT is produced by the liver and secreted in the blood where it is the second most abundant protein. Previously, it was demonstrated in wild-type mice that 5x10^12vg/kg AAVRh.10-A1AT mediated liver gene therapy achieved A1AT blood levels ([A1AT]) of >50 μM, well beyond the normal human level (22μM) and therapeutic threshold. However, when A1AD patients received vector doses ranging from 1x10^12 to 1.5x10^13vg/kg in the ADVANCE clinical trial (NCT02168686), only moderate increases of [A1AT], and no dose response, were observed (200nM). This is in stark contrast to the AAV5 serotype which reportedly achieves similar levels of coagulation factor across species, including mouse, NHP and patients (NCT02576795). To understand this discrepancy, we compared the pharmacokinetics and biodistribution of the AAVrh.10-CAG-A1AT. HIS vector in C57/B6J and humanized liver mice, following the intravenous administration of 5x10^12vg/kg. Strikingly, the humanized mice displayed >50-fold lower serum [A1AT] compared to wild-type mice, in line with the ADVANCE clinical trial outcome. Furthermore, AAVRh.10 capsid tyrosine mutants and alternative cassettes to CAG, including a liver specific promoter, did not provide increased expression over the original vector. Overall, these findings underscore transability caveats specific to AAVRh.10 for liver-targeted gene therapy. In conclusion, humanized liver mice more accurately predicted the [A1AT] levels observed in the human clinical trial, thus providing a valuable preclinical platform to investigate AAV-mediated liver gene therapy, poising for a successful transition to the clinic.

P427

Preclinical assessment of a hepatocyte-directed gene editing approach based on viral vectors and polymeric nanoparticles

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Neurogastrointestinal mitochondrial encephalopathy (MNGIE) is a rare metabolic disease caused by recessive mutations in the TYMP gene, which encodes the enzyme thymidine phosphorylase (TP). This causes a systemic accumulation of nucleosides that results in mitochondrial toxicity that is usually lethal during the first decades of life. The goal of the present work is to achieve an efficient integration of the human TYMP cDNA on introns of the Tymp and Alb genes of murine hepatocytes by the coordinated action of two elements: an RNA-based CRISPR/Cas9 system coupled to oligopeptide-modified poly (β-amino-ester) polymeric nanoparticles (PBAEs) and a TYMP cDNA template flanked by two homology sequences of 350 bp and delivered by a AAV2/8 vector. We developed two guide RNA targeting the two above mentioned target loci. The efficiency of sequence-specific cutting of the Cas9 nuclease is 30,88% ($\pm$10,19) for Tymp and 36,44% ($\pm$8,23) for Alb loci, measured with the Surveyor assay. We determined that the TYMP cDNA is successfully inserted in the desired genomic position and also assessed the presence of mRNA TYMP in the edited cells by Real-Time PCR. Finally, we confirmed the presence of active TYMP protein. All these results confirm that our genome editing approach is viable, although we need to increase the overall efficiency of the procedure to achieve sufficient expression in vivo. In summary, in our

experimental conditions our Cas9 nuclease is efficient at cleaving the target sequence, and the TYMP cDNA is correctly inserted and expressed.

P428

Specific gene correction of the AGXT gene and direct cell reprogramming for the treatment of Primary Hyperoxaluria Type 1

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Primary Hyperoxaluria Type 1 (PH1) is an inherited rare metabolic liver disease caused by the deficiency in the alanine: glyoxylate aminotransferase enzyme (AGXT), involved in the glyoxylate metabolism. The only potentially curative treatment is organ transplantation. Thus, the development of new therapeutic approaches for the treatment of these patients appears as a priority. We propose the combination of site-specific gene correction and direct cell reprogramming for the generation of autologous phenotypically healthy induced hepatocytes (iHeps) from skin-derived fibroblast of PH1 patients. For the correction of AGXT mutations, we have designed specific gene editing tools to address gene correction by two different strategies, assisted by CRISPR/Cas9 system. Accurate specific point mutation correction (c.853T-C) has been achieved by homology-directed repair (HDR) with ssODN harbouring wild-type sequence. In the second strategy, an enhanced version of AGXT cDNA has been inserted near the transcription start codon of the endogenous gene, constituting an almost universal correction strategy for PH1 mutations. Direct reprogramming of fibroblasts has been conducted by overexpression of hepatic transcription factors and in vitro culture in defined media. In vitro characterization of healthy induced hepatocytes (iHeps) has demonstrated hepatic function of the reprogrammed cells. PH1 patient fibroblasts and the gene edited counterparts have also been reprogrammed to iHeps, showing similar general hepatic characteristics as healthy iHeps. Restoration of the glyoxylate metabolism in the gene edited and reprogrammed iHeps is being demonstrated. The development of these advanced therapies will be useful as alternative cellular source to replace endogenous deficient hepatocytes with functional corrected cells.

P429

Cell therapy for Fabry disease using CellSaic technology

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Fabry disease is caused by decrease or loss in the activity of α-galactosidase (GLA), and its substrate globotriaosylceramide (Gb-3) accumulates in cells throughout the body, resulting in kidney injury and heart failure due to hypertrophy. Enzyme replacement therapy (ERT) has been used as a standard therapy, but it is a significant financial burden and the regular administration schedule is inconvenient for patients. In this study, we examined whether GLA secreted from donor cells could be an alternative to ERT. First, we established Gla-knockout (Gla-KO) mice (C57BL/6 background) by CRISPR. Gla-KO mice exhibited no Gla activity and accumulation of Gb-3, which is not recognized in the previous reported Gla-KO mice. Next, syngeneic wild-type mouse embryonic fibroblasts were cultured with a recombinant collagen peptide, called μ-piece, on 96U-bottom plate to produce cellular spheroid (hereafter referred to as CellSaic), and transplanted under the renal capsule of Gla-KO mouse. CellSaic protected central necrosis of cellular spheroid in long-term culture. One month after transplantation, CellSaic was clearly visible with vascular networks, and did not significantly show TUNEL-positive cells. LysoGb-3 in liver of transplanted mice significantly decreased depending on the number of CellSaic (Control vs 20 CellSaic: $p=0.0002$, vs 40 CellSaic: $p=0.0004$), suggesting that Gla secreted from CellSaic enters into the circulation and degrades Gb-3 in the liver. These results indicate the effectiveness of CellSaic to treat Fabry disease. We're planning ex vivo gene therapy in combination with CellSaic technology to enable the strategy practicable with a reasonable cell number.

P430

HNF4a promotes haepatic differentiation of human adipose tissue-derived stem cells

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Objective: Due to the high proliferation rate along with affinity to acquire hepatocyte functions, human adipose tissue-derived stem cells (hASCs) have been considering as an important candidate for cell therapy of liver diseases. In addition, hepatocyte nuclear factor a (HNF4a) has been redundantly reported as a key transcription factor during early liver development. This factor is essential for both hepatocyte differentiation and the maintenance of hepatic functions. Therefore, the present study seeks whether HNF4a can enhance hepatic differentiation of hASCs in the absence of any other stimulators.

Methods: Lentiviral transduction was applied to overexpress HNF4a in hASCs for up to 21 days. Then hepatic functionality was

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evaluated by analyzing specific hepatocyte genes and biochemical markers at different time points of differentiation induction.

Results: QRT-PCR revealed that stable overexpression of HNF4a in hASCs led to increased expression of hepatocyte genes such as ALB, AFP, CK18, and CK19. Furthermore, Urea production, glycogen staining, and immunocytochemistry indicated that the treated cells have differentiated toward hepatocyte-like cells.

Conclusions: Therefore, our findings demonstrate the significant role of HNF4a to induce hASCs into functional hepatocyte-like cells.Keywords: Human adipose tissue-derived stem cells (hASCs), hepatocyte nuclear factor a (HNF4a), Hepatocyte-like cells.

P431

Human hepatocytes encapsulated in injectable hydrogels for their use in liver cell therapy

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Cell-based liver therapy is envisaged as a potentially useful therapeutic option to recover and stabilize the lost metabolic function in different liver diseases to ameliorate the clinical outcome. Currently, only marginal livers, not suitable for organ transplantation, can ethically be used to isolate cells. However, transplanted hepatocytes cannot grant the best clinical results due to their low engraftment efficiency and survival. The main objective of this research is to improve the clinical results of liver cell transplantation by the use of strategies which optimize both delivery and retention of the cells in the host liver and which mimic cell-cell and cell-matrix interactions in vivo. To this end, we evaluated the benefits of using an injectable hydrogel to encapsulate human hepatocytes for their use in liver cell therapy. We used natural hydrogels based on the components of the extracellular matrix, gelatin and hyaluronic acid, to better mimic the natural environment of the cells and evaluated the metabolic performance and in vivo efficiency of human hepatocytes. As expected, human hepatocytes encapsulated in gelatin-hyaluronic hydrogels presented advantages over ‘‘2D-hepatocytes’’, which could help to overcome some current limitations of liver cell therapy and that would be potentially suitable for future therapeutic applications.

P432

CD160 serves as a negative regulator of NKT cells in acute hepatic injury

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CD160 and BTLA binds to herpes virus entry mediator (HVEM). Although the function of BTLA as a negative regulator has been well documented, the role of CD160 in NKT cells remains unclear. Upon analysis of CD160-/-mice and their mixed bone marrow chimeras, we found no apparent developmental defects of NKT cells. However, CD160-/-mice demonstrate severe liver injury after in vivo challenges with α-galactosylceramide (α-GalCer) and a large proportion of CD160-/- mice died following Con A challenges with elevated serum AST and ALT levels, due to hyperactivation of NKT cells with significantly elevated levels of IFN-γ, TNF-α, and IL-4. Importantly, inhibition of BTLA by anti-BTLA mAbs aggravated α-GalCer-induced hepatic injury, highlighting that both CD160 and BTLA serve as non-overlapping negative regulators of NKT cells. Our data presents CD160 as an important co-inhibitory receptor that delivers antigen-dependent signals in NKT cells that dampen cytokine production during early innate immune activation processes.

P433

Elucidating the mechanism of species specificity of recombinant AAV-LK03 transduction

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Recombinant adeno-associated viral (rAAV) vectors are of great interest as a potent vehicle for gene therapy, however, there can be quite a bit of discordance between rAAV transduction properties amongst different species making it difficult to select the optimal capsid for human clinical trials based on preclinical animal studies. Thus, we elected to study the mechanisms responsible for the observed species discordance in rAAV transduction. Previously, we selected several rAAV capsids from screening DNA-shuffled capsid libraries and identified some potent chimeric rAAV capsids (e.g. AAV-LK03) (Lisowski etal. Nature 2014), that are robust at transducing human liver but show very poor transduction of mouse liver. In this study, we investigated each step of rAAV-LK03 transduction from cellular uptake to transgene expression in cultured human and mouse hepatoma cells. While there was a >10- fold difference in transgene expression from AAV-LK03 in human versus mouse cells, both cell binding and internalization were similar. Furthermore, the nuclear vector copy number also showed no significant difference between the two species. Our data implicate that uncoating steps of AAV capsid proteins or other capsid processing mechanisms may be involved in the differential transduction efficiency of AAV-LK03 in human and murine cells. Further studies are ongoing and are required to resolve the molecular events in the nucleus responsible for transduction differences. Unraveling these differences may allow for better predictions of how to select optimal vectors for a given human application.

P434

Haemostatic efficacy of cryopreserved nanostructured fibrin agarose hydrogel

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Uncontrolled hemorrhage is still an important concern in surgical interventions being responsible of higher rates of mortality, morbidity and longer hospital stays. Several surgical techniques are used to decrease/reduce bleeding, however, such methods have transient or incomplete effect that often require the use of a topical hemostatic agent to prevent postoperative blood loss. We have recently demonstrated the superior hemostatic efficacy of a novel nanostructured fibrin-agarose hydrogel (NFAH) patch compared to two commonly-used commercial hemostatic agents in an animal model of liver resection. Results showed not only higher hemostatic success rate but also lower postoperative adhesions to adjacent tissues, no presence of hematoma and lower grades of hemorrhage, inflammation and necrosis in histological analysis. Unfortunately, due to its degradation and consequent loss of functionality, it is difficult and costly to keep a ready to use NFAH supply. To overcome these limitations, it is necessary to find a long-term storage condition that maintains the hemostatic properties and, at the same time, allows immediate availability of NFAH for any unscheduled surgery. To this end, we have cryopreserved NFAH following a protocol previously published. Subsequently, we have studied its hemostatic efficacy compared with fresh NFAH in a model of severe hepatic resection. Our results show no significant differences in time to hemostasis between fresh and 3 months cryopreserved NFAH. We conclude that cryopreservation does not affect the hemostatic properties of NFAH enabling clinical translation of this innovative hemostatic agent for multiple surgical conditions.

P435

Using AAV vectors to deliver ATP7B as a treatment for Wilson Disease

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Wilson disease is a genetic disorder of copper metabolism, caused by mutations in the ATP7B gene. ATP7B encodes for the copper-transporting ATPase2 protein (ATP7B), a copper binding enzyme found primarily in hepatocytes and responsible for maintaining proper cellular copper homeostasis. Although copper is essential for many cellular functions, it is toxic in excess. ATP7B clears copper via two pathways. TGN localized ATP7B loads copper onto ceruloplasmin, which is then secreted into the plasma; cytoplasmic ATP7B binds excess copper and excretes it into the bile. Loss of ATP7B causes high levels of copper accumulation in the liver, brain and other organs, leading to cellular damage. Our therapeutic approach is to restore ATP7B function by delivering a recombinant AAV vector encoded ATP7B transgene. To achieve this, we have engineered codon optimized and metal binding domain deletion variants of the ATP7B transgene, and tested their functionality and manufacturability in AAV vector preparation. The ATP7B variants generated properly localize to the TGN, and are being further characterized in vitro. In addition, we have conducted a series of studies in the tx-j mouse model of Wilson disease using AAV vectors encoding our deletion variants. In these studies, a single infusion resulted in a dose-dependent increase in serum ceruloplasmin activity, a decrease in total liver copper, and improvement in liver pathology. Administration of our vector in both wild-type mice and non-human primates revealed serotype-dependent gene transfer differences. This work will be valuable to better determine dose scaling from mouse to human in clinical trials.

P436

Durable CYP21A2 gene therapy in non-human primates for treatment of congenital adrenal hyperplasia

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Severe Congenital Adrenal Hyperplasia (CAH) is most commonly caused by genetic defects in CYP21A2 gene, which leads to a deficiency of the 21-hydroxylase enzyme and disruption in the biosynthesis of Adrenal corticosteriods. Despite treatment with corticosteroids, patients remain at significant risk for adrenal crisis, experiencing a 3-fold higher mortality rate than age matched controls. They also suffer from significant infertility, bone, metabolic, and cardiovascular disease, and hyperandrogenism in women leading to genital abnormalities, hirsutism, and other complications. We are developing an AAV5- based gene therapy (BBP-631) that will provide a functional copy of the CYP21A2 gene to the adrenal glands of CAH patients. To determine the durability of this therapy we treated cynomolgus monkeys with increasing doses of BBP-631 via intravenous injection. At 4-, 12- and 24-weeks post treatment, expression of hCYP21A2 mRNA and vector genome copies (VGC) in the adrenals and other peripheral tissues was measured. VGC was present in the liver and adrenals at 4 weeks, with durable detection through 24 weeks and total vg levels were dose dependent. hCYP21A2 RNA expression in adrenal and liver tissues was also dose dependent and continued to increase from 4 weeks through 24 weeks. The hCYP21A2 expression increased 10-fold at the highest dose from 12 to 24 weeks. There were no adverse safety signals in any of the treated animals. This data combined with efficacy data of BBP-631 in a Cyp21-/- mouse model supports our continued clinical development of BBP-631 as a treatment for congenital adrenal hyperplasia.

P440

Lentiviral haematopoietic stem cell gene therapy (HSC-GT) for metachromatic leukodystrophy (MLD): Preliminary results from a clinical trial with a cryopreserved formulation of OTL-200

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Metachromatic leukodystrophy (MLD), an ultra-rare and fatal demyelinating lysosomal storage disease caused by mutations in the arylsulfatase A (ARSA) gene, currently has no approved treatment. We have presented 1–7.5 years of safety and efficacy results in 29 early-onset MLD patients treated with a fresh formulation of an experimental autologous lentiviral-mediated haematopoietic stem and progenitor cell gene therapy ('OTL-200'). Here we present engraftment and initial pharmacodynamic effects of OTL-200 in the first four early-onset MLD patients (two late infantile, two early juvenile) treated with a cryopreserved formulation as part of an ongoing clinical trial. Autologous CD34+ cells were transduced ex vivo with a self-inactivating lentiviral vector encoding for the functional human ARSA gene under control of the constitutive PGK promoter, and cryopreserved to allow for minimum quality control testing prior to initiation of treatment. Patients received myeloablative conditioning with busulfan before intravenous administration of the thawed drug product. With 1–12 months follow-up, all patients are alive with no reports of adverse events related to OTL-200. All patients engrafted, as evidenced by haematological reconstitution and gene-corrected CD34+ bone marrow cells. Restoration of ARSA activity was observed in peripheral blood mononuclear cells and cerebrospinal fluid (a surrogate indicator of metabolic correction in the brain). Preliminary data from four patients treated with the cryopreserved formulation of OTL-200 show a haematological reconstitution and ARSA activity comparable to those observed in patients treated with the fresh formulation. Patients continue to be evaluated for changes in gross motor function, cognitive development, and long-term safety.

P441

Liver-Targeted AAV Gene Therapy Vectors Produced at Clinical Scale Result in High, Durable Levels of α-GalA Enzyme Activity and Effective Substrate Reduction in a Mouse Model of Fabry Disease

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Fabry disease (FD) is caused by mutations in the GLA gene encoding alpha galactosidase A (α-GalA), leading to systemic accumulation of globotriaosylceramide (Gb3) and lyso-Gb3 in plasma and tissues, resulting in renal, cardiac and cerebrovascular disease. FD is commonly treated with enzyme replacement therapy, requiring lifetime biweekly infusions and which does not invariably clear substrate from kidney, heart and other organs. A liver targeted AAV2/6-hGLA gene therapy was evaluated in a GLAKO mouse model of FD, using a vector manufactured with a clinical scale production method. In a 3-month pharmacology/toxicology study, one-time intravenous administration of AAV-hGLA was well-tolerated in GLAKO mice, resulting in stable supraphysiological expression of plasma α-GalA (over 300-fold normal levels). Dose-dependent increases in tissue α-GalA activity levels were achieved. Gb3 and lyso-Gb3 levels in plasma and tissues were quantified using mass spectrometry, and generally were undetectable in plasma, liver, spleen and heart, and markedly reduced in kidneys. The initial expression cassette was compared to an improved version, ST-920, in a 28-day study in wild type mice. ST-920 was well-tolerated and animals treated with ST-920 produced levels of plasma α-GalA activity over 1,500-fold of normal. These studies provide preclinical proof-of-concept for AAV-mediated targeting of hepatocytes to express therapeutic levels of circulating human α-GalA. A Phase I/II clinical trial to evaluate ST-920 in Fabry subjects is expected to commence later this year.

P442

AAV-mediated gene therapy shows efficacy on the biochemical and neurological phenotype of the nucleoside-enhanced mouse model of MNGIE

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Preclinical studies have shown that gene therapy is a feasible approach to treat mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), a disease caused by toxic accumulation of thymidine (dThd) and deoxyuridine (dUrd). Our objective was to study the effect of the treatment with different liver-targeted AAV8 vectors on the phenotype of a murine model of MNGIE, enhanced by chronic dThd and dUrd administration. Tymp/Upp1 double knockout (dKO) mice were fed with both nucleosides in drinking water since weaning until the end of the study (22 months). At 8 weeks of age, mice were

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treated with different doses of AAV8 carrying the human TYMP coding sequence under the control of different liver-specific promoters (TBG, AAT, HLP), and the biochemical and functional phenotype was studied over the life of the animals. Nucleoside exposure resulted in 30-fold higher plasma nucleoside levels in dKO mice compared with non-exposed WT mice. AAV-treatment provided elevated thymidine phosphorylase activity in liver and lowered nucleoside systemic levels in exposed dKO mice. dThd and dUrd exposure was reduced by the AAV vectors in a dose-dependent manner. AAV-treatment also prevented the imbalances in the deoxyribonucleotide pools observed in exposed dKO mice. Exposed dKO mice had enlarged brain ventricles (assessed by magnetic resonance imaging) and motor impairment (rotarod test); both were prevented by AAV treatment. Among all promoters tested, AAT showed the best efficacy. Our results confirm that AAV-mediated gene therapy restores the biochemical homeostasis in the murine model of MNGIE and, for the first time, show that this treatment improves its functional phenotype.

P443

Pre-clinical development of SPK-3006, an investigational liver-directed AAV gene therapy for the treatment of Pompe disease

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Pompe disease is a lysosomal storage disease caused by loss-of-function mutations in the acid alpha-glucosidase (GAA) gene, which lead to significant accumulation of glycogen in many tissues resulting in multi-system pathology. Enzyme replacement therapy (ERT) increases survival, slows disease progression, and is the current standard of care for Pompe disease patients. However, ERT has several significant drawbacks such as immunogenicity of the recombinant GAA and high treatment burden. We have shown that investigational liver-directed adeno-associated viral (AAV) gene therapy expressing a novel secretable GAA transgene results in decreased glycogen accumulation, increased survival, and improvement of cardiac, respiratory, and muscle phenotypes in the *Gaa*$^{-/-}$ mouse model of Pompe disease. Moreover, secretable GAA vectors demonstrated greater efficacy in restoring *Gaa*$^{-/-}$mouse muscle strength when compared to the standard of care regimen of 20 mg/kg ERT. Further optimization of the expression cassette and selection of a highly hepatotropic capsid led to the development of the clinical candidate, *SPK-3006*, an investigational liver-targeted gene therapy for the treatment of Pompe disease. In support of clinical translation, a single infusion of *SPK-3006* in non-human primates at three ascending doses demonstrated dose-dependent expression of GAA in plasma with no associated adverse histopathological findings and no significant changes in clinical pathology parameters. Additional *in vitro* and *in vivo* studies are ongoing to further characterize *SPK-3006* and to determine the biochemical properties of the transgene-derived protein product following liver expression of secretable GAA.

P444

Characterization of hematopoietic system reconstitution *in vivo* in metachromatic leukodystrophy gene therapy patients

ABSTRACT WITHDRAWN

P445

AXO-AAV-GM1 for the treatment of GM1 gangliosidosis: Program Overview

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GM1 gangliosidosis is a rare, inherited neurodegenerative lysosomal storage disorder caused by mutations in GLB1, encoding lysosomal hydrolase acid β-galactosidase (βgal), and catalyzing the hydrolysis of GM1 ganglioside. Mutations in GLB1 result in impaired β-gal activity, leading to accumulation

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of GM1 ganglioside predominantly in the central nervous system (CNS), where its rate of synthesis is the highest. The toxic accumulation of GM1 ganglioside leads to the progressive loss of neurons in the brain and spinal cord. GM1 gangliosidosis is uniformly fatal, and there are no disease-modifying treatments currently available. As a monogenic disorder, GM1 gangliosidosis is an ideal target for gene therapy to restore function, prevent neurodegeneration, and ameliorate symptoms. AXO-AAV-GM1 is an investigational gene therapy utilizing an adeno-associated virus (AAV) vector to deliver a functional copy of the GLB1 gene with the goals of improving neurological and neuromuscular function and extending survival by restoring β-gal activity and reducing GM1 ganglioside in the CNS and periphery. AXO-AAV-GM1 is being evaluated under a comprehensive clinical development program. The first patient was dosed in May 2019. Here we provide an overview of GM1 gangliosidosis, a summary of relevant preclinical information to support the rationale for the AXO-AAV-GM1 development program, and an overview of the clinical trial design for evaluation of GM1 patients.

P446

Liver-targeted, lipid nanoparticle encapsulated mRNA therapy corrects serum total bilirubin levels in a mouse model of Crigler-Najjar syndrome

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Crigler-Najjar (CN) syndrome is a rare disorder of bilirubin metabolism caused by mutations in the uridine diphosphate glucuronosyl transferase 1A1 (UGT1A1) gene, characterized by hyperbilirubinemia and jaundice. Currently, no cure exists for CN and treatment options are limited to phototherapy, which becomes less effective over time, and the potential for liver transplantation, highlighting the unmet clinical need to develop a novel therapeutic approach. Previous strategies have utilized AAV vectors for expression of UGT1A1 following systemic administration. However, this approach is limited by the presence of pre-existing neutralizing antibodies to the AAV capsid in patients, and the potential for readministration following a subtherapeutic dose is low. Here, we evaluated the therapeutic potential of a liver-targeted, lipid nanoparticle encapsulated mRNA therapy for expression of UGT1A1 following systemic administration in a mouse model of CN. UGT1 KO mice were rescued from lethal postnatal hyperbilirubinemia by phototherapy treatment. These adult UGT1 KO mice were then administered with a single dose of UGT1A1 mRNA encapsulated in lipid nanoparticles. Within 24 hours, serum total bilirubin levels rapidly decreased from 15 mg/dl (256 μmol/L) to less than 0.5 mg/dl (9 μmol/L), which is slightly above wild type mouse levels. Reduction in total bilirubin levels was sustained for two weeks at which time bilirubin levels began to rise, returning to baseline levels of hyperbilirubinemia by day 42 post-administration. Sustained reductions in total bilirubin levels can be achieved by repeated administration. Therefore, liver-targeted, lipid nanoparticle encapsulated UGT1A1 mRNA may be a potential treatment for patients with CN.

P447

Human genome-edited hematopoietic stem cells phenotypically correct Mucopolysaccharidosis type I

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Lysosomal enzyme deficiencies comprise a large group of genetic disorders that generally lack effective treatments. A potential treatment approach is to engineer the patient's own hematopoietic system to express high levels of the deficient enzyme, thereby correcting the biochemical defect and halting disease progression. Here, we present an efficient ex vivo genome editing approach using CRISPR/Cas9 that targets the lysosomal enzyme iduronidase to the CCR5 safe harbor locus in human CD34+ hematopoietic stem and progenitor cells. The modified cells secrete supra-endogenous enzyme levels, maintain long-term repopulation and multi-lineage differentiation potential, and can improve biochemical and phenotypic abnormalities in an immunocompromised mouse model of Mucopolysaccharidosis type I. These studies provide support for the development of genome-edited CD34+ hematopoietic stem and progenitor cells as a potential treatment for Mucopolysaccharidosis type I. Furthermore, the safe harbor approach constitutes a flexible platform for the expression of lysosomal enzymes making it conceivably applicable to other lysosomal storage disorders.

P448

Adeno-associated viral gene therapy for propionic acidemia (PA)

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Propionic Acidemia (PA) is a rare autosomal recessive metabolic disorder caused by reduced activity of the enzyme propionyl-CoA carboxylase (PCC). The PCC enzyme is composed of two subunits, alpha and beta, encoded by the *PCCA* and *PCCB* genes, and is responsible for the catabolism of propionate. PA is equally likely to be caused by mutations in the *PCCA* or *PCCB* gene. Patients diagnosed with PA typically present in the early newborn period with a metabolic crisis, which can be fatal if not promptly recognized and treated. While medical management can decrease the severity of symptoms, patients with PA still have high rates of morbidity and mortality, underscoring the need for new therapies. We have generated new mouse models of Pcca and Pccb deficiency by CRISPR Cas-9 gene editing. Other than a *Pcca (A134T)* knock-in allele, homozygotes for all Pcca and Pccb mutant alleles displayed neonatal lethality and increased 2-methylcitrate, similar to severely affected PA patients. A series of AAV cassettes designed to express the human orthologues of *PCCA* or *PCCB* in the liver of the mutant mice were prepared and pseudoserotyped with an AAV8 or AAV9 capsid. *Pccb*$^{-/-}$ or *Pcca*$^{-/-}$ mice treated at birth showed improved survival and metabolic correction. In summary, we have created new murine models of PA caused by Pcca

or Pccb deficiency, developed candidate AAV vectors that express the human orthologues, and tested them for efficacy in the mutant mice. Our results provide critical preclinical data for the development of AAV gene therapy for propionic acidemia.

P449

Novel neuronal and astrocytic models for Sanfilippo C syndrome using CRISPR/Cas9- edited iPSC. Their use in therapeutic approaches

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Sanfilippo C syndrome is a rare, autosomal recessive, lysosomal storage disorder caused by mutations in HGSNAT, a gene involved in heparan sulfate (HS) degradation. It is characterized by a progressive and severe neurodegeneration for which no treatment exists. Our objective was to generate human cellular models to test new therapies for this disease. We obtained two different HGSNAT-mutated induced pluripotent stem cells (iPSC) lines from a wild-type iPSC line, using CRISPR/Cas9 gene edition. Their stability, pluripotency and differentiation potential were confirmed. Optimizing previously stablished protocols, we differentiated these iPSC into neurons and astrocytes, due to the neurodegenerative involvement of the disease. We confirmed the presence of cell specific markers and the recapitulation of HS storage found in patients' cells. We also compared neuronal branching using isolated GFP+ neurons, to investigate potential differences. These cellular models are a useful tool to test therapeutic approaches. Previously, our group demonstrated on patients' fibroblasts the effectiveness of siRNAs as a substrate reduction therapy (SRT). Now, we are evaluating this strategy using the novel neuronal and astrocytic models obtained. After 3 days of transfection, siRNAs caused a reduction of around 80% in the EXTL2 expression levels, a gene involved in HS biosynthesis. At present, the HS levels and neuronal branching are being compared between the treated and not treated cells. In conclusion, we have generated neuronal and astrocytic models from two different CRISPR/Cas9-edited isogenic iPSC lines. Our results suggest that siRNAs are a promising approach for SRT in relevant human cell types.

P450

GCDH gene therapy ameliorates glutaric aciduria type I phenotype in mice

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Glutaric aciduria type I (GA-I) is a rare metabolic inherited disorder in the catabolic pathways of lysine, hydroxylysine and tryptophan. It is caused by the deficiency of glutaryl-CoA dehydrogenase (GCDH). The enzymatic defect results in the accumulation of glutarate, hydroxyglutarate and glutarylcarnitine in tissues and body fluids. Clinically, GA-I patients display macrocephaly, progressive dystonia and dyskinesia. Dietary lysine restriction, carnitine supplementation and intensified emergency treatment during catabolism are effective for some individuals. Unfortunately, one-third of affected children do not respond to therapy and experience irreversible brain damage. We have explored the feasibility of in vivo gene therapy in the preclinical model of the disease, the Gcdh -/- mice. Young adults and newborns at postnatal day 2 (P2) received an intravenous delivery of AAV9-GCDH in the tail vein or the temporal vein respectively. Mice were exposed to a lysine overload to enhance the severity of the disease, that results in lethality in young animals. Our results show that in adults, GCDH expression was restored in liver and the accumulation of glutarylcarnitine and glutaric acid was partially rescued in liver and serum, both in animals receiving standard or high lysine diet and last for at least 6 months after treatment. GCDH expression was also rescued in liver and brain of P2 treated mice. Interestingly, all P2 mice receiving AAV9-GCDH therapy, exposed to high lysine diet survived whereas 40% of non-treated mice died. In summary, these data suggest the potential of an AAV-mediated gene therapy approach for glutaric aciduria type I.

P451

A novel AAV-based therapy in combination with tolerogenic ImmTOR nanoparticles for a sustained treatment of ornithine transcarbamylase deficiency

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Ornithine TransCarbamylase deficiency (OTCd) is the most common urea cycle disease, with a prevalence of 15,000–60,000 worldwide. The disease results in accumulation of the neurotoxic ammonia in the blood, with detrimental effect for the brain. Despite dietary restriction and ammonia scavenging drugs, many pediatric patients die early in life due to ammonia crisis and neurotoxicity. In this study, we developed a novel therapeutic to treat OTC deficiency, which is based on Adeno-Associated virus (AAV)-based gene therapy in combination with biodegradable synthetic particles containing rapamycin (ImmTOR). ImmTOR has been recently shown to mitigate the formation of neutralizing anti-AAV antibodies and inhibit capsid-specific T cell responses in mice and non-human. This tolerogenic AAV strategy has two potential benefits: 1) ability to treat pediatric patients with the possibility to re-dose to maintain therapeutic levels, and 2) prevent liver damage associated with cellular immune reaction to the therapeutic virus. We generated a novel therapeutic ssAAV8 vector containing a Codon-Optimized (CO) human OTC transgene under the transcriptional control of a liver specific promoter, which is particularly efficient in correcting the OTC-spfash phenotype. A dose finding experiments was performed in the OTC-spfash mouse model, identifying the dose of 5e11 vg/kg as the therapeutic dose, resulting in restoration of

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physiological levels of urinary orotic acid and serum ammonia. ssAAV8 vector co-administered with ImmTOR particles improved efficacy, mitigated the humoral immune response to AAV-hOTC and enabled repeated dosing of gene therapy treatments.

P452

Correction of Gb3 levels in alpha-Galactosidase A deficient mice by enzyme produced *in vivo* by shielded engineered cells

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Introduction. Lysosomal storage diseases (LSDs) are a heterogeneous group of disorders caused by mutations in lysosomal enzyme processing proteins. Fabry disease is an X-linked LSD caused by a defective GLA gene resulting in a deficiency of alpha-galactosidase A (aGal-A) and accumulation of globotriaosylceramide (GB-3) in cells. This results in progressive, life-threatening, multi-organ involvement, including kidney failure, gastrointestinal symptoms, strokes, and heart disease at a young age. We used our Shielded Living TherapeuticsTM technology to create spheres containing a proprietary anti-fibrotic biomaterial and enclosing human cells genetically modified (GM) to secrete aGAL-A. We hypothesize that implanting these GAL-producing spheres into mice with a mice model of Fabry disease will result in a sustained production of aGal-A and a reduction in GB3 and Lyso-GB3. Methods. We implanted a variable number of GAL-A-producing spheres into the intraperitoneal (IP) space of a Fabry knockout mice model. Mice were followed for 3 weeks post-implantation. Results. After implantation of GLA-producing spheres, we observed good enzymatic activity in mouse plasma, liver and spleen. We detected significant reduction of renal, liver, spleen and heart Gb3 and Lyso-Gb3 levels. Conclusions. These preliminary data confirm that persistent aGAL-A secretion and reduction of GB3 and Lyso-Gb3 was obtained in plasma and in relevant tissues of Fabry mice after a single implantation of GAL-producing spheres. The Shielded Living TherapeuticsTM platform is a promising alternative to frequent, life-long administration of protein therapeutics, gene therapy and other therapeutic approaches, with several potential important advantages.

P453

The combination of ImmTOR with AAV Anc80 is therapeutically effective, safe, and repeatable in mice with methylmalonic acidemia while also being compatible with the low seroprevalence of Anc80 Nabs in the patient population

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Methylmalonic acidemia (MMA) is a common and severe organic acidemia most frequently caused by mutations in methylmalonyl-CoA mutase (MMUT). Here, we describe a therapeutic approach in which ImmTOR™ (nanoparticle-encapsulated rapamycin) is delivered in combination with an AAV vector expressing human MMUT and pseudoserotyped with the synthetic capsid, Anc80. We have previously shown that free rapamycin, chronically dosed, is tolerated by MMA patients. Here we demonstrate that ImmTOR, at doses up to 300 μg rapamycin/mouse, is well tolerated in a murine model of MMA ($Mut^{-/-}$;$Tg^{INS-MCK-Mut}$) that recapitulates a clinically severe disease phenotype. Since vector re-administration may be required for effective MMA treatment, we evaluated the ability of ImmTOR to enable therapeutic re-dosing in MMA mice. Mice was first injected with $5x10^{10}$ vg/kg of non-therapeutic Anc80-CB-Luc combined with 300 μg ImmTOR followed by $2.5x10^{-12}$ vg/kg of therapeutic Anc80-hAAT-MUT alone or combined with 300 μg ImmTOR after a 47-day interval. ImmTOR first treatment prevented the formation of anti-Anc80 IgG allowing for effective therapy, presented a pronounced reduction in metabolites by day 12 after repeat treatment ($p < 0.0001$). This shows that ImmTOR is efficacious and well-tolerated in MMA mice, and allows therapeutic redosing of AAV by blocking the formation of anti-capsid IgG. Finally, we surveyed 25 plasma samples from non-transplanted MMA patients. Nabs were detected in only 4/25 (16%), using an estimated 1% cut-point. Collectively, the low Anc80 NAb seroprevalence in the patient population coupled with ImmTOR-induced tolerance suggest high clinical promise for repeat treatments of MMA patients by a combination of ImmTOR and Anc80-MUT.

P454

Therapeutic efficacy of VTX-801 Wilson's disease mice in mice with at an advanced stage the disease

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Wilson disease (WD) is a disorder of copper metabolism that can present with hepatic, neurologic, or psychiatric disturbances, or a combination of these. Left untreated, WD is a life-threatening condition. Recently, we have demonstrated that an AAV vector carrying a reduced version of the human ATP7B (mini-ATP7B) provides long-term correction of copper metabolism in 6-week old WD mice.1 Here we provide further evaluation in animals with a more advanced disease. Unlike 6-week old WD mice, untreated 16 and 20 weeks old mice showed clear liver histological abnormalities, with a significant inflammatory infiltrate, hepatocyte hypertrophy and necrosis. Such older mice received two different doses of VTX-801, the dose previously identified as therapeutic in young animals and a dose 3 times higher. Animals were monitored monthly and sacrificed 6 months after VTX-801 administration. A time of sacrifice, VTX-801 administration achieved a reduction of copper urinary excretion rate and normalization or improvement

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of all relevant biochemical and hematological parameters. Furthermore, hepatic copper content was reduced groups in all treatment. Liver histology was clearly improved in all the animals receiving VTX-801 with reduced inflammation and hepatocyte hypertrophy and necrosis compared to untreated animals. Transduction efficiency was dose-dependent and only slightly lower than in VTX-801-treated young animals. Our data demonstrate that VTX-801 administered to animals showing significant liver histological alteration and sustained liver damage was able to prevent the progression of Wilson's disease and improve a majority of WD pathological features, indicating that gene therapy is efficient even at an advanced stage of the disease.

P455

Skeletal muscle-directed FGF21 gene therapy counteracts obesity and type 2 diabetes

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Obesity and type 2 diabetes are strongly associated and a major health problem because of their alarmingly growing prevalence worldwide. Currently available therapies are not suited for all patients in the heterogeneous obese/T2D population, and there is a need for novel treatments. Fibroblast growth factor 21 (FGF21) is considered a promising therapeutic agent for T2D/obesity. Native FGF21 has, however, poor pharmacokinetic properties, making gene therapy an attractive strategy to achieve sustained circulating levels of this protein. The aim of this study was to evaluate the potential of the anti-obesogenic and anti-diabetic effects of the long-lasting secretion of FGF21 into the bloodstream following a single administration of adeno-associated viral (AAV) vectors to the skeletal muscle (Skm). Treatment of adult mice, fed a high-fat diet for a long time, with AAV-FGF21 vectors resulted in marked increase in circulating FGF21 levels, which was parallel to high expression of vector-derived FGF21 in skeletal muscle. In contrast to HFD-fed null-injected animals, mice treated with AAV-FGF21 showed marked reductions in body weight, adipose tissue hypertrophy and inflammation, hepatic steatosis, inflammation and fibrosis and insulin resistance. In addition, treatment with AAV-FGF21 vectors reduced the incidence of liver neoplasms associated to long-term-HFD-feeding. Altogether, these results demonstrate that intramuscular administration of AAV vectors that lead to therapeutically-relevant levels of circulating FGF21 is safe in the long-term and highlight the therapeutic potential of this approach to treat T2D and obesity in the future.

P456

Modelling Tyrosine Hydroxylase Deficiency using induced pluripotent stem cells

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Tyrosine Hydroxylase deficiency (THD) is an inherited metabolic disorder caused by a defect in the TH enzyme, which catalyses the rate-limiting step in the biosynthesis of dopamine (DA). Two clinical phenotypes have been described: ''Type A'' tends to be L-Dopa responsive and ''Type B'' which produces a severe encephalopathy of early-onset with sub-optimal L-Dopa response. Although the genetic mutations are known, there is little understanding of the molecular basis for THD brain anomalies. We generated induced pluripotent stem cell (iPSC) lines from Type A and B patients, age-matched healthy individuals (Ctrl) and isogenic corrected iPSC line (THD-A-GC). We used non-integrative episomal vectors to deliver Oct4, Sox2, Nanog, Klf4, c-myc and Lin28 to generate 2–4 independent iPSC lines per individual, that showed a fully pluripotency. Upon DAn differentiation, neurons harbouring TH mutations (A and B) reproduced the disease-associated phenotype: reduction of TH-immunoreactive cells and decreased in enzyme activity. Mutation in R233H (THDA) causes a TH stability defect revealed by TH intensity increase in the cell body that was decreased along the axons to the axon terminal. CRISPR/Cas9 correction of the R233H mutation abolished the THD A deficit, restoring both the number of TH+ cells and TH protein levels. TH immunoreactivity (fiber density and axon gradient) and the presence of dopamine in TH+ cells was also recovered, confirming the robust consistency of mutation-related changes in THD A-derived DAn. This new human THD stem cell model offers a unique option for understanding THD physiopathology and opens the path for exploring novel therapeutic strategies.

P457

Preclinical validation of radioactive copper excretion as a translational tool for evaluating the pharmacodynamics of gene therapy VTX-801 for Wilson's Disease in future first in Human

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Wilson's disease (WD) is an autosomal recessive metabolic defect of hepatocyte copper excretion into the bile, caused by absent or reduced ATP7B copper transporter function. The ATP7B transporter has a dual role: it transports copper into the trans-Golgi compartment for incorporation into the plasma protein ceruloplasmin and into the bile for excretion of copper excess. Recently, we have demonstrated that the administration of an adeno associated vector (AAV) encoding a mini version of the human ATP7B cDNA (VTX-801) provides long-term correction of copper metabolism in Atp7B-/- (WD) mice. In anticipation of a future gene therapy clinical trial, we evaluated copper excretion as a pharmacodynamics biomarker. For that purpose, WD male mice were intravenously injected with one of 3 VTX-801 dosing regimen at 6 weeks of age. Three months later, radiocopper was injected intravenously in treated WD and age-matched control untreated WD or healthy mice Abdominal PET analyses were performed together with radioisotope quantification in feces and urine over a period of 72h, organs were collected at sacrificed and. post- Faecal radiocopper excretion was significantly higher in healthy mice in comparison to untreated WD mice, importantly VTX-801 treatment resulted in the restoration of faecal radiocopper elimination in a dose-dependent manner. Furthermore, hepatic radiocopper retention was significantly lower in WD treated mice and in WT animals in comparison to untreated WD mice. In conclusion, faecal radiocopper excretion represents a very promising biomarker to evaluate the pharmacodynamics of ATP7B gene supplementation in mice and possibly in WD patients for supporting therapeutic efficacy.

P458

Manufacture of an ATMP for *ex vivo* gene therapy of mucopolysaccharidosis IIIA (MPSIIIA)

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Mucopolysaccharidosis IIIA (MPSIIIA) is rare metabolic disorder of monogenic origin. There is currently no curative treatment for MPSIIIA and enzyme replacement therapy has been ineffective at penetrating the blood brain barrier. An alternative therapeutic strategy is ex vivo gene therapy using lentiviral vector (LV) to transduce haematopoietic stem cells. In preparation for an ex vivo clinical trial, we have conducted several validations of a manufacturing technique to create an ATMP consisting of autologous CD34+ cells transduced with an LV containing the human SGSH gene. The optimised 3-day manufacturing procedure consists of CD34+ enrichment using COBE and CliniMACS® Plus selection from mobilised peripheral blood stem cells; pre-stimulation culture in SCGM media without IL-3 but including SCF, Flt-3L and TPO cytokines; transduction with CD11b.SGSH LV and transduction enhancers protamine sulphate and LentiBOOSTTM. Following transduction the cells are formulated in CryoStor CS5 and filled into cryobags and QC vials. Drug product and QC vials are cryopreserved and stored in vapour phase LN2 (VP LN2). Batch release testing is performed from a thawed QC vial >24hrs after cryopreservation. A stability study using transduced MPSIIIA patient cells demonstrates that the cryopreserved transduced cells are stable for 6 months in VP LN2. An ATMP was manufactured for a patient with unmet clinical need under the terms of the GOSH Pharmacy 'specials' licence. The drug product had a VCN of 3.79, viability 84.30%, 212 CFUs per 1000 cells. Furthermore, the SGSH activity measured in CFUs of 3.7 μM 4MU/μg/17hr was significantly higher than healthy human controls.

P459

The Alpha-1-Antitrypsin Promoter Improves the Efficacy of an AAV Vector for the Treatment of MNGIE

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MNGIE (mitochondrial neurogastrointestinal encephalomyopathy) is a devastating disease caused by mutations in TYMP, encoding thymidine phosphorylase (TP). As a result of TP dysfunction, MNGIE patients accumulate toxic concentrations of the nucleosides thymidine (dThd) and deoxyuridine (dUrd), which interferes with mitochondrial DNA replication. Preclinical studies conducted in mice have shown that gene therapy using an adeno-associated vector (AAV) transcriptionally targeted to liver provides enough TP enzyme to this organ to enable effective clearance of dThd and dUrd excess from the whole body. Here, we have studied the effect of several promoters (thyroxine-binding globulin [TBG], phosphoglycerate kinase [PGK], hybrid liver-specific promoter [HLP], and alpha-1-antitrypsin [AAT]) and DNA configurations (single stranded or self-complementary) on the expression of the TYMP transgene, using the AAV8 serotype in a murine model of MNGIE. All vectors provided high levels of TP activity to the liver and normalized nucleoside homeostasis in mice. Liver-specific TBG, HLP, and AAT promoters were more effective than the constitutive PGK promoter, and the self-complementary DNA configuration did not provide any therapeutic advantage over the single-stranded configuration. Among all tested constructs, AAV-AAT was the most effective one, as judged by its capacity to normalize plasma dThd and dUrd levels in all mice treated at the lowest dose (5x10^10 vector genomes/kg). Based on these results, we propose using the AAT promoter when a vector is designed for clinical purposes.

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P460

Gene-cell therapy for Pompe disease

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Pompe disease is a rare autosomal recessive multisystem disease caused by mutations in the gene coding for the acid alpha-glucosidase enzyme (GAA) that leads to accumulation of glycogen in most tissues, producing tissue damage, especially in cardiac and skeletal muscle. In the absence of treatment, patients die in their first years due to cardiac or respiratory failure. Current treatments, based on intravenous infusion of recombinant human GAA (ERT), are only palliative. As an alternative to ERT, gene therapy is becoming a realistic possibility that could theoretically cure Pompe patients. In this work we aim to achieve this goal combining cell and gene therapy. Firstly, we have performed engraftment experiments with hematopoietic stem cells (HSCs) transduced with lentiviral vectors (LVs) expressing eGFP in a Pompe murine model, showing a long term expression and distribution, reaching several target tissues including the central nervous system (CNS). We have designed a modified murine GAA (mGAAopt) in order to achieve high expression levels, secretion and improved uptake by target cells. Myeloid cells (K562) transduced with LVs-mGAAopt expressed and secreted high GAA protein that was efficiently captured by murine muscle target cells. We have performed comparative experiments in order to study the improvement in expression, secretion and uptake between different designs of mGAA; mGAAopt and IGF2-mGAA. We are currently studying the therapeutic efficacy of our strategy in a murine model of pompe disease by intravenous inoculation of lin-GAAKO cells transduced with LVs expressing mGAAopt, IGF2-mGAA and mGAA.

P461

Phenylalanine hydroxylase (PAH) liver distribution and characterization following AAV5-hPAH gene therapy in Pahenu2 mice

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Phenylketonuria (PKU) is causally linked to the deficiency of phenylalanine hydroxylase (PAH), and characterized by hyperphenylalaninemia, and neurocognitive and neuropsychiatric deficits. Recent advances in AAV based gene therapy provide an opportunity for sustained amelioration of the PKU phenotype by correction the PAH deficiency. The effects of AAV5-PAH gene therapy were assessed in the well characterized classical PKU, PAHenu2, mouse model. AAV5-human PAH (hPAH) vectors were administered to Pahenu2 male mice at 2E13 and 2E14 vector genomes per kilogram body weight via tail vein injection. Experiments sought to address: 1) Bio-distribution of AAV5-hPAH vector genomes and transgene derived PAH protein in liver tissue. 2) Effects of AAV5-PAH gene therapy and transgene derived PAH protein on liver safety. A dose-dependent increase in the number of hepatocytes staining positive for both hPAH DNA and protein was detected with the majority of hepatocytes transduced at high dose. Comprehensive analyses consisting of histopathological examination, TUNEL and activation of the monocytic cell lineage were performed. No hepatic histopathological evidence of increased cell death or marked signs of inflammation following AAV5-hPAH vector administration were detected. These data support that AAV5-mediated gene delivery of hPAH may be a viable option as a potential therapeutic for PKU.

P462

Phenotype rescue by GCDH targeted gene edition in a cellular model of Glutaric Aciduria type I

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Glutaric Aciduria type I (GA-I) is a rare autosomal recessive disorder caused by the deficiency of the enzyme glutaryl-CoA dehydrogenase (GCDH). Current therapeutic strategies include dietary lysine restriction, carnitine supplementation and anti-catabolic emergency treatment during acute episodes. Unfortunately, despite the adherence to the diet about one third of the patients develop acute encephalopathic seizures with severe neurological consequences. In this work we develop a gene-targeting strategy to rescue the GCDH activity in neuroblastoma SH-SY5Y cells, through targeted insertion of the GCDH gene in the AAVS1 safe harbor genomic locus. First we generated a GCDH-deficient SH-SY5Y cell line using CRISPR/Cas9 (SH-SY5Y-GCDH-KO). Then, a GCDH expression cassette was inserted in the AAVS1 locus by CRISPR/Cas9 homologous recombination. Molecular analysis of gene-edited SH-SY5Y-GCDH cells confirmed the specific deletion in the GCDH region and integration of the GCDH cassette in the AAVS1 locus. Expression analysis of GCDH demonstrated loss of expression in the KO cells and restoration of GCDH in the edited cells. Exposure of KO cells to glutaric acid, a toxic metabolite from lysine catabolism, or to lysine, resulted in decreased viability of the culture that was rescued in the GCDH edited cells. Thus, our results provide a relevant human neuronal cellular model of GA-I to study the molecular pathogenesis of the disease and evaluate the potential benefit of gene-targeting correction.

P463

Lentivirus mediated insulin promoter directed insulin gene expression is effective in suppressing postprandial glucose excursions in Type 1 diabetes

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Autoimmune destruction of pancreatic beta cells in conjunction with insulin deficiency is the distinctive feature of Type 1 diabetes (T1DM). Achieving glucose regulated insulin gene

expression by gene therapy is a great area of interest to meet the demand for fluctuating daily glucose levels and necessary to avoid multiple daily injections of insulin. Because previous insulin gene delivery to non-beta cell types did not produce successful results in dealing with postprandial glucose increase, a new lentiviral vector (LentiINS) with insulin promoter hooked up to proinsulin encoding gene was generated using Multisite Gateway Technology to test its therapeutic potential in T1DM animal model of diabetes. Intraperitoneal delivery of HIV-based LentiINS vector resulted in 2-fold increase in serum insulin levels, lowering both of fasting and non-fasting hyperglycemia, and improved glucose tolerance. Furthermore, reconstitution of insulin gene expression in insulin gene knock out pancreatic beta cell line generated by CRISPR/Cas9 technology temporarily reduced plasma blood glucose levels following pancreatic beta cell transfer under kidney capsule of STZ-induced diabetic rats. In conclusion, insulin promotor directed insulin gene expression via lentivirus mediated gene delivery is effective against postprandial raise in glucose levels. TÜBİTAK-215S820 [1]: Sanlioglu, A. D., et al. (2012). ''Insulin gene therapy from design to beta cell generation.'' Expert Rev Mol Med 14: e18.

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P464

Codon optimization of the TYMP sequence for the gene therapy of MNGIE

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Preclinical studies have shown that liver-targeted AAV-mediated gene therapy is a feasible approach to treat mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). Optimizing the vector is a critical aspect because it helps minimizing the vector dose needed to reach the efficacy of the treatment. We designed four different codon-optimized sequences of the TYMP coding sequence (encoding the enzyme thymidine phosphorylase, TP), using 4 different algorithms. We cloned each optimized sequence in a lentiviral vector under the control of the phophoglycerate kinase promoter, and transduced 4 different human cell lines (Huh7, HEK293, and 2 lymphoblastoid cell lines from TP deficient patients), to analyse the degree of expression improvement achieved by each algorithm, as compared with the non-optimized wild-type TYMP sequence. Six days after transduction, we evaluated TP activity, vector copy number, and mRNA levels in the cell lines. Among all experiments performed, only one optimized sequence (algorithm A) resulted in significantly higher TP activity when expressed per vector copy number, and only in one cell line (Huh7). However, the ratios mRNA levels/vector copy number were also significantly elevated in this experiment. Remarkably, when normalized by TYMP mRNA copy number, TP activity values were lower for this codon-optimized sequence than those observed with the wild-type sequence. Taken together, these results suggest that the TYMP sequence codon-optimized according to algorithm A improves the results due to improved transcription and/or mRNA stabilization rather than translation improvement.

P465

Gene edited cells in Mitochondrial Recessive Ataxia Syndrome (MIRAS)

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Mitochondrial Recessive Ataxia Syndrome (MIRAS) is a mitochondrial disease, in which the identical mutation shows a wide range of phenotypes and onset times in different patients. The nuclear-encoded POLG c.2243g>c (p.W748S) mutation has a Finnish origin, and the early onset disease phenotype includes epilepsy and severe valproate hepatotoxicity. The mechanisms underlaying the disease remain unknown partially due to lack of a proper model system. The patient cell-derived iPS cells can be cultured and differentiated to study the disease in vitro, as they mimic the epileptic phenotype of the patients. In this study, we used CRISPR/Cas9 gene editing with homologous recombination to correct the disease mutations in patient iPS cells. Our results show that CRISPR/Cas9 editing system is effective for the MIRAS mutation correction. The generation of isogenic lines of MIRAS and measuring the consequent phenotypic changes of gene editing allow the studies of the pathogenesis and potential therapeutic interventions.

P466

Generation and functional analysis of plasmid DNA encoding β-hexosaminidase gene for Tay-Sachs disease therapy

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Tay-Sachs disease belongs to the group of autosomal-recessive lysosomal storage metabolic disorders. TSD is caused by mutations in the HEXA gene. The incidence of this disease is one in 100,000 live births. This disease results from a deficiency of lysosomal enzyme β-hexosaminidase A (HexA), which is responsible for GM2 ganglioside degradation. Tay-Sachs disease is characterized by acute neurodegeneration preceded by activated microglia expansion, macrophage and astrocyte activation along with inflammatory mediator production. Currently, TSD treatment is based on symptom relief and, in case of the late-onset form, on the delay of progression. pLX303 lentiviral expression plasmid encoding HexA enzyme gene was developed in this work. HEXAgene was cloned from donor plasmid into pLX303 vector by LR recombination. Genetic modification of HEK293T cells was carried out with the recombinant plasmid with HEXAgene. pLX303-Katushka2S construct encoding a red fluorescent protein was used as a control. The conditioned medium and modified cells were collected to analyze HexA enzymatic activity and enzyme expression, respectively, in 48 hours after transfection. MUGSfluorescent substrate was used to determine the HexA enzymatic activity. The level of fluorescence was measured using a InfinitePro 200 reader. The activity of HexA

enzyme was higher by 10 times in cells modified with HEXAgene compared to control cells. WB analysis showed the presence of HexA enzyme in genetically modified cells. Thus, the expression and functional activity of HexA enzyme was shown. The generated plasmid construct can be the basis for the development of new methods for Tay-Sachs disease gene-cell therapy.

P467

Lentiviral-mediated TRAIL transfer decreased serum alkaline phosphatase, total cholesterol, and glucose levels in high-fat diet-fed obese mice

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TNF-Related Apoptosis-Inducing Ligand's (TRAIL) putative protective role in obesity and diabetes distinguishes it from other TNF superfamily members, such as TNF-alpha and FasL. Soluble TRAIL (sTRAIL) application was implicated in attenuation of some metabolic abnormalities in high-fat diet-fed mice. We applied TRAIL-encoding lentiviral vectors to obese C57BL6 mouse models and tested for alterations in various liver and kidney marker levels. All mice were fed ad libitum until 8 weeks of age with chow (containing 5–10% fat). High fat diet (HFD, 60% fat) was started in the experimental groups and continued for at least 8 weeks. Weekly measurements of weight and non-fasting blood glucose levels were done in all animals. TRAIL-encoding 3rd generation lentiviral vectors were applied intraperitoneally. Blood was collected from the animal groups and sera was seperated to test for differences in the levels of albumin, glucose, total bilirubin, gamma-glutamyl transpeptidase, alkaline phosphatase, alanine aminotransferase (liver panel); total protein, creatinine, inorganic phosphorus, blood urea nitrogen, calcium levels (kidney panel); and total cholesterol levels, in experimental and control groups, by a biochemistry autoanalyzer. Our results revealed decreased serum alkaline phosphatase, total cholesterol, and glucose levels in experimental groups treated with Lenti-TRAIL vectors. These findings potentiate protective/therapeutic features attributed to TRAIL in obesity as well as in diabetes, and although further investigation is required, lentiviral vector-mediated TRAIL application might be an effective candidate for long-term alleviation of metabolic abnormalities in obesity (TUBITAK Grant No: 112S450).

P468

Liver–directed Pdx1 expression using recombinant AAV-2 and AAV-DJ/8 vectors in diabetic mouse model

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Type 1 diabetes (T1D) is a disease that is resulted from autoimmune destruction of the insulin-producing β-cells in pancreas. Recently, the generation of insulin-producing cells has emerged as a promising approach for the treatment of T1D. A transcription factor, pancreatic duodenal homeobox1 (Pdx1), plays an important role in the development of pancreatic β-cells. In this study, we compared the serotype 2 and DJ/8 adeno-associated viral vectors encoding the human Pdx1 gene to generate insulin-producing cells in the liver. When AAV2-Pdx1 was injected, the gene transfer efficiency into liver was 20 $\sim$ 30%, and the blood glucose levels were decreased in diabetic mice, but did not reach to the normal level. When AAV-DJ/8-Pdx1 was injected into diabetic mice, diabetic symptoms were alleviated than the control group. Interestingly, however, the blood glucose level was not decreased. We analyzed the differences in liver tissues transduced by the different types of AAV. We will discuss the transduction and insulin production patterns of both AAV-2 and AAV-DJ/8 vectors in the mouse livers.

P469

S1P prevent irradiation induced parotid injury in miniature pig model

ABSTRACT WITHDRAWN

P470

Reaction of liver cells to streptozocin-nicotinamide-induced diabetes mellitus in mice

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One of diabetes mellitus (DM) animal models is performed by injection of streptozocin-nicotinamide (SZC-NA) – agents that via GLUT-2 transporter blockage affect glucose transport into cells. GLUT-2 is highly present on pancreatic β-cells that explain toxicity of STZ to them and application of SZN-NA to model DM. GLUT-2 is present on hepatocytes, which participate in maintaining glucose levels and deposit it as glycogen. Damage of hepatocytes is always

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accompanied by activation of hepatic stellate cells (HSC) that transform into collagen-producing myofibroblasts, resulting in liver fibrosis. The aim of the research was to study reaction of liver cells to SZN-NA-induced DM in mice. Experimental groups: 1) control mice (C57Bl/J); 2) mice with intraperitoneal injection of SZC-NA (100mg/kg). Liver paraffin sections (40 days after) were stained by Mallory's trichrome (connective tissue), PAS (glycogen content), immunohistochemically (IHC) with antibodies to CD163 (macrophages), desmin (HSC), α-SMA (myofibroblasts). Diabetes development was proved by increase of blood sugar and decrease of insulin-secreting β-cells in pancreas. In the liver there was hydropic degeneration of hepatocytes with reduction of glycogen content, mixed polymorphonuclear-mononuclear infiltration including CD163+macrophages. IHC to desmin demonstrated reduced number of desmin+HSC in compare to control group. IHC to α-SMA showed no myofibroblasts. Features of fibrosis were excluded by Mallory's trichrome staining to connective tissue. Thus, SCZ-NA-induced DM is accompanied by damage of glycogen content in hepatocytes, inflammation with Kupffer macrophages activation. The HSC are inhibited, they don't transform into myofibroblasts, there is no liver fibrosis development. Work supported by Program of Competitive Growth of KFU.

P471

TRAIL increases glucose-stimulated insulin secretion in Min6 cells

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TNF-Related Apoptosis-Inducing Ligand's (TRAIL) protective effect against diabetes in animal models is now well-acknowledged. In accordance, TRAIL does not induce apoptosis in primary pancreatic beta cells, and claimed to protect these cells from the destructive effects of proinflammatory cytokines. We hypothesized that this protective effect may also include increased glucose-stimulated insulin secretion in beta cells. We tested our hypothesis in Min6 mouse beta cell lines. Cultured Min6 cells were treated with three different concentrations of soluble TRAIL (sTRAIL; 1/10/100 mg), along with increasing concentrations of D-glucose (0/2,8/15/25 mM). Insulin ELISA test revealed increased insulin secretion in response to 15 and 25 mM of glucose applications at 10 mg TRAIL concentration. The fact that TRAIL increased glucose-stimulated insulin secretion at 10 mg concentration is in accordance with our previous findings where TRAIL induced proliferation in beta cells selectively at the same concentration. While this effect should be tested on different cell lines and primary cells as well, it potentiates TRAIL as a candidate therapeutic molecule for gene therapy approaches in diabetes (TUBITAK grant no: 112S450).

P472

Biochemical, histological and behavioural characterization of a new mouse model of Niemann-Pick C2 disease

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The Niemann-Pick C2 (NPC2) disease is an ultra-rare autosomal recessive Lysosomal Storage Disease (LSD) caused by deficiency of Niemann-Pick C2 protein. NPC2 is involved in the egress of unesterified cholesterol from the late endosome/lysosome compartment to other parts of the cell. As a consequence, pathologic accumulation of cholesterol in lysosomes occurs, which leads to cellular damage. Clinically, patients exhibit neurodegeneration due to progressive neuronal death, hepatosplenomegaly, lung deficiency and reduced life expectancy. To date, only substrate reduction therapy is approved for the treatment of NPC2, with limited efficacy. Here, we report the characterization of a new NPC2 mouse model generated by targeted disruption of the Npc2 gene. Starting at 1 month of age, NPC2-deficient mice showed signs of CNS pathology which included accumulation of unesterified cholesterol, lysosomal distension and dysfunction, hypomyelinitation, neuroinflammation and reduced brain weight. Evidence of impaired autophagy was detected in the cerebellum, which led to a drastic reduction in Purkinje cell density. In the periphery, cholesterol accumulation was observed in most organs analysed, but lysosomal pathology was most severe in the liver. NPC2-deficient mice also showed progressive hepatosplenomegaly and body weight loss. Finally, these mice had severely shortened lifespan as well as evident behavioural alterations, ataxic gait and reduced exploratory activity, all of which worsened over time. Altogether, these results demonstrate that this animal model recapitulates human NPC2 disease, providing a valuable tool to gain insight into the physiopathology of the disease and for the development of novel gene therapy strategies to treat NPC2 patients.

P473

Long-term correction of visual impairment after AAV-NAGLU-mediated gene therapy in a mouse model of mucopolysaccharidosis type IIIB

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Mucopolysaccharidosis type IIIB (MPSIIIB) is a rare autosomal recessive lysosomal storage disease caused by the deficiency of the lysosomal enzyme N-acetyl-alpha-glucosaminidase (NAGLU), involved in the stepwise degradation of the glycosaminoglycan heparan sulfate (HS). The lack of NAGLU is responsible for the pathological accumulation of undegraded forms of HS inside the lysosomes, that leads to cellular dysfunction and eventually, cell death. MPSIIIB is characterized by progressive and severe neurodegeneration and neuroinflammation, with mild somatic involvement. With age, patients also develop marked photoreceptor degeneration causing severe vision loss. We have previously demonstrated that intra-cerebrospinal fluid (CSF) administration of AAV9 vectors encoding the murine NAGLU enzyme (AAV9-Naglu) corrected both brain and somatic

pathology in a mouse model of MPSIIIB. Here, we examined the long-term ability of this gene therapy to counteract photoreceptor loss and to preserve visual function in MPSIIIB mice. To this end, AAV9-Naglu vectors were administered into the CSF of 2-month-old MPSIIIB mice and the therapeutic effect on visual function was evaluated 10 months after vector delivery. At 12 months of age, electroretinography and histopathological evaluation of retinas from MPSIIIB-treated mice showed a complete restoration of cone function and an amelioration of rod-driven responses. This correlated with an improvement in visual acuity in the water maze and optomotor response tests. Therefore, this study demonstrates that this AAV9-Naglu gene therapy, in addition to revert brain and somatic pathology, would be able to improve the eye disease observed in MPSIIIB patients.

P474

Generation of a rat model of Mucopolysaccharidosis type IVA (Morquio A disease) that reproduces the human pathology

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Mucopolysaccharidosis Type IVA (MPSIVA) is an autosomal recessive Lysosomal Storage Disease caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS). The absence of this enzyme leads to accumulation of the glycosaminoglycans (GAGs) keratan sulfate (KS) and chondroitin-6-sulfate. The pathological accumulation of these undegraded GAGs, mainly in chondrocytes and extracellular matrix of cartilage, has a direct impact on cartilage and bone development, leading to skeletal dysplasia, early cartilage deterioration and life-threatening complications. The availability of animal models that closely reproduce the human disease is key to assess the efficacy of new therapies. None of the existing MPSIVA mouse models develop the skeletal and cartilage pathology, the main features of the disease. To overcome these limitations, we have generated a new MPSIVA rat model using the CRISPR/Cas9 technology to introduce the most frequent and severe human missense mutation (c.1156C>T). As a result of the lack of GALNS activity, MPSIVA rats developed the main pathological signs of the disease, such as alterations in body size, early loss of articular cartilage, chondrocyte hypertrophy, skeletal alterations, enamel hypoplasia and accumulation of KS in serum and peripheral organs, mainly in the liver and cardiovascular and respiratory systems. MPSIVA rats had a considerable shorter lifespan compared to healthy WT littermates. Therefore, the MPSIVA rat constitutes the first animal model that recapitulates the pathological phenotype of Morquio A patients. This rat model would be a very useful tool to assess new therapeutic approaches to revert the skeletal and peripheral alterations in Morquio A disease.

P478

Do Mesenchymal Stromal Cells require genetic engineering to enhance their osteogenic commitment?

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Genetic engineering of multipotent mesenchymal stromal cells (MSC) is being explored to circumvent inefficiencies in differentiating into fully functional tissues. Particularly in Wharton's jelly (WJ)-MSC, numerous in vitro experiments have evidenced that their commitment into the osteogenic lineage is considerably less pronounced than that observed in BM-MSC, making them candidates for genetic engineering prior to clinical use. However, in the present study we demonstrate that such delay in WJ-MSC's bone differentiation is related to insufficient osteogenic induction with current in vitro methods rather than incompetence to differentiate properly. Particularly, our results evidence that in vitro stimulation of the BMP-2-pathway with BMP-2 plus tanshinone IIA matches WJ-MSC osteogenic commitment with that of their BM-MSC counterparts. Additionally, we prove that conditioned media from differentiating BM-MSC strongly enhances WJ-MSC osteogenesis and shortens time for differentiation. This suggests that WJ- and BM-MSC's therapeutic effects may be equivalent when administered within bone microenvironment. To demonstrate this hypothesis, we injected human WJ- and BM-MSC intra-tibially in NSG immunodeficient mice. Four weeks after injection, new bone formation was detected in subjects from both groups but not in PBS treated animals, therefore confirming that osteogenic priming of WJ-MSC ex vivo is not required. In view of this results, we conclude that despite genetic engineering is indeed a powerful tool allowing to ''customize'' MSC for specific purposes, the risks associated to its clinical use are not justified when there is still a major lack of knowledge regarding the behaviour of this type of cells in response to specific microenvironments.

P480

Feasibility of gene therapy for Friedreich Ataxia-associated cardiomyopathy in non-human primates: Evaluation of delivery route, biodistribution and expression following AAVRh.10.FXN administration

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Friedreich ataxia (FA) is a rare autosomal recessive genetic disease, resulting from frataxin (FXN) deficiency causing mitochondria and bioenergetic impairment. FA is characterized by spinocerebellar and sensory ataxia as well as hypertrophic cardiomyopathy which is the main cause of premature death in FA patients. Previously, it was demonstrated that AAVRh.10-hFXN administration can fully rescue the heart function and morphology, at early and late stages of the disease, in an FA cardiac mouse model, when transducing ≥50% of cardiomyocytes while increasing moderately the FXN protein level globally throughout the heart at ≥57% the endogenous level. To translate this therapeutic strategy into the clinic, a short-term feasibility study was conducted in cynomolgus monkey to compare the biodistribution and expression of AAVrh.10-hFXN administered by three routes. The vector was delivered by intravenous (i.v.) infusion (6E+12vg/kg, n = 3), retrograde infusion through the coronary sinus (1E+13vg/kg, n = 3) or directly by multiple epicardial injections (2 sites; 2E+12vg each in 65–125μL volume, n = 3). The vector biodistribution was quantified and mapped in the left and right ventricles and atria, in addition to the CNS and major peripheral organs. The i.v. group displayed superior vector biodistribution throughout the heart, with transgenic protein levels close to human and NHP endogenous levels, thus exceeding the predicted therapeutic thresholds identified previously. Importantly, the hFXN protein was correctly targeted and processed in the cardiomyocyte mitochondria. In conclusion, this study demonstrates the feasibility and therapeutic potential of FA cardiac gene therapy, following i.v. delivery of AAVRh.10-hFXN vector at dose clinically relevant, i.e. 6E+12 vg/kg.

P482

A new rat model of Pompe disease generated by CRISPR/Cas9 technology mimics the human disease phenotype

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Glycogen storage disease type II (GSD II or Pompe disease) is a life-threatening autosomal recessive genetic disorder that affects approximately one in 40,000 births. GSD II results in deficiency of the lysosomal enzyme acid α-glucosidase (GAA) that leads to a progressive glycogen accumulation in lysosomes. GSD II patients show myopathy with progressive muscle weakness and finally die because of cardiorespiratory failure. Although mouse models of Pompe disease develop ubiquitous glycogen storage, they do not completely reproduce human disease severity as they generally display an intermediate phenotype between the severe infantile and milder adult forms of Pompe disease. Here, a new knock-in rat model of Pompe disease (KI GAA rat) was generated by introducing a nonsense mutation in the rat GAA exon 7 using the CRISPR/Cas9 technology. Two different lines of homozygous KI GAA rats were obtained and phenotyping was performed at different ages to fully characterize the progression of the disease. In male and female KI GAA rats of both lines, expression of GAA was marginal and glycogen accumulation was highly increased in different tissues such as skeletal muscle, diaphragm, heart and brain. Moreover, KI GAA rats showed cardiomegaly, decreased body and tissue weight and histological evaluation revealed marked skeletal muscle pathology. In agreement, KI GAA rats showed severe impaired muscle function during grip strength test. This new rat model fully mimics the clinical phenotype of Pompe patients and represents a useful tool to assess novel treatment strategies for the disease.

P483

Modulating cell fate determinants for muscle cell therapies: Notch and PDGF signaling enhance stemness and migration of satellite cells and human iPS cell-derived myogenic progenitors

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Satellite stem cells sustain skeletal muscle regeneration by generating transit-amplifying progenitors named myoblasts, most of which then fuse into regenerating myofibers. Despite their skeletal myogenic differentiation potential, satellite cells have a limited migration ability, which hampers their clinical use in cell therapies for forms of muscle diseases requiring widespread muscle targeting, such as Duchenne muscular dystrophy. Conversely, skeletal muscle pericytes are less myogenic but more migratory than satellite cells; notably, they can cross the endothelial wall upon intravascular delivery. Here we show that modulation of the Notch and PDGF signaling pathways with DLL4 and PDGFBB, involved in the developmental specification of pericytes, induce perivascular cell features in adult mouse and human satellite cell-derived myoblasts. DLL4 and PDGFBB-treated myoblasts express markers and acquire functional properties of pericytes while up-regulating markers associated with satellite cell self-renewal. This phenomenon was entirely reversible upon removal of the inducing factors and inhibition of the Notch signaling pathway. Treated cells acquire trans-endothelial migration ability in vitro and in vivo, while remaining capable of engrafting skeletal muscle upon transplantation. Importantly, we provide also transcriptomic and functional evidence that this mechanism is conserved across species and developmental stages, including in human induced pluripotent stem cell-derived myogenic progenitors (promising candidates for muscle cell therapies owing to virtually unlimited supply and controllable differentiation dynamics). These results extend our understanding of muscle stem cell fate plasticity, providing also a druggable pathway to enhance migration and facilitate delivery of myogenic cells for gene and cell therapy of severe and incurable muscle disorders.

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P484

Wild-type lamin A overexpression combined with mutant Lmna knock-down extends lifespan in a murine model of LMNA-congenital muscular dystrophy

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Lamin A/C, encoded by LMNA, is one of the components of the nuclear lamina, a meshwork underlying the inner nuclear envelope. Lamin A/C plays a key role in nuclear resistance to mechanical stress, thus maintaining cell integrity. They regulate gene expression through interaction with chromatin, nuclear histones and transcription factors. Mutations in LMNA gene lead to a variability of phenotypes, including LMNA-related congenital muscular dystrophy (L-CMD), an autosomal dominant, severe and early-onset disorder characterised by muscular atrophy and weakness, joint contractures sparing elbows, severe respiratory insufficiency and cardiomyopathy. To date, there is no treatment available. In the present study, we assess the therapeutic potential of wild-type human lamin A/C overexpression combined or not with mutant Lmna mRNA knock-down in a L-CMD mouse model harbouring the p.LMNA Lys32del mutation. After in vitro validation, intramuscular AAV2/9-mediated transfer of a flagged human lamin A coding sequence (F-hLaminA) resulted in a broad diffusion leading to a partial reversion of the metabolic defects. In addition, systemically administration in new-born homozygous mice resulted in an extended lifespan. We selected two shRNAs that specifically down-regulate the expression of the mutant Lmna mRNA without affecting the wild-type mRNA. The combination of wild-type F-hLamin A overexpression with the shRNA targeting mouse Lmna mRNA resulted in an even greater extended lifespan in homozygous mutant mice. These results demonstrate for the first time that the combination approach of mutant Lmna knock-down with wild-type Lamin A overexpression could be proposed as a potential therapy for L-CMD.

P485

Study on the therapeutic potential of HITI for the treatment of LMNA-associated congenital muscular dystrophies

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LMNA-associated congenital muscular dystrophies (L-CMD) are rare monogenic diseases that appear in childhood or youth and are characterized by progressive muscle weakness, anomalies in the atrio-ventricular conduction system, cardiac tissue fibrosis and respiratory failure. They are produced by mutations in the LMNA gene that encodes the A and C lamin proteins, which are part of the nuclear lamina. Our goal is to find new therapeutic approaches, based on gene editing, for the treatment of L-CMD. In this work, we explore the use of homology-directed independent integration (HITI) mediated by CRISPR / Cas9. Our strategy consisted in the integration of a wild type, LMNA minigene in myoblasts carrying one of the mutations causing the disease (R249W). The technology used for this purpose, HITI, requires the expression of Cas9 endonuclease and integration site-specific guides, in this case for a region of LMNA intron 2. Myoblasts nucleofected with expression vectors for EGFP, Cas9 and the guides together with a minicircle carrying the minigene were sorted and processed to obtain edited clones that were characterized at the molecular, cellular and functional level (integration analysis of the minigen, study of nuclear morphology and analysis of myogenic differentiation capacity). The integration of the wild type minigene in the LMNA-R249W myoblasts had three main consequences: (i) inhibition of the expression of the mutated allele LMNA-R249W, (ii) recovery of wild type nuclear morphology, (iii) rescue of the myogenic differentiation. Our results support the hypothesis that HITI technology has a high therapeutic potential for the treatment of L-CMD.

P486

Transduction efficiency of AAV serotypes after local injection in mouse and human skeletal muscle

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Adeno-associated viral vector (AAV) is an efficient tool for gene delivery in skeletal muscle. AAV-based therapies show promising results for the treatment of various muscular dystrophies. These dystrophies represent an heterogeneous group of diseases affecting muscles and typically characterized by progressive skeletal muscle wasting and weakness and development of fibrosis. The tropism of each AAV serotype has been extensively studied using systemic delivery routes, but few studies have compared their transduction efficiency via direct intramuscular injection. Yet, when only a few muscles are primarily affected, a local intramuscular injection to target these muscles would be the most appropriate route. A comprehensive comparison between different rAAV serotypes is therefore needed, as well as an evaluation of the impact of surrounding fibrosis on transduction. Here, we first investigated the transduction efficiency of rAAV serotypes 1 to 10 by local injection in skeletal muscle of control C57BL/6 mice. We used a CMV-nls-LacZ reporter cassette allowing nuclear expression of LacZ to localize targeted cells. Detection of beta-galactosidase activity demonstrated that rAAV1, 7, 8, 9 and 10 were more efficient than the others with rAAV9 being most efficient in mouse. These serotypes were also efficient in dystrophic and fibrotic Scid/beige/sgca-null mice. None of these serotypes showed a tropism for interstitial cells within fibrosis. Furthermore, using a model of human muscle xenograft in immunodeficient mice, we observed that rAAV8 and rAAV9 had similar transduction efficiency in human muscle. These findings demonstrate that human muscle xenograft can be used to evaluate AAV-based approaches in a human context.

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P487

Effect of Smad7 gene transfer in a Duchenne muscular dystrophy mouse model

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Duchenne muscular dystrophy (DMD) is a rare genetic disease characterized by a progressive muscle loss. While the majority of therapeutic approaches are only targeting the genetic defect, combining such an approach to the targeting of the secondary pathological cascade could improve the efficiency of this therapy. The TGFβ/Smad2/3/4 pathway plays a central role in the pathological cascade, in particular the onset of fibrosis following the binding of TGFβ1 and the regulation of muscle mass following the binding of myostatin. Therefore, by regulating the TGFβ/Smad2/3/4 pathway, we could combine the decrease of fibrosis and the increase of muscle mass. In this study, we assessed the effect of an AAV9 vector encoding Smad7 cDNA, a negative regulator of this pathway, in DBA-mdx mice, mouse model of DMD. We first evaluated the effect of Smad7 overexpression on fibrosis following intramuscular injection of our vector. Histological and molecular analyses did not show any improvement of fibrosis following treatment. Overexpression of Smad7 led to hypertrophy of injected muscles as demonstrated by the increase of fibre diameter. We also assessed Smad7 AAV transfer following systemic injection to investigate its effects at the functional level. In this experiment, Smad7 AAV transfer was also combined with the injection of an AAV8-microdystrophin vector. Microdystrophin expression improved muscle strength of DBA2-mdx mice. Surprisingly, Smad7 overexpression induced decrease of strength in wild-type and mutated treated mice and abolished microdystrophin positive effect at the functional level in double-injected animals. Further experiments are being performed to understand the deleterious effect of Smad7 overexpression.

P488

CRISPR/Cas9 mediated gene editing of Duchenne muscular dystrophy with a single gene deleted adenoviral vector

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Advances in gene editing enable the correction of mutations causing Duchenne muscular dystrophy (DMD) by developing personalized CRISPR/Cas9 treatments for the different mutations underlying the disease. Recent studies showed efficient in vivo genome editing following AAV delivery of a DMD specific CRISPR/Cas9 machinery. Nevertheless, viral delivery of all required CRISPR/Cas9 components including multiple guide RNA (gRNA) expression units within one single vector has not been fully exploited yet. Gene deleted high-capacity adenoviral vectors (HCAdVs) can transport up to 35 kb of foreign DNA and allow e.g. to deliver the complete CRISPR/Cas9 machinery including several gRNAs within a single vector. We produced a CRISPR-HCAdV containing two gRNAs specific for intronic sequences flanking the DMD mutation hotspot exon 51. We used this DMD specific CRISPR-HCAdV to transduce immortalized dystrophic skeletal myoblasts carrying a Δexon 48–50 mutation leading to a frameshift and premature stop codon in DMD exon 51 and absence of full-length protein. Upon transduction, locus-specific PCR and sequencing confirmed efficient locus-specific deletion of DMD exon 51 on genomic level and seamless splicing of DMD Exon 47 to exon 52 on mRNA level. Reconstitution of DMD expression after treatment of dystrophic muscle cells was shown using immunofluorescent DMD staining. Moreover, we quantified DMD protein following treatment of DMD myoblasts using in-cell western. We believe that besides, or as an alternative to AAV, HCAdVs could be used as efficient delivery vehicles for CRISPR/Cas9 based gene editing in the context of DMD and other muscular disorders.

P489

Effects of exercise on the efficacy of microdystrophin gene therapy

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We tested the hypothesis that running wheel exercise would complement microdystrophin gene therapy in mdx mice, a model of Duchenne muscular dystrophy. Mdx mice injected with AAV9-CK8-microdystrophin or excipient were assigned to three groups: mdxRGT (run, gene therapy), mdxGT (no run, gene therapy), or mdx (no run, no gene therapy). Wildtype (WT) mice were assigned to WTR (run) and WT (no run) groups. WTR and mdxRGT performed voluntary wheel running for 21 weeks; remaining groups were cage-active. Both weekly running distance and final treadmill fatigue time for mdxRGT and WTR were similar. In contrast, mdx final treadmill time was $\sim$4-fold less vs. both run groups. WTR and mdxRGT produced greater in vivo maximal plantar flexor torque vs. mdx at every time point after treatment, and mdxRGT vs. mdx in vitro diaphragm force-drop after an eccentric injury protocol was significantly less. Notably, rates of mitochondrial respiration from permeabilized red quadriceps fibers revealed ADP-stimulated respiration was approximately 30–40% lower in mdx than WTR, was significantly improved with gene therapy alone, and the mdxRGT combination provided the greatest bioenergetic benefit. These data demonstrate that running wheel exercise complemented AAV-CK8-microdystrophin gene therapy, improving muscle function and mitigating energetic defects in mdx mice.

P490

Transplantation of scf-expressing mesenchymal cell sheet activates epicardium and attenuates adverse cardiac remodeling in acute myocardial infarction

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Cell sheet technology has opened new avenues of cell-based therapy for myocardial infarction (MI) and heart failure. However, there is still scope for improvement stem cell regeneration properties before this treatment can be routinely applied in clinical settings. In this study, we investigated whether stem cell factor (SCF) hyperexpression enhances the therapeutic efficacy of mesenchymal stem cell (MSC) based cell sheets. MSC were isolated from fat tissue of Wistar rats and transduced with adeno-associated virus encoding SCF or control vector. Four groups of animals: sham, MI induction only, epicardial transplantation of control MSC sheet (cCS) and SCF produced MSC sheet transplantation (SCF CS) were compared by using acute MI models. After transplantation cell sheet's MSC and SCF–MSC engrafted, migrated across the epicardium into the myocardium and showed signs of differentiation into endothelial-like cells. Transplanted SCF CS retained robust and prolonged (>14 days) overexpression of SCF in vivo. SCF CS transplantation stimulated WT1-positive epicardial cells activation, their differentiation into endothelial cells and smooth muscle cells and vascularization of heart tissue. We showed more effective c-kit+cells recruitment into the infarcted myocardium after transplantation of the CSF CS, compared with cCS and MI groups. Additionally, cCS and SCF CS transplantation attenuated adverse cardiac remodeling after MI. The results suggest that epicardial transplantation of the SCF produced MSC sheet promotes repair of the infarcted myocardium and improves cardiac functions by locality paracrine action, activation of the epicardium and attraction of c-kit+ PC. Work was supported by RSF №17-15-01368, RFBR № 18-015-00438, 19-015-00231 grants

P491

Expression of L-type Ca +2 channels in m. Soleus and m. EDL of rats at early stages of hind-limb unloading and upon their subsequent re-adaptation.

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Atrophy of skeletal muscles accompanies disuse caused by immobility as well as weightlessness during spaceflight Hindlimb unloading reproduces functional unloading and muscle atrophy in spaceflight (in m. Soleus by 3 days, and in m. EDL by 28 days in rats), as well as decrease in speed-strength characteristics. Muscular contractile activity is triggered by calcium intake through high-threshold L-type Ca2+ channels. Increase in stationary level of calcium leads to transformation of slow phenotype m. Soleus. The purpose of this work was immunofluorescent and gene expression study of L-type Ca2+ channels in m. Soleus and m. EDL rats in 7 groups: control, hindlimb unloading 1, 3, 7 days and re-adaptation 1, 3, 7 days after 7 days hindlimb unloading based on NGS data (CAGE RNA expression) and immunocytochemistry (supported by the grant 17-00-00243 by RFBR). The samples were stained with primary antibodies to alpha1S-subunit of L-type Ca2+ channel and fluorescent secondary antibodies. Immunofluorescence intensity of muscle sarcolemma was evaluated. In m. Soleus rats after 1, 3, 7 days of hindlimb unloading and after 3, 7 days of re-adaptation increases of fluorescence intensity. In m. EDL fluorescence increased in all groups. Thus, early stages of functional unloading leading to atrophy and changes in speed-strength characteristics of muscles are accompanied by increase in expression of L-type Ca2+ channels in both muscles. Re-adaptation does not lead to restoration of Ca2+ channels expression in both muscles.

P492

Investigation of the mechanisms of reorganization of motor control in the period of postgravitational readaptation

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At present time, there is not enough information about investigations of the mechanisms of restoring movements in the period of postgravitational rehabilitation. The study was performed on nonlinear laboratory rats weighing 180–200 grams in accordance with the rules of bioethics. The unloading method by the model of E. R. Morey-Holton was used as a simulation of gravitational unloading. With the support of the grant of the RFBR № 19-04-01067, it was shown, that the reflex excitability of the spinal motor centers of the rat calf muscle decreased during the posthypogravitational readaptation, as well as the velocity of excitation of the efferent paths of the rat calf muscle and the synchronicity of muscle fiber contraction also decreased, neuromuscular transmission was facilitated. Recovery of the soleus muscle, in contrast to the gastrocnemius muscle, was less pronounced, so we assumed that it was due to the fact that the soleus muscle was more susceptible to weightlessness than other muscles. Thus, a study of the mechanisms of the reorganization of motor function in the period of postgravitational readaptation will allow to offer adequate ways to increase the efficiency of recovery processes.

P493

Macrophage secreted factors as novel therapeutics to promote stem cell-mediated muscle repair

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Skeletal muscle injuries are extremely common and can result directly from lacerations, contusions and strain, as well as, indirectly from ischemia and neurological impairments. While current treatment options remain sub-optimal, recent work in the cell therapy and tissue-engineering fields have focused on enhanced therapeutics that may yield better clinical outcomes. In order for these to be successful they need to be based on a thorough understanding of muscles inherent stem cell-mediated repair response. As such, there is a focus on understanding the role of the inflammatory process that ensues post injury, and its

ability to present novel pathways for therapeutic manipulation. Studying these processes through in vivo imaging has the potential to reveal phenomena that might not be observed by in vitro studies. In vivo cell tracking in zebrafish larvae identified distinct macrophage populations based on cell behaviour. A long-term injury responding macrophage population ''dwelled'' in the wound site throughout the repair process and functionally interacted with injury responding muscle stem cells. Genetic ablation of these dwelling macrophages resulted in a muscle regeneration deficit, which was attributed to a loss of stem cell proliferation. Single cell-RNA sequencing of injury-located macrophages informed the genetic signature of the stem cell-interacting macrophage subset along with their secreted factors that potentially regulate stem cell proliferation. These factors were confirmed to be pro-mitogenic in in vitro myoblast culture systems. Currently, these candidates are being applied to mouse muscle injuries to assay their potential to promote full functional muscle regeneration and present as successful pro-regenerative therapeutics.

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P494

A new rat model for Duchenne muscular dystrophy generated by CRISPR-induced deletion of Exon 45

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Duchenne Muscular Dystrophy (DMD) is a genetic neuromuscular disorder with a prevalence of one in 4500 male births. It is characterized by a progressive loss of muscle mass leading to ambulatory, respiratory and cardiac functions impairment. No treatment has been found yet for DMD, but many ongoing studies are focusing on pharmacological, cellular and gene therapies. Multiple disease models are present for DMD, some of which are expensive and difficult to use in large-scale experiments, like dogs, and others do not represent accurately the human disease, such as mdx mice. Here, we report the creation and characterization of a new DMD rat model holding a deletion of exon 45, the most common single exon deletion in DMD patients. The model was generated by CRISPR/Cas9 and presents major DMD characteristics such as lack of full-length dystrophin expression, muscle degeneration/regeneration cycles followed by muscle wasting and inflammation. The model also shows the differential presence of revertant fibers in its skeletal muscles correlating with the level of disease severity, an observation also found in DMD patients holding the same deletion. Finally, we are testing different gene therapy approaches on the rat such as exon skipping and exon deletions by CRISPR/Cas9, starting in vitro with rat cell models.

P495

Development of a novel preclinical gene therapy approach for congenital muscular dystrophy 1A

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Congenital muscular dystrophy type 1A (MDC1A) is an early onset, life-threatening autosomal recessive disease and one of the commonest congenital neuromuscular disorders. MDC1A is caused by mutations in the laminin $\alpha2$ (LAMA2) gene, encoding the $\alpha2$ heavy chain of the laminin 211 isoform, a heterotrimeric complex that mediates myocyte-anchorage to the extracellular matrix. Because LAMA2 cDNA (9,4 Kb) largely exceeds the packaging limit (4,7 Kb) of adeno-associated vectors (AAVs), it is necessary to use alternative vector systems. The fact that laminin 211 exerts its function extracellularly and must be necessarily secreted opens a window of opportunity for innovative therapeutic approaches in this disease. We postulate that the protein does not need to be secreted by the myocytes themselves, but it could be synthesized and released from a distant tissue such as the liver, from which it will reach the muscular extracellular matrix via circulation. Our approach involves the use of High-Capacity (helper-dependent, ''gutless'') adenoviral vectors, which are able to deliver such large transgenes. To demonstrate proof of principle we have investigated whether this protein can reach the muscular extracellular matrix upon systemic administration. We injected recombinant human laminin 211 (Biolamina, Sweden) via i.p. in merosin-deficient mice and we found that seven days later the protein is localized in the skeletal muscle, showing a typical pattern of extracellular matrix distribution. These results strongly support our proposed therapeutic strategy targeting extra-muscular tissue for laminin 211 synthesis and secretion in this disease.

P496

The CB1 enhanced the osteogenic differentiation ability of mesenchymal stem cells in the inflammatory environment

ABSTRACT WITHDRAWN

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P497

Humoral immune response against allogeneic equine mesenchymal stem cells (MSCs) mediated by the major histocompatibility complex (MHC): an issue to take into account for the safety and efficacy of treatment with MSCs

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Allogeneic mesenchymal stem cells (MSCs) present several advantages, but recipient immune response needs to be further elucidated. Proinflammatory priming of MSCs activated their in vivo regulatory capacity, but repeated administrations led to slight inflammatory reaction in an osteoarthritis equine model. This may be associated with higher major histocompatibility complex (MHC) expression, which would increase MSC immunogenicity potentially inducing humoral mediated immune memory. This study aimed at assessing allo-antibody production against donor's equine MHC (equine leukocyte antigen, ELA) in animals that received intra-articular repeated administration of allogeneic MSC-primed. For this purpose, we used stored samples from a previous study. Donor and recipients ELA-haplotypes were stablished by microsatellite typing and complement-mediated microcytoxicity assays were carried out by exposing target cells from the donor (unstimulated MSCs [MSC-naïve], MSC-primed or lymphocytes [control]) to sera collected at different time-points from 10 recipients: ELA-mismatched MSC-naïve recipients, ELA-mismatched MSC-primed recipients or ELA-partially matched MSC-primed recipients. All animals receiving allogeneic MSCs produced allo-antibodies after the first injection, regardless of the matching degree. However, antibody peak production after second administration was only observed in ELA-mismatched recipients, both of MSC-naïve and MSC-primed. Horses injected with MSC-primed produced fewer antibodies but MSC-primed were more targeted in the microcytoxicity assay. Thus, activated immunomodulatory profile of MSC-primed could have led to slighter humoral response after first administration, but these cells would be more easily targeted by existing antibodies post-second injection. Allo-antibody production against allogeneic equine MSCs could explain their time-limited efficacy and may affect the safety and efficacy of this cell therapy.

P498

IL-4 gene overexpression promotes repair of the ischemic skeletal muscle

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Peripheral arterial disease is caused by atherosclerosis that leads to obstruction of arteries and inflammation. Macrophages and monocytes sub-types participate in different stages of repair and regeneration of the ischemic limb, controlling angiogenesis, myogenesis, fibrogenesis and proliferation of local cells. Here we aimed to evaluate effects of exogenous IL-4 gene expression over monocytes/macrophages and in the ischemic skeletal muscle. Limb ischemia was induced by electrocauterization in the left femoral artery of male Balb/c mice at 10–12 weeks old age. Three days later, this limb was electroporated with 50 μg of IL4-expressing plasmid vector (uP-IL4). In the blood, Ly6C+ monocytes decreased 34.6 ± 3.6 % (before ischemia) to $15.2 \pm 7.2\%$ and $24.8 \pm 0.8\%$ 4 days after electroporation with uP-IL4 and uP (empty vector), respectively. The tissue macrophages (F4/80+/CD206+/MHCII-) were $8.2 \pm 1.4\%$ prior to ischemia that changed to $8.3 \pm 4.0\%$ and $22.5 \pm 4.3\%$ 2 days after electroporation with uP and uP-IL4, respectively. Visual analysis of the mouse pads showed improvement with uP-IL4 treatment in comparison to the untreated group, but no significant superficial blood flux was found between them. However, the ratio of muscle force and muscle mass after 4 weeks showed a complete recovery with uP-IL4 treatment, while the control groups recovered only 50%. In histology, we found that uP-IL4 treatment reduced adipocytes and myofibers with peripheral nuclei, showing healthier and normalized skeletal muscle. Collectively, it seems that the overexpression of IL-4 in the ischemic limb recruited more anti-inflammatory monocytes and macrophages temporarily, which promoted ischemic muscle repair and regeneration with less adipocytes.

P499

Differentiation potential of stem cells cultured on glass surfaces coated with magnetic nanoparticles

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Cell-matrix interaction plays a major role in cell differentiation alongside growth factors and cell-cell interactions. Investigation an effective nanomaterials promoting adhesion, proliferation, and differentiation of cells is crucial in tissue engineering. Iron oxide magnetic nanoparticles (MNPs) is one of the promising nanomaterials that may be used to modify cell substrate. The manipulation of MNPs by an external magnetic field allows constructing complex surface for cell culturing. In this study, we investigate the influence of MNPs-based surface on the differentiation potential of adipose-derived mesenchymal stem cells. The study was performed according to Program of Competitive Growth of KFU and funded by the subsidy allocated to KFU (project 16.2822.2017/4.6), the Russian Presidential grant MK-4498.2018.4 and RFBR project № 18-53-80067. MNPs were synthesized by the method of chemical reduction and characterized by both atomic force (AFM) and hyperspectral dark-field microscopy (HSM). Next, the surface of MNPs at concentrations 0.3–1.2 mg/ml was constructed by the method of colloid immobilization. The roughness of samples was analyzed by AFM. Biocompatibility of samples was measured by MTT-assay. Differentiation of cells into chondrocytes,

adipocytes, and osteoblasts was done using specific media with corresponding growth factors. Differentiation potential during 14 days was studied with the bright-field microscopy and HSM. Results of MTT-assay suggests good biocompatibility of samples coated by MNPs with concentration <600 μg/ml. In addition, MNPs-based coating does not prevent differentiation into chondrocytes, adipocytes, and osteoblasts. In conclusion, the results allow us to consider MNPs-based surface as a promising approach in regenerative medicine, but further studies are required.

P500

Impact of nucleated cell isolation method on the manufacturing of mesenchymal stromal cell-based medicinal product

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Advanced therapies medicinal products (ATMPs) based on mesenchymal stromals cells (MSCs) play an important role in regenerative medicine nowadays. Protocols used for ex vivo MSC expansion may determine the characteristics of the finished product obtained for clinical application. Several protocols based on density gradient are used to isolate nucleated cells (NC) as a first step of the manufacturing process. We compare manual (centrifugation in transfer bags) versus automatic (SEPAX, GE Healthcare) isolation of NCs from bone marrow samples and their impact on MSC final dose, viability, phenotype, differentiation capacity to mesodermal lineages and adherence potential to bone particles. NCs were seeded at 2E5 cells/cm2 and cultured for 10 days in a GMP compliant facility. Then, MSCs were tripsinized, reseeded at 1-4E3 cells/cm2 and expanded during 10 extra days. We obtained a MSCs product meeting the specifications required (40 ± 10E6 viable hMSCs; viability ≥70%; CD45-/CD105+, CD31-/CD73+, CD90+≥90%; sterile, endotoxin ≤0.5 EU/mL), independent of which NC isolation method was used. Furthermore, they maintain their differentiation potential and the capacity to adhere to bone particles. Automatic NC isolation operates with a closed system using single-use kits allowing a sterile environment during processing. Nevertheless, recovery performance of NC was 4.2-fold higher when using the manual system. As a consequence, the volume of bone marrow required to be harvested from the patient is significantly lower. Furthermore, the manual NC isolation is less-time consuming and also less expensive. Therefore, we decided to use the manual NC isolation as a first step of our GMP compliant MSC manufacturing process.

P501

Periostin splice variant expression in skeletal muscle fibrosis and effective anti-fibrotic action of antisense oligonucleotides that block periostin expression

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Fibrosis contributes to the fatal pathology of muscular dystrophies, worsening disease pathology and poor efficacy of potential gene therapies. Periostin, a component of the transforming growth factor (TGF)-β1 signalling pathway has been implicated in fibrosis progression and could provide a more-specific anti-fibrotic target than the pleotropic cytokine. We have found periostin protein to be highly expressed in diaphragm muscle of the mdx mouse, an established DMD model, compared to wild type (WT) controls, and that this expression increases with disease progression. We have therefore designed antisense oligonucleotides (AO) to skip out-of-frame exons of Postn pre-mRNA with subsequent knockdown of periostin protein expression. We examined the phenotypic effect of an optimised AO in an in vitro wound healing assay and show significant inhibition of TGF-β1-induced wound closure by the AO, supporting the concept that periostin knockdown reduces fibrosis via modulation of the TGF-β1 signalling pathway in muscle. Postn pre-mRNA undergoes C-terminal alternative splicing and we have identified four Postn mRNA splice variants in mouse skeletal muscle; those containing exon 17 had significantly higher expression levels in mdx diaphragms compared to WT controls. This suggests particular periostin isoforms contribute to skeletal muscle fibrosis, in line with reports of fibrosis in other tissues. In light of this, we are using AOs to induce the alternative splicing of Postn pre-mRNA to switch protein expression from a pro-fibrotic isoform to one that enables tissue repair, and provide evidence of an anti-fibrotic effect of an optimised AO in terms of skeletal muscle fibroblast activation.

P502

Structural analysis of neuromuscular junctions in aging: effect of Sox2 heterozygosity

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AIMS The neuromuscular junction (NMJ) is the synaptic interface through which motor neurons innervate fibers enabling muscle contraction. It is composed of nerve terminals, endplates (organized in characteristic pretzel-like structures), and terminal Schwann Cells (tSCs) that ensure functionality and maintenance of NMJs. During aging, synaptic activity is impaired, followed by muscle atrophy and declined muscle mass and function (sarcopenia). Pretzel structures dissolve and nerves disconnect from fibers. The transcription factor Sox2 is a key regulator of the Schwann cell lineage. Since Sox2 levels decrease during aging, we hypothesized that this decrease might impact the structure and function of tSCs capping NMJs during sarcopenia and aging. METHODS Skeletal muscles of young, adult and old WT and heterozygous Sox2GFP mice were analyzed by confocal microscopy. Qualitative and quantitative analysis of NMJs were performed. RESULTS We detected relevant degeneration in NMJs during aging. Differences in NMJ structure were observed in WT and Sox2GFP endplates during aging. A higher frequency of denervated-NMJs and NMJs without capping-tSCs was observed in Sox2GFP compared with WT. CONCLUSIONS These results suggest an essential role of Sox2 in the maintenance of the NMJ-structure during aging, possibly through tSC modulation, and shed light into the mechanisms underlying sarcopenia. Key words: aging, neuromuscular junction, terminal Schwann Cells, Sox2.

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Assessment of exon-skipping feasibility for treatment of dysferlinopathy

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Limb girdle muscular dystrophy type 2B and Miyoshi myopathy, which are among the most prevalent muscular dystrophies, are caused by mutations in DYSF gene. There is no effective treatment for these diseases to date. Exon-skipping (ES) is one of the most promising approaches to treat muscular dystrophies. We have assessed the feasibility of ES for several exons and mutations. Plasmid constructs lacking exons 3+4 (for mutation c.TG573/574AT; p. Val67Asp) and 26+27 (for mutation c.2779delG Ala927-LeufsX21) were generated. Artificial patient myoblasts (obtained via MyoD-mediated direct myogenic differentiation of patient fibroblasts) were transfected. The subsequent analysis has demonstrated a truncated dysferlin mRNA (rtPCR) and protein expression (Western Blotting, immunocytochemistry), supporting the hypothesis that exon-skipping could be used to treat patients with dysferlinopathy. Further, we have generated three antisense oligonucleotides design candidates for each to induce exon-skipping in corresponding exons. Later on, we will perform in vitro screening to select the best design and will continue the development of novel therapeutic approach for this disease.

P504

Influence of species-specific plasmid pBUDK-ecVEGF164-ecBMP2 on osteogenesis MSC horse «*in vitro*»

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Bone regeneration is one of the major focus point in the field of regenerative medicine. We investigated the possibility of achieving osteogenic differentiation of horse mesenchymal stem cells (MSCs) in vitro after gene modification as a result of prolonged presence of bone morphogenetic protein (BMP-2) and vascular endothelial growth factor (VEGF164). MSCs were extracted from horse fat tissue and transfected with DNA plasmid encoding two therapeutic horse species-specific growth factors (VEGF164 and BMP2) using lipofectamine. MSCs were cultivated in growth medium without biologically active additives for osteodifferentiation. Native MSCs cultivated with growth medium without biologically active additives was used as a negative control for osteodifferentiation while MSCs cultivated with induction medium was used as a positive control. After fixation of the MSCs in 4% formalin, the cells were stained with Alizarin Red and washed with PBS. Next the stained MSCs were solubilized with 10% acetic acid by shaking for 15 min. The absorbance of the released Alizarin red stain was measured using microplate reader (TECAN, Switzerland) at 405 nm wavelength. The absorbance from negative control was 0,064 ± 0,002, while for positive control it was 0,243 ± 0,007 and in the case of MSCs gene modification it was 0,237 ± 0,006. In conclusion, we showed that the recombinant species-specific plasmid pBUDK-ecVEGF164-ecBMP2 stimulates osteogenesis of horse MSCs in vitro. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University. This study was funded by Russian Foundation for Basic Research and the government of the Republic of Tatarstan (grant #18-415-160010).

P505

Mesenchymal stem cells from gingiva as a new source of autologous myogenic progenitors

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A search of available autologous cell sources with myogenic potential remains one of the key problems. This work aimed to study the myogenic and regenerative potential of human MSC from gingiva (MSC-g) in 2D (monolayer) and 3D (spheroids) culture. In 2D culture, after induction MSC-g formed multinucleated myotubes and expressed MyoD – a marker of early stages of myogenesis. In 3D culture in 7 days MSC-g formed compact spheroids that did not contain early progenitor cells, there was no expression of MyoD and no single myotubes. At the same time, we observed more differentiated well-formed myofibrils with characteristic peripheral nuclei arrangement and sarcomeric alfa-actinin expression in the form of cross-striation, which is characteristic of mature muscle tissue. Moreover, comparison of secretome profile in 2D and 3D cultures revealed an increased level of proangiogenic factor VEGF in media conditioned by spheroids. In in vivo study, on the model of rabbit calf muscle injury, suspension of MSC-g reduced the size of the scar, whereas spheroids from MSC-g promoted full organotypic recovery of muscle tissue. Thus, 3D culturing of MSC-g can stimulate effective spontaneous myogenic differentiation with myofibrils formation and activates secretion of VEGF, which can increase spheroids' resistance to hypoxia and their survival in sites of injury. Therefore, spheroids from MSC-g can become an alternative accessible and less invasive source of myogenic cells and can find its place in personalized medicine for the therapy of muscle tissue dysfunction and dystrophy. The study was financially supported by Russian Science Foundation (grant № 17-75-30066).

P506

Quantitative index of cell fusion for cell therapy development

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The muscular dystrophies are the group of genetic diseases which lead to progressive degeneration of the muscle tissue. One of developing approaches for pathogenetic therapy is a gene- and cell-based therapy. But a very little fraction of myoblasts fuses with muscle fibers, which is not sufficient for phenotype correction. Therefore, novel approaches to increase the efficiency of cell fusion for cell-based therapy are being developed. We have designed a technique to quantify the processes of cell fusion. The fusion index (FI) is defined as Fi = (Nm-Qm)/(Nt-1)*100%, where Nm-number of nuclei in multinucleated structures, Qm-total number of multinucleated structures and Nt is a total number of nuclei in a field of view. We used C2C12 myoblasts as an experimental object. Cells were cultivated on DMEM/F12 medium with addition of 20% FBS, PenStrep and glutamate. To induce muscle cell fusion it was replaced with differentiating medium (DMEM HG +2% FBS + glutamate + penstrep). After 4 days of cultivation, the Fi was $8.43 \pm 4.33\%$ (n = 2). On day 8 on differentiation medium, the average Fi was $52.04 \pm 2.6\%$ (n = 3). Thus, it is possible to create a technique that allows quantitative assessment of the FI in cell cultures. This data will be used to measure the induction of cell fusion in the development of cell therapy for inherited muscular diseases.

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P507

Visualization of woven bone structure through analysis of biopsy specimens using synchrotron radiation and conventional X-ray micro-CT

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This study explores the application of synchrotron radiation (SR) and conventional micro-CT (C-μCT) in evaluating bone biopsy specimens. Bone biopsy specimens were obtained using a trephine bur during bone graft removal for implant placement 6 months after performing maxillary sinus bone graft procedure. Image data of specimens were obtained using SR and C-μCT. SR-μCT was performed using a beamline 6C biomedical imaging at Pohang Accelerator Laboratory with a monochromatic X-ray beam of 23 keV; C-μCT was performed using a table-top CT scanner (Skyscan 1272). Reconstruction images obtained using the two methods were qualitatively compared with 2D images evaluated under 3D visualization. SR-μCT images, especially of the new bone (NB)-graft-woven bone formation, were less noisy and sharper than C-μCT images. To evaluate NB-graft-woven bone formation, only SR-μCT images showed areas of NB formation with bone substitutes (BS; Bio-Oss®) and woven bone (WB) contact and correctly visualized true 3D structures of bone formation. Hence, μCT techniques are non-destructive and provide detailed images of bone biopsy. SR-μCT could particularly obtain improved image quality with contrast of NB, BS, and WB, demonstrating the level of detail comparable with bone formation. SR-μCT could be an available, unbiased 3D alternative to WB formation and high-throughput analysis.

P508

Cell based screening of different adenoviral serotypes to identify candidates for the development of improved vectors for the treatment of muscular disorders

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Recent advances in gene therapy allow for the treatment of inherited muscular diseases such as myotubular myopathy (MTM), limb girdle muscular dystrophy (LGMD), Becker muscular dystrophy (BMD) or Duchene muscular dystrophy (DMD). Several studies showed efficient in vivo genome editing strategies following AAV delivery of a therapeutic transgene or specific CRISPR/Cas9 machineries for gene correction approaches. Nevertheless, viral delivery of large transgenes or Cas9 together with multiple guide RNAs using one single vector has not been fully exploited yet. Gene deleted high-capacity adenoviral vectors (HCAdVs) can transport up to 35 kb of foreign DNA and allow to deliver large transgenes involved in muscular diseases or the complete CRISPR/Cas9 machinery including several gRNAs within a single vector. As adenovirus (AdV) serotype 5, which is commonly used as gene therapy vector, has limitations for in vivo applications, we used a library of 19 different reporter gene expressing AdV serotypes and tested their efficiency to transduce various human and rodent skeletal and cardiac muscle cells. In rodent cells, no other AdV could compete against AdV5. In sharp contrast, we observed higher transduction efficiencies in human cells after infection with AdV37, 35 and 21 when directly compared to AdV5. We conclude that these identified AdV types are promising candidates for vectorization to establish novel gene therapeutic strategies to treat muscular diseases including DMD. We believe that besides AAV those future HCAdVs could be efficient delivery vehicles for gene therapy approaches to treat muscular disorders especially when complex or large transgenes are required.

P509

Histopathological analysis of gingiva-derived MMSCs transplantation effect on skeletal muscles regeneration

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Searching for an affordable, safe and easy-to-use method for obtaining myogenic cells with high regenerative potential and therapeutic efficacy is an important task to cure acquired and inherited myopathies. Previously we described myogenic potential of gingiva-derived multipotent mesenchymal-stromal cells (GMMSC). To evaluate effect of GMMSCs on skeletal muscles regeneration decellularized muscle extracellular matrix (DMECM) seeded by GMMSCs cultivated in 2D and 3D conditions or 2D and 3D cultivated GMMSCs alone were implanted into the rabbit's m.gastrocnemius after surgical intersection-

stitching. GMSCs were committed to myogenic differentiation before seeding and transplantation. Paraffin sections of operated muscles 20, 28 and 35 days after injection were stained with H&E, Mallory's trichrome, immunohistochemically with antibodies against α-SMA, myogenin. Enrichment of the DMECM with cultivated GMMSCs led to increased vascularization and inflammatory infiltration of the implanted matrix, promoted faster resorption of the DMECM and consolidation with muscle tissue compared with unseeded DMECM. The transplantation of GMMSCs cultured under three-dimensional conditions contributed to the complete organotypic restoration of the structure of muscle tissue after intersection-stitching with active reparative rhabdomyogenesis. The transplantation of GMMSCs cultivated under routine conditions contributed to the formation of a thinner and orderly formed scar at the site of injury. This work was funded by a Russian Science Foundation (grant #17-75-30066).

P510

Chitosan/gelatine/agarose nanostructured scaffolds promote angiogenesis

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Formation of blood vessels at the implantation site is crucially important for the effective growth of cells on implants used for the restoration of damaged tissues. Growth of newly formed blood vessels is essential to supply oxygen and nutrients to developing tissues. Porosity of scaffolds play important role in the formation of blood vessels. In our study, we designed porous biopolymer scaffolds doped with 3–6wt% of halloysite nanotubes for subcutaneous implantation to determine their rate of resorption and biocompatibility. During histological analysis, we observed the neo-vascularization in connective tissue penetrated the scaffold pores indicating that scaffolds are biocompatible and can induce the angiogenesis without growth factors and introduced cells. To determine the blood perfusion in implantation area we employed the laser Doppler analyzer. During the scanning, moving blood cells shift the light frequency according to the Doppler Effect. The analysis was performed in 3 and 6 weeks after implantation. Implantation of both types of nanostructured implants led to reduction in blood supply to a level below 100 approximate perfusion units (APU). After 6 weeks blood flow was completely restored to normal values above 100 APU. The varying rate of scaffolds resorption depending on the presence of the nanotubes allows modulating the properties that can be used in development of various tissue-engineering structures. The work is performed according to the Russian Government Program of Competitive Growth of KFU and funded by the subsidy allocated to KFU (MD-6655.2018.4). Also, this study was funded by RFBR and the government of the RT (#18-415-160010).

P511

Evaluation of collagen membrane as a pulp capping agent in swine

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Objective: This study aims to investigate the effects of the highly biocompatible collagen membrane as pulp capping agent in miniature swine.

Method: 20 premolars of 2 miniature swines were randomly assigned into 3 groups, the experimental group was collagen membrane and the control group were MTA and iRoot BP Plus. Class I cavities were prepared with 1mm diameter diamond round burs. 8 weeks later, teeth were harvested, fixed and processed histologic analysis.

Results: In collagen membrane group, 10 samples all showed as even and completed calcium bridge formation. The coronal side of new formed dentin presented as osteoid dentin and accounted for the majority part. While, the pulp side of new formed dentin presented as tubules dentin layer with polarizing odontoblast layer occurred.. Several osteoblasts were observed in the new formed dentin bridage in one sample. There was no severe inflammatory reaction, over calcification or dentin resorption in dental pulp tissue. Both MTA group and iRoot BP group can form even and complete dentin bridage too. The thickness of the dentin bridge was about 1.12 ± 0.28mm, 0.60 ± 0.03mm and 0.58 ± 0.14mm in collagen membrane group, MTA group and iRoot BP Plus group, respectively. The thickness of new formed dentin in collagen membrane group was significant higher than MTA group and iRoot BP group. There was no statistical difference between MTA group and iRoot BP Plus group.

Conclusion: This study demonstrated that collagen membrane is a good candidate for maintaining pulp tissue in case of small dentin defect and without obvious infection.

P512

Introduction of mesenchymal stem cells reduced the effect of ischemia on nitric oxide content in the hippocampus and restored the approximate motor activity of rats after modeling of cerebral stroke

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We studied the effects off intranasal mesenchymal introduction of stem cell (MSC) on the approximate motor activity and the level of NO in hippocampus of the rats after modeling of ischemic stroke caused by ligation of common carotid arteries. Experiments were partly supported by subsidy to Kazan Federal University for the state assignment in the sphere of scientific activities (No 17.9783.2017/8.9). It was implemented the analysis by EPR spectroscopy the dynamics of the NO content in the dentate gyrus (CA4 region of the rats hippocampus before and after modeling of cerebral ischemia. A significant decrease in NO production 1 day after modeling of ischemia was found. It was shown that intranasal introduction of MSC partially increased the level of NO in hippocampus, reduced after brain stroke. It is shown that intranasal administration of MSC in the acute period after occlusion of the common carotid arteries is accompanied by a more rapid restoration of the approximate motor activity in experimental animals.

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P513

Chondrogenesis of stem cells on substrate coated with magnetic nanoparticles

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Cellular differentiation is a key process in regenerative medicine. Differentiation is often carried out in 3D culture to prevent dedifferentiation. Nevertheless, there is a possibility to make a fairly rough surface that promotes differentiation in monolayer culture. The promising candidate to produce surface coating is iron oxide magnetic nanoparticles (MNPs). MNPs can be manipulated by the external magnetic field for constructing unique coating. In this work, we investigate the chondrogenesis of adipose-derived mesenchymal stem cells (ADMSCs) on MNPs-coated surface. The coating was done by the method of colloidal immobilization of DNA (50 μg/ml) and MNPs (600 μg/ml). Differentiation of cells into chondrocytes was done using StemPro™ Chondrogenesis Differentiation Kit. ADMSCs were analyzed during 14 days of differentiation with a bright-field microscope. On the 14th day, cells were fixed with 0.1% glutaraldehyde and stained with 0.1% safranin dye. Then, samples were analyzed with hyperspectral-enhanced dark-field microscopy. Hyperspectral signatures of cells were collected with hyperspectral imaging technology. According to the results, there is a difference in signatures of the cells cultivated in chondrogenic media and the cells in the negative control. Also there are the same peaks in the samples with MNPs coating and the positive control group. Thus it was shown that surface coating produced by MNPs was not prevent differentiation of cells into chondrocytes. The study was performed according to the Program of Competitive Growth of KFU and funded by the subsidy allocated to KFU (project 16.2822.2017/4.6), the Russian Presidential grant MK-4498.2018.4 and RFBR project № 18-53-80067 and № 18-29-25057.

P514

Novel rat model of hindlimb ischemia for assessment of the efficiency of gene and cell therapy

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Background: The prognosis for patients with critical limb ischemia is rather disappointing. The problem of modeling of limb ischemia in animals is one of the main reasons why the clinical trials have been failed to demonstrate efficacy. The aim of our investigation is the development of novel model of hindlimb ischemia in rats to assess the effectiveness of gene and cell therapy.

Methods: All procedures were approved by the local ethics committee and study was supported by Grant NSC-3076.2018.4. At the first stage the external iliac artery was ligated immediately after bifurcation of the common iliac artery. Then femoral artery was ligated before its branching with following dissection of arteries between ligatures. At the second stage (after 7 days) the branches of popliteal artery to gastrocnemius muscle and formed collaterals were ligated and dissected.

Results: Ischemic limb was half in size compared to intact limb. Histopathological analysis showed a continuous hindlimb ischemia of operated limb with significant interstitial fibrosis ($11.89 \pm 5.53\%$ vs $2.55 \pm 2.13\%$ in intact limb by day 42, $p < 0.05$). Polymorphic muscle fibers in operated limb were significantly smaller in diameter and capillary density was reduced (0.82 ± 0.03 vs 1.91 ± 0.06 in intact limb by day 42, $p < 0.05$). Laser Doppler flowmetry revealed reduction of blood flow in the operated limb (0.67 ± 0.22 of intact limb blood flow by day 42).

Conclusion: Our results reflect the picture of prolonged ischemia followed by increased fibrosis intensity without any native skeletal muscle structure restoration. This study was supported by the Russian Government Program of Competitive Growth of Kazan Federal University.

P515

Adipose stromal cell sheet producing hepatocyte growth factor (HGF) effectively stimulates recovery of ischemic skeletal muscle in mouse hind limb ischemia model

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Adipose derived stromal cells (ADSC) are considered as the most promising cell source for cell therapy. However, direct injections of cell suspensions showed limited efficacy putatively due to low cell viability as a result of cell-to-cell interaction loss, compression damage during administration by injection as well as unfavorable microenvironment in ischemic and inflammatory tissue. In this study we simultaneously used two approaches to increase the regenerative capacity of ADSC. First, the cells were modified with adeno-associated virus encoding HGF gene to enrich ADSC secretome and enhance therapeutic potential. Second, gene-modified ADSC were transplanted to ischemic limb as engineered constructs known as ‘‘cell sheets’’ (CS) to enhance their survival, preserve viability and regenerative functions. We found superior blood flow restoration, tissue vascularization and innervation, fibrosis reduction after transplantation of HGF-producing ADSC sheet compared to HGF-ADSC suspension or ADSC sheets. We suggest that observed effects are determined by pleiotropic effects of HGF along with multifactorial paracrine action of ADSC which remain viable and functionally active within engineered cell construct. Thus, we demonstrated high therapeutic potential of utilized approach for skeletal muscle recovery after ischemic damage associated with complex tissue degenerative effects. This therapeutic strategy that could be applied during vascular surgery holds great promise for the treatment of ischemic peripheral vascular disease, particularly in patients with diabetes mellitus complicated by neuropathies. Study was supported by the Russian Science Foundation grant #19-15-00384

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P516

Functional characterization of a proprietary GMP human platelet lysate

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Human platelet lysate (hPL) has been used as a xeno-free culture supplement to expand different cell types since the 80s, but its use has recently thrived due to the requirement of safely replacing fetal bovine serum (FBS) for clinical scale manufacturing. Furthermore, hPL has been described to exert therapeutic properties in bone and cartilage regeneration or autoimmune diseases such as graft-versus-host disease. We have previously standardized the production of a clinical grade hPL for clinical applications within a public health system setting, taking advantage of platelets that are routinely discarded in blood transfusion centres. Quality control tests and a robust manufacturing protocol were validated, ensuring safety, efficacy and traceability of our product. Here, we have further characterized our in-house manufactured hPL by means of a proteomic study. Interestingly, after high-abundance protein depletion, 910 common proteins were identified. Enrichment analysis showed biological processes related to protein activation cascade, vesicle-mediated transport and immune system process, among others. Additionally, to study potential effects on fibroblast growth, we performed a high-throughput microarray analysis comparing explant-derived fibroblast cultured with FBS (10%) or with our hPL (at 5 and 15%, n = 3, respectively). 129 differentially expressed genes were obtained when comparing hPL versus FBS, 38 were upregulated and 89 downregulated. No significant differences were found between hPL at 5 and 15%. In conclusion, we present a new step forward towards an in-depth characterisation of an in-house-GMP hPL that can be a medicinal product itself or a raw material for the manufacturing of cell-based therapies.

P517

microRNA 21 Promotes Orthodontic Tooth Movement via TNF-alpha/ RANKL pathway in T cells

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Orthodontic tooth movement (OTM) occurs when under the mechanical forces. The alveolar bone is remodeled, and OPG/RANKL/RANK axis plays an important role during the process. In this study, the orthodontic tooth movement model was set up in C57BL/6 and microRNA-21 knock out mice. We discovered that the speed of orthodontic tooth movement was slowed down in miR-21-/- mice. TRAP staining showed that osteoclasts number was decreased, RANKL in serum was also much at a lower level in miR-21-/- mice. Further more, we found CD4+ T cells could partially rescue the OTM in miR-21-/- mice. Further studies showed TNF-alpha/RANKL pathway involved in miR-21-/- reduced OTM. It suggested that miR-21 promote RANKL secretion by active T cells then influence the maturity of osteoclasts and orthodontic tooth movement. These findings provided an important evidence to understand the systemic factors in OTM.

P518

Vegf and fgf2 genes stabilizes angiogenic effect in hind limb ischemia model in rats

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Background: Peripheral arterial diseases are usually caused by atherosclerosis and limits blood supply to the lower limbs. There is significant interest in developing new therapeutic approaches to manage peripheral arterial disease.

Methods: All procedures were approved by the local ethics committee and study was supported by Grant NSC-3076.2018.4. On the 7th day after modeling of two-stage ischemia, heads of the gastrocnemius muscle were injected by plasmid solution in amount of 200μg of cDNA plasmid in 200μl of 0.9% sodium chloride. Experimental groups: «Control» - 200μl of 0.9% sodium chloride solution without plasmid; «VEGF» - pVax1-VEGF-Fu2A-DsRed; «FGF2» - pVax1-FGF2-pFu2A-DsRed; Group «VEGF-FGF2» - pVax1-VEGF-Fu2A-FGF2-Fu2A-DsRed.

Results: On the 3rd day after ischemia modelling a picture of ischemic damage with reduced capillary density(by 45.3 ± 12.7%) and replacement of dead muscle fibers by connective tissue(up to 9.73 ± 6.32%)was observed in all the groups. Histopathological analysis showed an increase in the capillary density, a higher rate of muscle tissue regeneration to the 21th day of experiment in «VEGF-FGF2» group. The effect of plasmids injection was firstly observed on the 7th day of the experiment when 2-3-fold increase in the expression of vegf and fgf2 mRNA in «VEGF-FGF2» group as compared with «Control» was detected. And this effect maintains up to 14th day.

Conclusion: Combination of vegf and fgf2 genes caused a higher rate of neoangiogenesis. The molecular analysis indicates a higher effect of double gene constructions in the induction of vascular growth factor expression compared to control group. This study was supported by the Russian Government Program of Competitive Growth of Kazan Federal University.

P519

The rat skeletal muscle under the simulated weightlessness

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In the simultaneous recording in vivo isometric contraction curves of the rat gastrocnemius and soleus muscles evaluated the effect of hindlimb unloading on the amplitude-time parameters. With the support of the grant of the RFBR № 19-04-01067, it was shown increased strength and decreased the time a single muscle contraction. And in the gastrocnemius muscle, this trend continued until the final observation on 5-week period hindlimb unloading. At the soleus muscle from the fourth week, there was an incomplete reversal of force and time reductions. We are observed an increase in fusion frequency tetanic contractions the gastrocnemius and soleus muscles on the three weeks of hindlimb unloading. Further increase the unloading time no change in the frequency of fusion of these muscles. When comparing the curves of tetanic contractions in intact and posted animals were seen, along with the initial increase in the amplitude of the summed contractions characteristic pessimal pattern of decline, which correlates with the decrease in the time of single reductions and can explain how an increase in force of single muscle contraction may be a general pattern of muscle weakness. Analysis of tetanus caused by indirect and direct stimulation, showed that in the case of hanging tetanus can lead to an optimal form for direct stimulation of skeletal muscle, indicating that the neuronal component observed during the hindlimb unloading. The data obtained supplement the body of knowledge about the processes occurring in states close to weightlessness.

P520

Influence of unilateral deforming arthrosis on the condition of contralateral limb

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The purpose of this research was to study the effect of unilateral pathology of the coxofemoral and knee joint on the functional state of the muscles and their spinal centers of the contralateral limb. The study was funded by the subsidy allocated to Kazan Federal Unversity for the state assignment in the sphere of scientific activities №17.9783.2017/8.9 and conducted with participation of the 29 healthy subjects volunteers, 77 patients with unilateral coxarthrosis and gonatrosis with their consent. M-and H-responses of the quadriceps muscle were recorded during stimulation of the femoral nerve projection in the groin area. The threshold and maximum amplitude of M- and H-responses of the pathological and contralateral limb were determined. The thresholds of M- and H-responses and their maximal amplitudes recorded on the right side did not significantly differ from the left in healthy people. A significant increase in thresholds and decrease in the amplitude of M-and H responses of affected and intact limbs were found in all patients. Consequently, an asymmetrically located pathological focus rendered inhibition on the activity of the peripheral and central link of the locomotor apparatus not only in the side of damage, but also in the intact one. Changes in the central link of the intact limb may occur when there are no obvious clinical signs of joint damage. Thus, it is necessary to make corrections to the tactics of treatment of arthrosis of large joints, in particular, the early implementation of therapeutic measures on the conditionally intact limbs.

P521

Personalized gene-activated implants are effective to reconstruct large bone defects in experiment

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Currently, the treatment of patients with extensive bone defects remains extremally challenging and based on bone autografts. An effective alternative can be personalized bone grafts and substitutes. We have developed a technology for making 3D-printed gene-activated implants consisting of OCP and plasmid DNA carrying gene of vascular endothelial growth factor (VEGF). The study was performed on pigs with an average weight of 50 ± 2 kg ($n = 4$). We planned tibial bones defects of the right hind limbs (T-shaped, with circular central part 10 mm in length and 2 peripheral parts $10 \times 5 \times 5$ mm in size) and bilateral defects of the mandible in the corners area ($25 \times 15 \times 10$ mm). 3D-printed gene-activated implants were implanted into the defects, the same scaffolds without plasmid DNA were controls. According to CT data, no bone defects were detected, bones were filled with high-density regenerate and implants were fully integrated with bone walls. Histological examination confirmed that the implants underwent partial biodegradation, and newly formed bone tissue were identified into the pores and on the surface of the implants. Thus, personalized gene-activated materials is effective for reconstruction of skeletal bones. However, some optimization of the implants' microstructure is needed to increase their biodegradation rate.

P522

Correction of bone and cartilage pathology and other somatic alterations by AAV-mediated gene therapy in a new rat model of Morquio A

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Mucopolysaccharidosis Type IVA (MPSIVA) or Morquio A disease is a Lysosomal Storage Disorder caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS). This alteration leads to an accumulation of the glycosaminoglycans keratan sulfate (KS) and chondroitin-6-sulfate, mainly within chondrocytes and extracellular matrix of cartilage, leading to systemic skeletal dysplasia, early cartilage deterioration and life-threatening complications. There is not effective treatment for MPSIVA, as current treatment with enzyme

replacement therapy (VimizimR) is ineffective at correcting abnormal growth. Therefore, there is an important unmet medical need. Here, we have developed an adeno-associated virus (AAV) vector-mediated gene therapy approach to treat MPSIVA in a rat model that closely reproduces the human disease. Single intravascular administration of AAV-GALNS to MPSIVA rats increased GALNS activity in serum, bones and in all peripheral organs. As a result, normalization of body weight gain, naso-anal length and survival rate was observed in the AAV-treated MPSIVA rats. Moreover, at the skeletal level, this treatment led to correction of KS accumulation in bones, improvement of articular cartilage pathology and amelioration of dental alterations observed in non-treated MPSIVA rats. In addition, AAV-GALNS therapy led to long-term (1 year) normalization of KS levels in serum and liver and corrected respiratory and cardiovascular alterations. This is the first study in a relevant new rat model that provides clear evidence that gene therapy can contribute to correct the severe clinical phenotype of Morquio A patients. Our results provide a strong rationale for the future clinical translation of AAV-GALNS vector-mediated gene therapy to treat MPSIVA.

P523

AAV-mediated gene therapy to treat mucopolysaccharidosis type IVA in a new mouse model of the disease

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Mucopolysaccharidosis type IVA (MPSIVA) or Morquio A disease is an autosomal recessive Lysosomal Storage Disease caused by the deficiency of galactosamine (N-acetyl)-6-sulfatase (GALNS) enzyme, involved in the stepwise degradation of the glycosaminoglycans (GAG) keratan sulfate (KS) and chondroitin 6-sulfate. Accumulation of undegraded GAG leads to lysosomal pathology, which in turn results in severe skeletal dysplasia as well as somatic disease affecting several organs. Patients with more severe forms of the disease die between the second and third decades of life. Since there is no effective cure for MPSIVA, new therapeutic approaches are needed. Here we first developed a new mouse model of MPSIVA disease using ESCs, carrying a disrupted version of Galns gene, obtained from International Mouse Phenotyping Consortium. MPSIVA mice did not develop skeletal alterations. However, similarly to human patients, these mice showed progressive KS accumulation in serum and in peripheral tissues that resulted in hepatic, tracheal, heart valve and corneal pathology. MPSIVA mice were treated with an adeno-associated viral (AAV) vector-mediated gene therapy to express Galns (AAV-GALNS) in several tissues. After AAV-GALNS vector administration, MPSIVA-treated mice showed increased GALNS activity in bone, liver, heart, adipose tissue and serum, which led to long-term (>6 months) normalization of KS levels in serum, peripheral organs and CNS. Moreover, the corneal clouding characteristic of MPSIVA disease was also corrected. These results demonstrated that gene therapy was able revert MPSIVA disease in mice. Hence, this work represents the first step towards the development of a treatment for Morquio A disease.

P524

New uses for existing technologies: automated enumeration of mesenchymal stem cells with haematology analysers

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Mesenchymal stem cells (MSCs) are a key component in many cell therapy products. Their expansion is crucial to achieve the required cell dose to treat patients, and cell number is a critical process indicator. There are several cell-counting methods, but rapidly determining cell quantity with accuracy and precision remains a challenge. Although low cost, manual cell counting by optical microscopy (OM) with a haemocytometer has inherent limitations such as inaccuracy. Moreover, it is time consuming and labour intensive. Flow cytometry (FC) is an accurate method. However, it is more expensive than OM and equally time consuming. Dedicated cell counters have been commercialized to try to overcome these problems: they achieve better accuracy than OM but their main inconvenient is the cost and the inefficiency of buying a specific instrument just for one purpose. Since most laboratories performing quality control for cell-therapy manufacturing also provide services to blood and blood cord banks, we considered worthwhile to explore the use of existing technology to count MSCs cells. We present an innovative approach to determine MSC cell count with the body fluid module of Sysmex XN-550 (XN), which is a haematology analyser. We validated XN for MSCs cell concentration measurement, by comparing its results to OM and FC. We also evaluated the linearity, detection limits, carryover, and precision of XN. XN displayed excellent performance and good correlation was found between the three methods. XN is a reliable, economical, fast and practical alternative to OM and FC for the quantification of MSCs.

P525

Diversity of mesenchymal stromal cells: secretome in focus

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Mesenchymal stem/stromal cells (MSC) can stimulate tissue regeneration and regulate tissue-specific stem cell functions. They include small subpopulation of stem and progenitor cells (less than 1%) that can differentiate in adipogenic, osteogenic, chondrogenic and some other lineages. Additionally, many key MSC functions are realized through the secretion of paracrine factors, extracellular vesicles (EV) and extracellular matrix (ECM). So other cell subtypes are present in heterogeneous

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population of MSC, which have regulatory functions mediated by their secretome. However, secretome-based diversity of MSC is poor understood. In this study we used immortalized human MSC (ATCC). Stem cell subpopulation was assessed using CFU-test, and cell multipotency was confirmed by differentiation into adipogenic, osteogenic and chondrogenic lineages. To evaluate the ability to produce ECM components MSC were cultured as cell sheets, then decellularized using previously developed protocol (dECM). Deposition of fibronectin, collagen type I and IV, laminin was checked in dECM by immunohistochemical analysis. To mark MSC producing EV, cells were transduced by GFP-tetraspanin-CD63. Within MSC we observed subpopulations of multipotent stem cell by CFU-test and differentiation potency (<1%), stromal cell preferably deposed ECM and ''secreting'' cells (5–10%) actively produced EV. Cultured in cell sheets MSC formed tissue-like structures with ''hills and valleys'' growth patterns, and ''secreting'' cells concentrated in ''hills'', whereas stromal cell, which deposed fibronectin, distributed equally. These data highlights MSC diversity according to their secretory function in vitro and can be used to define the target MSC population for application for regeneration medicine. The study was supported by RSF (№19-75-30007).

P526

Effect of secreted frizzled-related protein2 on regeneration of dental pulp stem cells

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Objective: This study aims to clarify the effect of SFRP2 on the directed differentiation and regeneration of stem cells in vivo, and provides a theoretical basis for tissue regeneration of dental pulp stem cells.

Methods: 1.Human dental pulp stem cells were isolated, cultured and identified in vitro, transfected with retrovirus to obtain SFRP2 overexpressing dental pulp stem cells, and the overexpression efficiency was detected by RT-PCR. 2.Detect the osteogenesis and odontogenic differentiation of dental pulp stem cells by SFRP2 in vitro experiments: dental pulp stem cells were induced to differentiate in vitro, alkaline phosphatase activity (ALP) was used to detect early osteo-/dentinogenic differentiation; alizarin red staining and Calcium ion assay was used to detect the late osteo-/dentinogenic differentiation index, and the expression of osteo-/dentinogenic differentiation transcription factors and related genes BSP, DSPP and DMP-1 were detected by RT-PCR.

Results: After overexpression of SFRP2, alkaline phosphatase activity of dental pulp stem cells was enhanced, alizarin red staining and calcium ion determination were increased. RT-PCR detection revealed osteo-/dentinogenic-related transcription factors OSX, RUNX-2, related genes BSP, DSPP, DMP-1 expression was significantly improved.

Conclusions: The results of this study showed that after overexpression of SFRP2, the ability of dental pulp stem cells to differentiate into osteo-/dentinogenic direction was enhanced.

Keywords: Secreted frizzled-related protein2(Sfrp2); Dental Pulp Stem Cell(DPSC); osteo-/dentinogenic differentiation

P530

Highly efficient paired nickase-mediated correction of junctional epidermolysis bullosa via COL17A1 reframing

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The COL17A1 gene encodes the transmembrane protein type XVII collagen. Type XVII collagen forms the anchoring filaments, essential for maintaining connection between the plasma membrane of basal keratinocytes and the lamina lucida of the basement membrane zone. Mutations within COL17A1 typically result in reduced or absent expression of type XVII collagen in junctional epidermolysis bullosa (JEB), a genetic blistering skin disease. Pairing of Cas9 nickases has been shown to reduce off-target activity without impairment of on-target gene editing. Paired nicking ribonucleoproteins (RNPs) were designed to specifically target a frameshift mutation responsible for JEB. These proved highly efficient at allele-specific gene disruption (>88% efficiency) in immortalised keratinocytes, without selection. Highly efficient restoration of COL17A1 was confirmed at the RNA (>40% efficiency) and protein level (>42% efficiency) in bulk treated samples. FACS analysis of samples indicated a restoration efficiency of >51%, without selection. Subsequently, correct membrane localization of restored COL17A1 was observed in the majority of cells following immunofluorescent analysis of corrected patient keratinocyte monolayers. These studies demonstrate the development of an ex vivo gene editing therapy for JEB, using CRISPR/Cas9 paired nicking to permanently treat the genetic basis of the disease without the need for single cell expansion or selection for corrected cells.

P531

Knockdown of prothymosin α improves wound healing in diabetic mice

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Cutaneous wound healing is a complex process that requires collaborative efforts of different mediators as well as tissues and cells. Among many molecules known to influence wound healing, transforming growth factor-β (TGF-β) has the broadest spectrum of actions, affecting all cell types involved in all stages of wound healing. Prothymosin α (ProT) is a highly acidic protein involved in various cell functions, such as cell proliferation, apoptosis, immunomodulation, and acetylation. We have reported previously that ProT can increase Smad7 acetylation, thereby downregulating TGF-β/Smad signaling. In this study,

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we employed ProT transgenic mice to investigate the role of ProT in cutaneous wound healing. In mice undergoing dorsal wound surgery, ProT transgenic mice exhibited a significant delay in wound closure. Furthermore, they expressed lower levels of the fibroblast-specific protein S100A4, the myofibroblast marker α-smooth muscle actin (α-SMA), and inhibitor of matrix metalloproteinase-1 (TIMP-1), and collagen deposition in the wound tissue. Phosphorylated Smad2/3 was reduced, whereas protein acetylation was increased in the wound tissue of ProT transgenic mice. Furthermore, ProT suppressed α-SMA expression through acetylated Smad7 to inhibit myofibroblast activation. Furthermore, ProT decreased TGF-β-mediated migration in keratinocytes and fibroblasts. Notably, we show that lentivirus-mediated knockdown of ProT at the wound site improved cutaneous wound healing in streptozotocin (STZ)-induced diabetic mice. Collectively, our results elucidate the pathophysiological role for ProT in the process of cutaneous wound healing. Furthermore, we also identify ProT as a therapeutic target for wound healing.

P532

Safety criteria and patient selection in a cell-based clinical trial for the systemic treatment of recessive dystrophic epidermolysis bullosa: MesenSistem-EB

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Recessive dystrophic epidermolysis bullosa (RDEB) is due to biallelic mutations in COL7A1 gene encoding type VII collagen (C7). These mutations lead to a decrease or complete absence of C7, reducing epithelial-mesenchymal adhesion in the skin and mucous membranes. The continuous formation of blisters/erosions, together with overlapping cycles of healing and chronic inflammation, is associated with mutilating fibrosis and aggressive epidermoid carcinomas that drastically reduce the patient's life expectancy. Therapies to treat symptoms such as pain and itching are currently under research. Systemic administration of allogeneic bone marrow derived mesenchymal stromal cells (BM-MSCs) has shown transitory clinical benefits in RDEB, likely due to their anti-inflammatory effects. Aiming to extend benefits, we are conducting a phase I/II unicentric study in 9 RDEB patients (EudraCT-2017-000606-37; MesenSistem-EB) by intravenous administration of higher doses of BM-MSCs obtained, in this study, from haploidentical donors. The production of autoantibodies against C7 may be triggered after the treatment, especially in C7-null patients, raising safety and efficacy concerns. Thus, patients with minimal C7 expression (indirect immunofluorescence and western blot) and absence of antibody binding to the base of salt split skin are eligibility criteria for safety in MesenSistem-EB. Genetically diagnosed pediatric patients with RDEB were pre-selected at La Paz University Hospital. We have identified and recruited 7 patients, with moderate to severe clinical manifestation. Five of these patients have been already treated. The investigational medicinal product was manufactured at the Cell Therapy Unit (IIS-Gregorio Marañón). So far, no severe adverse effects or antibodies deposition at the skin have appeared.

P533

Preclinical models for *in vivo* gene editing of COL7A1 based on delivery of CRISPR/Cas9 to RDEB patient skin by adenoviral vectors

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Recessive Dystrophic Epidermolysis Bullosa (RDEB), a devastating skin fragility disease characterized by recurrent skin blistering, scarring and high risk of squamous cell carcinoma development is caused by mutations in COL7A1, the gene encoding anchoring fibrils-forming Collagen VII. Ex vivo correction of COL7A1 by gene editing in patient keratinocytes and fibroblasts (the skin cell types that produce and secrete Collagen VII) has been achieved before. However, in vivo editing approaches are necessary to address the direct treatment of skin lesions. We have generated helper-dependent adenoviral vectors for delivery of Cas9 protein and two gRNAs for excising COL7A1 exon 80, which contains a frameshift mutation highly prevalent in the Spanish RDEB patient population. This NHEJ-based editing strategy proved to be highly effective for restoration of Collagen VII expression when tested in cultured patient keratinocytes and fibroblasts. To model in vivo delivery of the viral vector to patient cutaneous tissue, we initially produced skin organotypic cultures using patient cells, and injected vector preparations into blisters generated by detachment of dermis and epidermis after gentle suction, resulting in efficient exon 80 deletion in a high number of patient fibroblasts. We then tried vector delivery in a skin-humanized mouse model generated by grafting patient skin equivalents onto immunodeficient mice. Blisters generated by controlled suction on regenerated patient

skins were injected with viral vectors and COL7A1 gene editing and Collagen VII expression restoration were assessed.

P534

Compassionate use of mesenchymal stromal cells for the treatment of graft-versus-host disease

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Graft-versus-host disease (GvHD) appears in approximately 50% of patients who receive allogeneic hematopoietic stem cell transplantation (HSCT) for the treatment of hematological disorders. Only 30–50% of the patients respond to steroids as first-line of treatment for GvHD and the second-line is not clearly established. In addition, the treatment with non-specific immunosuppressant is associated with a high risk of infection and toxic effects. Mesenchymal stromal cells (MSC) possess extensive immunomodulatory properties. Therefore, in order to find safer and more effective therapies, several clinical trials have used MSC for the treatment of steroid-resistant GvHD. Overall, the infusion of MSC is associated with a better outcome in turns of survival and response. Between March, 2008 and April, 2016, 52 consecutive patients suffering aGvHD (n = 33), cGvHD (n = 2) or both (n = 17) and resistant to two lines of treatment were infused with MSC as compassionate use in references centers in the Andalusian Health System. These patients received between 1 and 5 doses of MSC (1-2x106 cells/kg of bodyweight). MSC were derived from adipose tissue (n = 47) and/or bone marrow (n = 7) from healthy third-party donors in our GMP facilities. MSC infusions were generally well tolerated; only two cases presented serious adverse events. 63.5% of the patients responded to the MSC treatment, of which 32.7% achieved a complete response. Median survival time was significantly higher in responder patients compared with no responders (p = 0.0003). Overall survival was significantly higher in children (p = 0.02). These results corroborate that MSC are an effective and safe therapy for the treatment of steroid-resistant GvHD.

P535

CRISPR/Cas9-mediated correction of two recurrent COL7A1 mutations in primary and induced pluripotent stem cells from patients with recessive dystrophic epidermolysis bullosa

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Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a rare and severe genetic skin disease responsible for blistering of the skin and mucosa after minor trauma. RDEB is caused by a wide variety of mutations in COL7A1 encoding type VII collagen, the major component of anchoring fibrils which form key attachment structures for dermal-epidermal adhesion. Here, we achieved efficient COL7A1 editing through non-viral delivery of the CRISPR/Cas9 system in primary RBEB cells and in iPSCs developed previously. First, we designed different guide RNAs (gRNAs) targeting the specific mutation or sequences in close distance to the mutation to be corrected in exon 3 and in exon 80. Two of these gRNAs showed up to 70% cleavage activity in RDEB keratinocytes, fibroblasts and iPSCs, when delivered together with Cas9 as a ribonucleoprotein complex (RNP). We have also evaluated their off-target activity in HEK293 cells, as well as in RDEB keratinocytes, fibroblasts and iPSCs, and found no evidence for non-specific cleavage activity at the in-silico predicted sites. For gene correction purpose, we treated primary RDEB cells and iPSCs with site-specific RNPs together with the corresponding Donor delivered as ssODN. Up to 20% of genetic correction was observed in bulk-nucleofected RDEB iPSCs without selection, as assessed by Sanger sequencing and TIDER analysis. RT-qPCR and western blot analyses confirmed re-expression of type VII collagen in fibroblasts differentiated from gene-corrected RDEB-iPSCs. Currently, we are also differentiating gene-corrected RDEB-iPSCs into keratinocytes in the perspective of the development of transplantable skin models suitable for clinical application.

P536

Highly efficient gene-editing strategies for clinically-relevant ex vivo correction of Recessive Dystrophic Epidermolysis Bullosa in primary patient cells

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Mutations in COL7A1, the gene encoding the anchoring fibrils-forming type VII collagen, are the cause of RDEB, a skin fragility disease characterized by recurrent skin blistering, scarring and high risk of squamous cell carcinoma development. Our focus is on correcting a frameshift mutation in exon 80 of COL7A1 that is highly prevalent in the Spanish patient population. Recently, we demonstrated that a one-step NHEJ-based correction protocol based on dual gRNA/Cas9 RNPs delivered to patient keratinocytes by electroporation allowed highly efficient restoration of Collagen VII expression by removing mutation-bearing COL7A1 exon 80. Ex-vivo gene-corrected keratinocytes achieved normal human skin regeneration upon transplantation onto immunodeficient mice. Biosafety was assessed by NGS analysis of 244 in silico predicted off-target sites without detection of indel generation. Importantly, this exon removal strategy has been tested in different exons within the COL7A1 collagenous domain with remarkable efficiency, making this strategy suitable for a wider population of patients around the world. On the other hand, we have explored a marker free HDR-based approach for RDEB correction, using a modified sgRNA combined with a donor template-carrying AAV. This template

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encompasses 10 exons of COL7A1 gene, potentially enabling precise HDR-based correction for a larger cohort of RDEB patients. We have achieved gene correction efficiencies close to 40% in primary keratinocytes from RDEB patients with different mutations. Collagen VII expression in bulk populations of gene-corrected patient keratinocytes is comparable to that detected in normal keratinocytes, showing that this is a feasible strategy to restore dermal-epidermal adhesion in regenerated skin tissue.

P537

Extracellular vesicles secreted by mesenchymal stromal/stem cells reverse TGF-β induced myofibroblast differentiation

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Mesenchymal stromal/stem cells (MSC) are perspective tools for the treatment of fibrosis due to their specific paracrine activity. However, the potential possibility of MSC secretome to promote reverse of fibrosis, restoring the functional activity of the tissue, is more promising. So, we tried to uncover the probable mechanisms of MSC influence on redifferentiation of myofibroblasts, that are crucial ''players'' in fibrogenic progress. Previously, we demonstrated, that components of MSC secretome, both soluble factors and extracellular vesicles (EV-MSC), inhibit TGF-β induced differentiation fibroblasts into myofibroblasts in vitro. However, only EV-MSC promoted the changing of myofibroblast phenotype into fibroblast. To clarify the potential mechanism of this phenomenon we investigated the composition of EV-MSC using RNA-sequencing. We found that EV-MSC contain a large spectrum of RNAs, including lncRNAs and miRNAs. 270 miRNA were detected, and 52 among them had targets associated with fibrosis. In order to specify the role of major miRNAs that regulate fibrosis, we have chosen miRNA-21 (''profibrotic'') and miRNA-29 (''antifibrotic''), that are wide presented in EV-MSC. Thus, the suppression of the miRNA-21 within EV-MSC led to a decrease in the proportion of cells with the myofibroblast phenotype. At the same time, overexpression of the studied miRNAs did not significantly influence on the activity of the EV-MSC. Based on the data, we can conclude that the EV-MSC stimulate myofibroblasts dedifferentiation into fibroblasts. Presumably, this antifibrotic effect of MSC might be largely mediated by the balance between profibrogenic and antifibrogenic miRNAs in the composition of EV. The study was funded by RFBR grant#18-015-00525.

P538

Superoxide dismutase 3-introduced stem cells and their extracellular vesicles exert improved efficacy in the murine dermatitis model

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The immunoregulatory abilities of mesenchymal stem cells (MSCs) have been proposed and studied in a variety of immune disorders including autoimmune and allergic diseases. However, the unequivocal benefits observed in preclinical settings have not been reproducible in clinical trials. This discrepancy is presumably because of the functional inability of MSCs in harsh host microenvironment. Therefore, to achieve more beneficial outcomes from the clinical studies, it is necessary to better understand MSC features and to explore novel strategies to strengthen the therapeutic potency of MSCs. Genetic manipulation can be a strategy for the generation of highly efficient cell therapeutics. Previous studies have demonstrated that antioxidant enzyme can be introduced into cells to prolong their longevity and augment their anti-inflammatory effects. Therefore, in the present study, we explored the anti-inflammatory functions of extracellular superoxide dismutase 3 (SOD3)-introduced MSCs. SOD3-transduced MSCs (SOD3-MSCs) more efficiently down-regulated not only the differentiation and activation of various immune cells but also the symptoms of murine model of atopic dermatitis-like inflammation. More interestingly, the SOD3 protein was expressed even in extracellular vesicles (EVs) secreted from SOD3-MSCs. These SOD3-delivering EVs also exhibited superior therapeutic effects as their parent cells did. Our findings indicate that MSCs transduced with an antioxidant enzyme, as well as their EVs can be utilized for the development of next-generation cell therapeutics for inflammatory diseases.

P539

Antifibrotic effect of mesenchymal stromal cell sheets is mediated by interaction of stromal and endothelium cells

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Cell sheets (CS) are multi-layered cell structures containing living cells, native matrix and soluble factors produced by the cells themselves during formation of the construct. Cell sheets from stromal cells due to their active ability to produce extracellular matrix proteins and rich secretome have shown positive results in treatment of surface defects and deeper soft tissue lesions. However, exact mechanisms by which the action of the CS is implemented and explanation of their superiority to injected cells is yet to be clarified. Using an original mouse model of a pressure ulcer defect, we have shown that transplantation of a CS from murine MSC leads to earlier closure of the wound defect with a reduced scar tissue volume compared to transplantation groups of suspended cells and conditioned medium from MSC. Assuming that the action of CS can be mediated by interaction of stromal and endothelium cells with subsequent modulation of angiogenesis and scar formation, we carried out a number of studies on the course of vascular formation and remodeling after transplantation of CS. Analysis of CD31, van Gieson and staining for myofibroblasts have shown significant modulation in CS delivery groups compared to suspension of SC medium. Detailed results will be presented at the meeting indicating mechanisms of CS therapy by modulation of angiogenic response. Study was supported by Presidential grant #MK-1068.2019.7 and by RFBR grant #17-04-01452 (animal test).

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P540

A selection-free COL7A1 repair strategy based on homologous recombination

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RDEB is a severe rare blistering skin disease caused by mutations in COL7A1 resulting in total or near absence of type VII collagen within the basal membrane zone of the skin. Since previous studies indicate that ≥35% expression of type VII collagen is sufficient to provide skin stability we aimed to repair mutant COL7A1 in bulk populations of RDEB patient keratinocytes by homologous recombination (HR). Here, we present a double-nicking strategy for HR-mediated correction of a splice-site mutation in exon 3 of COL7A1, using a catalytically modified version of SpCas9, SpCas9 D10A, that can significantly reduce the risk of undesired off-targets and mediate improved HR outcomes. Whereas specificity, cutting activity and repair efficiency are mainly determined by the selected gRNAs, the presence of homologous DNA sequences for gene repair via HR is a prerequisite. We established and optimized a selection-free correction strategy, using paired SpCas9 D10A ribonucleoproteins (RNPs) and rationally designed single- and double-stranded repair templates with 5́overhangs. Upon delivery of the RNPs together with repair templates into RDEB keratinocytes via electroporation, we analyzed the gene editing efficiency via next generation sequencing (NGS) and immunofluorescence staining. The combined treatment of patient keratinocytes with RNPs and double-stranded 5́overhang repair template results in a correction efficiency of >20% of all alleles analyzed via NGS. In conclusion, our HR-mediated gene therapy approach represents a robust tool for the correction of gene mutations in COL7A1, showing high on-target efficiency, lowered off-target risk compared to SpCas9 usage, and improved HR rates.

P541

Production of clinical grade temporary epidermal substitute obtained from hESC derived keratinocytes for the treatment of sickle cell leg ulcers: a challenge for regenerative medicine

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Skin is the largest organ of the body involved in self-protection against external damages. Epidermis, the upper layer of the skin is mainly composed of keratinocytes organized to form a physical barrier at the interface of the environment. Some of diseases associated to genetic mutations or not could weaken this protection and lead to the disruption of skin integrity. Cell therapy approaches using adult keratinocytes are currently envisaged however these cells present limited proliferative capacities and variability in genetic background. Access to an unlimited source of embryonic pluripotent stem cells (hESC) will aim at overcoming these limitations since these cells are available in unlimited quantities thanks to their unlimited proliferation capacity and their pluripotency. In this context, a protocol allowing the generation of keratinocytes from hESC able to perform functional pluristratified epidermis was developed. In the perspective of a human clinical application, the entire protocol have been optimized and adapted following good manufacturing practice (GMP) conditions from a clinical grade hES cell line (RC9) obtained at the Biotech Company Roslin Cells. A quality control of the keratinocytes was established. These controls include the checking for contaminations, karyology, and viability. Specific controls such as the analyses of the expression of keratinocytes markers and the absence of pluripotency markers were performed to verify the quality of the keratinocytes cells bank. In addition, a clinical grade support was selected for this capacity to allow the formation of a pluristratified epidermis in vivo.

P542

Characterization of aged dermal stem cell phenotype: implications for skin homeostasis

ABSTRACT WITHDRAWN

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P543

Human breast milk exosomes accelerate mouse wound healing

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The healing of cutaneous wounds is a very efficient process despite being very complex. Under certain pathological conditions healing may be impaired, prolonging the healing process and eventually leading to medical intervention and the chronicity of the wound. Exosomes are secreted extracellular vesicles present in biological fluids where they play a key role in intercellular communication at the tissue, organ and organismal levels. Given that human breast milk contains abundant maternal extracellular vesicles (MEVs) with pro-regenerative and immunomodulatory properties, the aim of the present study was to evaluate if topical application of MEVs into open wounds would be beneficial for healing. Full-thickness excision wounds of 4-mm diameter were created in the dorsal skin of C57BL/6 mice and topical application of 20 micrograms of human MEV isolated at weeks 9, 11, 12 and 15 postpartum of breastfeeding were placed onto the open wounds. Control wounds were vehicle-treated. Macroscopic measurements up to 7 days post-wounding revealed that the area of the wound treated with MEV significantly decreased compared with controls. Histological analyses at day 7 post-wounding showed no differences in the granulation tissue area between groups. Nevertheless, dermal integrity of the wounds appeared to vary under MEV treatments isolated at different breastfeeding weeks. Our preliminary results suggest that MEV treatment of different breastfeeding weeks may induce a faster wound closure but also granulation tissue aberration.

P544

Development of matrixes and devices on the basis of inert materials by the method of ion-beam treatment to prototype cells and medical targets

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Matrix materials are in demand and promising in the field of cellular technologies. A properly created matrix will not be an inert environment, but an active medium regulating the basic processes of cell life. Created a biomatrix based on inert material for the cultivation of cells from various sources. In the course of the work, factors affecting the increase in adhesion properties were identified, the substrate toxicity and oncogenicity were excluded, the growth of mouse hepatocytes, human fibroblasts and Candida albicans was analyzed on this surface. It is proved: the effectiveness of modifying the surface of the glass to improve the adhesive properties, thus, an increase in biomass in a shorter period of time, the formation of a monolayer of cells. Using scanning electron microscopy, it was found that the 2D matrix has an ordered cellular structure that affects the increased adhesive properties. The 2D matrix is not cytotoxic and oncogenic. Hepatocytes of mice, on a 2D-matrix, are adsorbed in an amount (2.8 ± 0.2) times higher than on a smooth glass. On the treated glass ion formed cell aggregates. The number of human fibroblasts adsorbed on a 2D-matrix is (1.5 ± 0.5) times the number of cells than on a smooth glass. Fibroblasts adsorbed on modified glass have a normal configuration for this type of cell. Candida albicans cells are 5 times more actively adsorbed on the modified glass than on the control glass. Hepatocyte extract of mice incubated on modified glass contains protein fractions with molecular weights of 30, 43, and 120 kDa compared with the control.

P549

Target optimized variant of CRISPR associated nuclease enables allele-specific knock out of ELANE-related neutropenia

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We present here a novel and broad approach for allele-specific CRISPR gene editing which allows the targeting of different mutated alleles of a disease-associated gene using one CRISPR composition. This approach opens up unlimited opportunities for gene editing therapy in cases of dominant negative mutations, compound heterozygous mutations and haploinsufficiency. First, we analyze human haplotypes and identify either a single nucleotide polymorphism (SNP) or several SNPs that are predicted to be associated with most of the known mutated alleles. Then, we design a CRISPR based gene editing strategy targeting the SNP and optimize a CRISPR associated nuclease (CAS) for that sequence to enable editing only at the desired alleles. We demonstrate the utility of this approach for knocking out mutated alleles of ELANE. Heterozygous mutation in ELANE causes the majority of Severe Congenital Neutropenia (SCN). Over 100 heterozygous mutations in ELANE associate with the disease. We identified three SNPs that can be linked with the majority of mutated alleles and designed CRISPR based strategies to knock out these alleles. Then, we used our directed-evolution platform to optimize a CAS nuclease to effectively and specifically cleave at the SNPs. The optimized specificity of the nuclease allows the allele specific editing. Finally, we show that electroporation of human CD34+ cells with the optimized CRISPR composition knocked out the mutated ELANE allele and the edited cells mature to active neutrophils.

P550

In vivo genome editing of hAPOC3 in the liver of APOC3 transgenic mouse leads to a robust and stable reduction in serum triglyceride levels and normalization of lipid profiles

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Apolipoprotein C-III (APOC3) is a key player in triglyceride-rich lipoprotein metabolism and is strongly associated with elevated plasma triglyceride (TG) levels. Patients with severe hypertriglyceridemia are at risk for pancreatitis and cardiovascular disease. Loss-of-function mutations in the APOC3 gene are associated with low triglyceride levels and a decreased risk for cardiovascular disease. Meganuclease-mediated genome editing

in liver could disrupt the expression of APOC3, therefore achieving sustained reduction of serum APOC3 and triglyceride levels. In this study, we utilized AAV8 vectors to evaluate the potential of engineered meganucleases to inactivate liver expression of human APOC3 (hAPOC3), in hAPOC3 transgenic mice. The hAPOC3 transgenic mice in this study contain multiple copies of the hAPOC3 gene and have significantly elevated serum triglyceride levels (7964 ± 2435 mg/dL), 57-fold higher than the levels in WT mice (139 ± 27 mg/dL), as well as abnormal levels of total cholesterol, LDL, and HDL. Seven days after a single tail vein injection of an AAV8 vector expressing the hAPOC3-targeting meganuclease, we observed a dose-correlated and up to 95% reduction of serum hAPOC3 and triglyceride levels and normalization of lipid profiles. We analyzed the editing efficiency by measurement of hAPOC3 copy number using ddPCR and on-target indel analysis by deep sequencing on liver DNA samples collected 8 weeks after dosing. We observed significant and dose-correlated reduction of hAPOC3 gene copies and on-target editing. Currently, we are also evaluating the in vivo editing of APOC3 in non-human primates.

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P551

Efficient gene editing of the PKLR locus in human long-term haematopoietic stem cells to correct Pyruvate Kinase Deficiency

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Pyruvate kinase deficiency (PKD) is an autosomal recessive disorder caused by mutations in the PKLR gene that lead to a reduction of the erythroid pyruvate kinase protein (RPK) activity. This disease is associated with reticulocytosis, splenomegaly and iron overload, and may be life-threatening in severely affected patients. In selected cases, allogeneic Hematopoietic Stem Cell Transplantation (HSCT) has been shown to correct the disorder. Therefore, autologous HSCT of genetically corrected cells should offer a durable and curative therapeutic option. With this purpose we conducted a gene editing approach to correct PKD in human Long-Term Hematopoietic Stem Cells (LT-HSCs). We developed a knock-in gene editing strategy at the genomic starting site of the PKLR gene by combining RNP electroporation and two different adeno-associated viral vector (AAV6) donors to deliver either a TurboGFP expression cassette or a promotor-less therapeutic codon optimized RPK cDNA (coRPK), flanked by specific homologous arms. Up to 40% specific integration and stable expression of both donors were detected in colony forming units (CFUs) generated from gene edited CB-CD34+cells, in the absence of toxicity related to the procedure. Moreover, gene edited CB-CD34+ cells engrafted efficiently in both primary and secondary NSG mice, demonstrating that gene editing of Long-Term HSCs has been achieved. In addition, safety of our gene editing approach for its further clinical application is being assessed through GUIDE-Seq and rhAmpSeqTM combination. These results demonstrate the feasibility of gene edit the PKLR locus in LT-HSCs, and therefore its future potential clinical application for the treatment of PKD.

P552

CRISPR/Cas9-mediated gene knockout to address primary hyperoxaluria

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Primary hyperoxaluria (PH) is a rare genetic disease caused by mutations in one of three genes (AGXT, GRHPR and HOGA1) involved in the oxalate metabolic pathway, giving rise to PH types 1, 2, and 3, respectively. PH is characterized by excessive accumulation of the toxic waste product oxalate, which leads to formation of insoluble deposits in the kidney and other organs, resulting in renal failure and systemic oxalosis. Currently, the treatment of late-stage disease is limited to combined liver-kidney transplantation. Here, we report the effects of CRISPR/Cas9-mediated knockout of Ldha and Hao1, two genes involved in oxalate formation. Knockout of Hao1 is expected to cause accumulation of glycolate rather than oxalate, a metabolite which is safely excreted. Guide RNAs spanning the murine genes were screened for editing activity, and selected gRNAs were formulated with Cas9- encoding mRNA in lipid nanoparticles (LNPs). These LNPs were tested in a PH1 disease model (Agxt-/-) mice. Editing in total mouse livers was greater than 70% for both Hao1 and Ldha. We saw a significant and dose-dependent increase in the level of glycolate along with a significant decrease of the oxalate precursor glyoxylate with editing of Hao1, as well as a reduction of urinary oxalate levels by 57% and 63% with editing of Hao1 and Ldha, respectively. Oxalate reduction was sustained throughout the 15-week long observational phase. The data suggest that editing of genes in the glyoxylate detoxification pathway using a non-viral delivery approach constitutes a potential one-time treatment option for genetic forms of hyperoxaluria.

P553

Engineering of small and potent synthetic RNA-guided cytosine base editors

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CRISPR-Cas9 has gained prominence among methods employed for targeted genome editing. More recently, cytosine and adenine base editors (CBEs and ABEs, respectively) have been added to the CRIPSR toolbox. Both CBEs and ABEs can affect targeted base pair exchanges with only minimal introduction of DNA double strand breaks, making them an attractive alternative to the less-efficient donor template-based homology-directed repair (HDR). However, current state-of-the-art SpyCas9 CBEs are suboptimal for certain applications due to their large size (>1850 amino acids) and narrow targeting window of typically 4–5 nucleotides, with nucleotides outside of this range not being converted at all. We recently reported the characterization of several novel

small Cas9 proteins and the engineering of synthetic RNA guided nucleases (termed sRGNs) that all detect a short NNGG PAM motif and show DNA cleavage activity comparable to SpyCas9. By substituting the smCas9 of Staphylococcus lugdunensis (Slu) for SpyCas9, we generated a significantly size reduced (~20%) CBE with similar C:T conversion rate as SpyCas9 CBEs. Furthermore our SluCas9 CBE showed a markedly extended potential targeting range (~8–9 nucleotides) and a further reduced indel frequency as compared to state-of-the-art SpyCas9 BE4max. In summary, the developed Slu-CBE and sRGN-BEs allowed editing with high efficiency and enhanced targeting range and a reduction of unwanted by-products, while at the same time offering a compact size that renders them more favourable for therapeutic delivery as human gene therapy agents.

P554

A versatile reporter system to identify designer epigenome modifiers for effective multiplexed epigenome editing

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Multiplexed genome editing offers the opportunity to simultaneously edit multiple genomic loci in the same cell through the concurrent introduction of DNA double strand breaks (DSBs) by designer nucleases. This strategy has been explored, for example, to generate off-the-shelf chimeric antigen receptor (CAR) T cells by the concomitant disruption of the TCR, B2M and PDCD1 genes. While efficient, this procedure harbors a substantial risk of genotoxicity as the simultaneous introduction of DSBs drives chromosomal translocations. Multiplexed epigenome editing may represent a safer alternative as it leaves the genomic sequence intact. To simplify the cumbersome screening of designer epigenome modifiers (DEMs) capable of efficiently silence the intended target loci, we have generated reporter cell lines harboring up to three fluorescent marker genes each driven by a distinct promoter element. In a first step, we used this system to identify the best-performing DEM from three customized pools each designed to target and epigenetically edit a single promoter element. Since simultaneous DEM delivery may result in variegation of target locus silencing due to competition for the respective cellular factors, we have used the reporter system in a second step, to fine-tune the conditions to achieve highly effective multiplexed epigenome editing at the three chosen loci with similar potency. In conclusion, by simply exchanging the promoter element, our reporter system offers the opportunity to screen for the best-performing activating or repressing DEM targeting any promoter of interest in a multiplexed setting. This unique setup offers considerable benefit to researchers interested in multiplexed epigenome editing.

P555

Highly precise gene editing correction of a Pyruvate Kinase deficiency-causing mutation in patient-derived lymphoblastic cells using single stranded oligodeoxinucleotides

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Gene Editing is the next step in the refinement of Gene Therapy, where the disease-causing mutations can be reversed in patient's genome. Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disease, caused by mutations in the PKLR gene, which encodes the Erythroid Piruvate Kinase (RPK). More than 200 mutations have been described. RPK expression is highly regulated along erythropoiesis. These characteristics make the Precise Gene Editing a promising therapy for the treatment of PKD. We have explored the potential of a Precise Gene Editing Correction of mutations causing PKD using single stranded oligodeoxinucleotides (ssODN). We generated a PKD patient-derived lymphoblastic cell line (PKD-LCL) with two heterozygous mutations reported in exon 3 (359C>T) and exon 8 (1168G>A) of PKLR gene. Different mutation-specific guide RNAs were designed to target these PKD mutations. Guide RNAs were electroporated into PKD-LCLs as ribonucleoproteins. Targeting efficacy was assesed using Mosaic Finder, an in-house developed bioinformatic tool that detects allelic mosaicism on Next Generation Sequencing datasets. In exon 3, targeting was much more specific for the mutated allele than for the WT allele (around 30 times). Then, we designed a corrective ssODN to restore the wild type amino acid sequence coded by this exon. We achieved up to 2.5% precise correction maintaining a very high allele-specificity. Our results demonstrate the feasibility of a highly personalized gene editing therapy to treat PKD-causing mutations. New designs in our mutation-specific gene editing tools are being explored to increase efficacy and evaluate the clinical relevance of this approach to correct PKD.

P556

Allele specific repair of splicing mutations in cystic fibrosis through AsCas12a genome editing

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Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the CFTR gene. A significant number of mutations (~13%) alter the correct splicing of the CFTR gene, causing the production of aberrant mRNA transcripts and nonfunctional protein channels. Among the CFTR splicing defects, the 3272-26A>G and 3849+10kbC>T mutations lead to the formation of new acceptor and donor splice sites respectively, generating in both cases the inclusion of intronic portions, the consequent frameshift and the loss of CFTR expression. To permanently correct these genetic defects, we developed a genome editing approach using a single crRNA and the Acidaminococcus sp. BV3L6, AsCas12a, which removes essential splicing regulatory elements that form the aberrant 3' or 5' cryptic splice sites within intronic sequences. This genetic repair

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strategy is highly precise, showing very strong discrimination between the wild-type and mutant sequence (up to 100% of the editing exclusively in the mutated allele) and a complete absence of detectable off-target sites. We demonstrated that our AsCas12a-based gene correction strategy efficiently corrects the splicing pattern in human primary airway epithelial cells carrying the 3272-26A>G or 3849 + 10kbC>T mutations. Moreover, we observed the rescue of endogenous CFTR function, for both mutations, in patient derived intestinal organoids, which are recognized as a highly valuable preclinical model to predict ex vivo therapeutic efficacy in CF patients. These results demonstrate that allele-specific genome editing with AsCas12a can correct aberrant CFTR splicing mutations, paving the way for a permanent splicing correction in genetic diseases.

P557

Chimerization enables effective delivery of TALE-based effectors by lentiviral vectors

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Designer effectors based on the DNA binding domain of Transcription Activator-Like Effectors (TALEs) are among the most powerful sequence-specific tools and have an excellent reputation for their remarkable specificity in editing the genome, transcriptome and more recently the epigenome. However, the repetitive structure of the TALE arrays interferes with efficient packaging in lentiviral vectors (LVs), a widely used delivery system for human gene therapy. To overcome this limitation, we developed Chimeric TALEs (ChiTALEs). To this end, we replaced 3 out of 17 TALE repeat units with TALE-like units from the bacterium Burkholderia rhizoxinica in combination with an extensive codon optimization to maximize intra- and inter-repeat sequence variability. We demonstrate that ChiTALE can be easily generated using conventional gene synthesis and that ChiTALE-based transcriptome and epigenome editors targeting two human genes, CCR5 and CXCR4, showed comparable activities in human reporter cell lines as their canonical TALE-based counterparts. Moreover, transduction of multiple cell lines with an LV encoding the CXCR4-specific ChiTALE epigenome editor resulted in rapid and sustained silencing of endogenous CXCR4 expression. We believe that efficient lentiviral delivery of the highly effective ChiTALE platform facilitates long-lasting editing of clinically relevant primary cells, and that ChiTALEs further expand the existing toolbox to precisely edit the genome, transcriptome and epigenome ex vivo and potentially in vivo.

P558

Efficient delivery of mRNA and CRISPR in CNS using polymer nanoparticle

ABSTRACT WITHDRAWN

P559

LAG-3, but not Tim-3, disruption in TCR gene edited human memory stem T cells enhance the anti tumor activity against multiple myeloma

ABSTRACT WITHDRAWN

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P560

In vivo CRIPSR/Cas9 Ldha inhibition as universal treatment for primary hyperoxaluria

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Sequence-specific endonucleases, specially CRISPR/Cas9 systems, have substantially increased the efficiency and specificity of genome editing, enabling the precise modification of the genome. Genome editing technologies not only facilitate deciphering the contribution of a specific mutation(s) in particular genetic disease but also represent an invaluable tool for the development of innovative therapeutic strategies for genetic diseases. Our previous results clearly indicate that CRISPR/Cas9-mediated substrate reduction therapies (SRTs) targeting glycolate oxidase (GO) represents a promising therapeutic option for PH1. In this work, we have extended the use of this in vivo genome editing strategies to target other enzymes of the glyoxylate metabolism that would be widely applicable not only for PH1 but also for other PH subtypes. Thus, we have developed CRISPR/Cas9 systems targeting Ldha gene that encodes the hepatic lactate dehydrogenase (LDH). A single administration of AAV8 therapeutic vectors drastically reduced LDH levels in the liver of PH1 and PH3 mice, reducing urine oxalate levels and kidney damage without toxic symptoms. Genome wide off-target analysis revealed the safety of this approach with no indel detection in the liver of treated animals. Altogether, our data provides evidence that in vivo genome editing technologies would provide new tools for improved and more universal therapeutic approaches for PH.

ABSTRACT WITHDRAWN

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P561

Abnoba-seq: a sensitive in vitro assay to profile CRISPR-Cas nuclease off-target activity in a truly unbiased manner

P563

Improved methods for CRISPR HDR using Alt-R HDR Enhancer and modified ssDNA donors with optimized design

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CRISPR-Cas9 proteins introduce double-stranded breaks (DSBs) at targeted genomic loci. DSBs are repaired by endogenous cellular pathways such as non-homologous end joining (NHEJ) and homology-directed repair (HDR). Providing a ssDNA template during repair allows researchers to precisely introduce a desired mutation by utilizing the HDR pathway. However, rates of HDR are often low compared to NHEJ-mediated repair. Here, we describe methods to improve the rate of HDR vs NHEJ-mediated repair by careful selection of template characteristics, including homology arm lengths, placement of synonymous SNPs in the HDR template, and the

addition of chemical modifications to increase DNA stability against cellular nucleases. In addition, we demonstrate improved HDR rates when using Alt-R HDR Enhancer, a small molecule compound that increases the rate of HDR by inhibiting NHEJ-mediated repair. We implement our findings in the Alt-R CRISPR HDR Design Tool, a novel bioinformatics tool for ssDNA HDR template design. The Alt-R CRISPR HDR Design Tool supports single-stranded designs up to 3 kb, single- and dual-guide designs (i.e., for use with nickases), guide selection suggestions, insertion of synonymous SNPs in coding regions, and more.

P564

An engineered self-inactivating adeno-associated virus mediated Cas9 delivery system for therapeutic genome editing

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Gene therapy research has accelerated as a result of studies identifying disease-causing mutations and the emergence of genome editing nucleases. These nucleases, such as CRISPR-Cas9, can induce double-stranded DNA breaks to facilitate repair or knockout of disease-causing genes. Effective use of these nucleases requires efficient delivery to the cell, for which adeno-associated virus (AAV) has emerged as a promising delivery option. Its low immunogenicity, and efficient delivery to specific tissues has already led to FDA approval for two disease targets. However, a major safety concern with delivering Cas9 using AAV (AAV-Cas9) is the formation of stable episomal DNA that mediates persistent Cas9 expression. Stable Cas9 expression can lead to potential off-target editing and immune responses to the bacterial Cas9 protein. Due to Cas9's multiplexing capabilities, we tested the ability to edit a genomic site while simultaneously targeting the stable episomes to inactivate Cas9 expression at the transcriptional level. We compared several methods of inactivation in vitro, including excision-based strategies and direct editing of the Cas9 DNA. We transduced cells with AAV-Cas9 and characterized the levels of Cas9 mRNA over time. Cas9 mRNA levels decreased by more than 80% relative to a non-inactivating condition, and simultaneous editing of the genome target was confirmed by Surveyor. In order to enable efficient cutting of the target site prior to Cas9 loss, ongoing work is being conducted to control kinetics of inactivation. The result will be a precision self-inactivating Cas9 that will provide both effective and safer therapeutic genome editing approaches for the clinic.

P565

Cell cycle "push-up" to boost targeted genome editing in hematopoietic stem cells

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The scope of genome engineering in hematopoietic stem/progenitor cells (HSPCs) has broadened from random to precise genome insertions for treating genetic diseases of the blood lineages. Gene editing protocols have been significantly improved by delivering CRISPR/Cas9 as pre-assembled ribonucleoprotein and a donor template for homologous directed-repair (HDR) as adeno-associated virus serotype-6 (AAV6). However, this procedure still suffers from a low efficiency of HDR events, which is constrained into the most primitive cells and limits the yield of transplantable edited cells. Since we previously demonstrated that AAV6 transduction induced a p53 dependent cell cycle arrest in HSPCs, we exploit the electroporation step of the editing procedure to transiently co-deliver mRNAs encoding for a panel of AAV-helper adenoviral proteins to possibly counteract this response. We identify one short adeno-5 E4 variant (E4orf6/7) that transiently pushes cells into the S/G2 phases, thus boosting of 1.5-fold HDR efficiency and the yield of edited cells in comparison to our standard protocol. Since this stimulus immediately activates a negative-feedback loop, it does not induce toxicity or affect clonogenicity and engraftment capacity of HSPCs after primary and secondary xenotransplantation in NSG mice. In combination with a p53 inhibitor, it results in 3-fold higher long-term engraftment of edited clones (up to 60% of human graft), as characterized by clonal tracking with a barcoded-AAV6 template. Overall, this study will be instrumental for broadening applicability of this technology to genetic blood disorders that require a high level of corrected cells.

P566

Kill-switch and self-inactivating AAV vectors based on CRISPR/Cas9 for precise control of *in vivo* gene expression

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Despite an excellent safety record of AAV vectors, undesirable toxicity resulting from permanent gene expression represents a clinical concern. Here, we aimed to resolve this issue by creating AAV vectors that harbor a kill-switch based on the bacterial CRISPR II system. This approach has two components: (i) a guide RNA expressed from the AAV vector and (ii) the Cas9 endonuclease, which is supplied in trans and directed by the gRNA to a target site in the vector. Remarkably, we found that transgene expression could be potently suppressed (~50–80%) in vitro and in vivo after expressing Cas9 in trans. To further increase the safety of our new vector design, we implemented means to temporally restrict Cas9 expression. Therefore, we constructed and validated versatile self-inactivating Cas9 vectors based on a full-length or split Cas9 expressed from self-complementary (sc) or single-stranded (ss) AAV vectors. As hoped for, both systems permitted potent reduction of Cas9 expression in vitro and in vivo, without impeding Cas9 on-target activity. Moreover, we consistently detected a robust expression and activity of Cas9 from scAAV vectors, which is very encouraging for future gene editing applications in cells and tissues in which AAV second-strand synthesis is rate-limiting. Here, we present new and versatile AAV vectors with a powerful built-in safety feature that allows for deliberate cessation of transgene expression. Moreover, we report an original self-inactivating split Cas9 that can be

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packaged as sc or ssAAV genomes and that should increase the safety of future in vivo AAV applications including in humans.

P567

A novel λ integrase-mediated transgenesis platform for biologics production

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Genome engineering is an important component of gene/cell therapy and molecular medicine. In this respect, several tools like ZFNs, TALEN and CRISPR-Cas system are currently being used for transgenesis. However, issues like insertional mutagenesis, off-target events, and small cargo size have limited their usage. We have developed a λ-bacteriophage based tool where a novel λ-integrase variant can be used to catalyze conservative site-specific integration of large plasmid into a safe harbor site(s) of human genome, termed attH4X, found in 1000 human Long Interspersed Elements-1 (LINE-1). To improve this tool with respect to safety concerns and targeting efficiency, we have further refined the platform by initially producing seamless vector using λ-Int mediated in vitro intramolecular recombination and then subsequently target the seamless vector specifically at LINE-1. To broaden the utility of our platform, we have explored the tool in the field of biologics testing and production. In this context, we randomly inserted linear 'landing pads' (4X attH4X) sequences along with a selectable marker in HEK293T followed by targeting the seamless supercoiled target vector specifically into the landing pad. This has resulted in the high transgene expressing clones harbouring target vector in one of the four attH4X sequences of the landing pad. This platform could allow integration of large transgene(s) at a specific genomic locus which can be used to test different variants of the therapeutic protein to select the best performing variant. Currently, we are utilizing this platform for FVIII therapeutic protein production to demonstrate its application in biopharmaceuticals production.

P568

Using CRISPR base editors to perform targeted mutagenesis screenings for mechanism of action discovery

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The CRISPR/Cas9 system is a revolutionary technology that enables rapid introduction of genetic perturbations at targeted genomic regions. Our purpose is to develop a high-throughput targeted multiplexed mutagenesis workflow using a dCas9-AID system coupled with pooled functional selection and NGS to elucidate the mechanisms of G protein-coupled receptor (GPCR) pharmacology and ligand-protein allosteric interactions in endogenous cellular context. The aim of the project is to create point mutations in the genes to better understand the compounds mechanism of action (MoA). We used the CRISPR-X technology from the Bassik lab at Stanford University, which couples the inactive form of Cas9 (dCas) with AID (Activation-induced cytidine deaminase) that converts C to U. Our initial experiments targeted GFP (using a single plasmid per cell), resulted in a high mutagenesis rate that correlates with the loss of GFP signal measured by Flow Cytometry. This test system showed that the mutagenesis using a single plasmid per cell worked efficiently and allows us to proceed with the GPCR MoA studies. In the first of these studies we targeted the GPCR Beta 2 Adrenergic Receptor (B2AR) with a library of sgRNAs tiling all along the gene. We monitor the GPCR activity with a reporter system containing 6xCRE-mCherry, which allows us to sort for cells that have an increased or decreased GPCR activity after the mutagenesis.

P569

All-in-one delivery using LentiFlash technology, a MS2-chimeric RNA delivery tool designed for clinical applications

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1: ICS-PHENOMIN 2: Flash Therapeutics

Gene editing, regenerative medicine and immunomodulation offer great promises for a wide range of therapeutic. Nevertheless, many obstacles exist especially for clinical development which require efficient and safe delivery technologies including gene expression level and duration tailoring. The transfer of gene-editing systems, transcription factors or antigens mediated by DNA delivery technologies has been widely used for research purposes but they pose major drawbacks for clinical applications, such as toxicity, phenotype modifications, chromosomal integrations, and long-term expression. RNA technologies enable to overcome most of these issues but certain challenges remain, such as safe intracellular delivery, RNA stability and immune responses. As a game-changing RNA carrier, LentiFlash, a non-integrative bacteriophage-lentivirus chimera, opens up new avenues by delivering multiple and different RNA species into the cell cytoplasm. RNA-mediated expression level and duration must be managed according to applications. Gene-editing requires low/short-term expression to avoid off-target effects while immunomodulation or regenerative medicine imply high/mid-term expression. Ex vivo or in vivo administration may also impact delivery requirements. Here, we show that LentiFlash (i) delivers RNA in entire cell populations such as immune or stem cells, keratinocytes, or muscle cells with various expression levels and duration depending on cell type, culture and RNAs species (ii) preserves viability and cell phenotype, (iii) provides a therapeutic solution for ex vivo and in vivo applications. All these properties, as well as the ability to produce LentiFlash using lentiviral production platforms already validated in clinical settings, offer additional safety considerations for clinical development and human use.

P570

Designer recombinase for precise excision of HTLV-1

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Human T-cell lymphotrophic virus type 1 (HTLV-1) is a pathogenic retrovirus whose infections frequently lead to adult T-cell lymphoma (ATL), a disease that is currently incurable.

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Worldwide more than 10 million people are infected and approximately 5% of those develop ATL, a highly aggressive cancer. In the last years genome editing tools have emerged as promising antiviral agents. Here, we used substrate linked directed evolution to engineer a Cre-derived site-specific recombinase (HTLVrec) to excise the HTLV-1 proviral genome from infected cells. While Cre naturally targets the loxP sequence, we identified a conserved loxP-like sequence (hoxLTR) present in the long terminal repeats of the virus. HTLVrec efficiently recombines the hoxLTR sequence in bacteria and human cells without detectable activity on predicted off-target sites. Our data suggest that recombinase mediated excision of the HTLV-1 provirus represents a promising strategy to reverse HTLV-1 infections that bears potential for future clinical applications to treat HTLV-1 infections.

P571

Mesenchymal Stromal Cell support to optimize HSPC-Gene Editing

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Hematopoietic stem and progenitor cells (HSPCs) are localized in a specialized microenvironment in the bone marrow (BM) named hematopoietic niche. Mesenchymal stromal cells (MSCs) are key elements of the BM niche. They support HSPC quiescence and self-renewal by direct contact and releasing specific factors. Gene editing (GE) of HSPCs represents an attractive therapeutic strategy for genetic diseases. A current limitation to its therapeutic efficacy is the low yield of engrafting edited cells achievable with current GE protocols. In order to exploit the HSPC supportive capacity of MSCs in the context of GE, we isolated and characterized human MSCs from BM aspirates of healthy donors. We found that MSCs express and release several HSPC supportive factors, including CXCL12, SCF, ANGP1 and that they are able to attract and support HSPC in vitro and in vivo in humanized ossicle models. We developed a 2D co-culture system to favour the expansion of GE-HSPCs. MSCs increased the number of total and edited primitive HSPCs, defined as Lin-, CD34high, CD90+, CD45RA-, 24h post editing and, more robustly, after 4 days of culture. Furthermore, the expansion of HSPCs on MSCs before GE preserved primitive HSPCs. Edited HSPCs have been transplanted into sub-lethally irradiated NSG mice. Preliminary results showed a faster repopulating capacity of edited cells co-cultured with MSCs. In conclusion, we defined a novel strategy to favour expansion and maintenance of GE-HSPCs in vitro. Further experiments to optimize our protocol are ongoing, in order to identify novel factors responsible for preserving primitive edited cells.

P572

Direct transgene expression and mAb production by using new generation minimised UCOEs on human iPS and CHO cells

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It has recently been shown that efficient and stable expression of transgenes from the A2UCOE is at least in part due to its resistance to DNA methylation- mediated silencing. Analysis of a deletion series from the CBX3 end of a fully functional 2.2kb A2UCOE where expression of an eGFP reporter gene is directly driven off the HNRPA2B1 promoter, revealed a 1.2kb and 1.7kb truncation that retained full UCOE activity following transduction of P19 and F9 cells in both undifferentiated and differentiated states. These 1.2–1.7 and a further deletion 0.5kb A2UCOEs, were also able to retain stable expression in murine embryonic stem cells and now in human iPS cells during differentiation into embryoid bodies and tissue specific cell types. In addition, from a practical perspective our finding that the 0.5-1.2-1.7A2UCOEs retain the same stability of expression as the larger 2.2A2UCOE patent suggests that it can effectively replace the latter within therapeutic LV constructs allowing a greater capacity and also more safety for the gene of interest by cutting off the enhancer elements which carries a potential mutation risk. A further project now on human mAb production with recombinant CHO cells by using our new UCOE models has been started and the early results show that they are more productive than the control A2UCOEs and able to produce human recombinant mAbs in mg level even under two months of cultivation. The results already encouraging to provide a new better UCOE model for recombinant protein production in mammalian cells.

P573

Assessment of the tumorigenicity potential of CRISPR/Cas9 genome editing using an innovative *in vitro* cellular transformation assay

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CRISPR/Cas9 is a programmable RNA-guided nuclease being identified as a potential game changer in precise genome editing (PGE) therapies. The two major safety concerns about CRISPR/Cas9 are OFF target editing, unspecific cuts due to mismatched pairing between the guide RNA and the DNA, and ON target DNA recombination, a consequence of the genomic instability generated by Cas9-induced double strand breakages. Both these undesired effects can potentially have disruptive phenotypical long-term consequences if they involve, for instance, oncogenes or oncosuppressors. Development of robust assays to test the safety of CRISPR/Cas9 is paramount to support its application in gene editing therapies. We assessed the malignant transformation of CRISPR/Cas9 edited cells by measuring their ability to grow in an anchorage independent fashion as a hallmark of oncogenic transformation. For this purpose, we applied an assay strongly correlated to the gold standard soft agar, in which growth in low attachment (termed GILA) is determined by measuring ATP in lysed cells. We edited the immortalised (but non-transformed) liver cell line NeHepLxHT with therapeutic gRNAs, along with positive controls consisting of knock-out (KO) of several tumour suppressor genes. The GILA assay was validated to delineate the phenotypic, and potentially oncogenic, changes associated with ON and OFF target toxicity: no evidence of in vitro oncogenic changes was associated with the therapeutic guide RNAs, whilst the combined tumor supressor knock-out showed the pre-transforming phenotype represented by loss of anchorage dependent growth.

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P574

CRISPR-Cas9 genome editing induces megabase-scale deletions

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Only in less than a decade after CRISPR-Cas9 characterization, up to a dozen of clinical trials have been proposed. However, CRISPR-Cas9 approach still faces concerns regarding unintended alterations (on- and off-targets). Recently, two teams of researchers have used Cas9-nuclease to trigger several double-strand breaks (DSBs) all along a unique chromosome and that led to the full deletion of this last. In a way, these works have warned us on how toxic can be several DSBs on chromosome integrity. So, in sight of gene therapy, we asked ourselves if a single DSB can impair the targeted chromosome. We therefore decided to carry cytogenetic analysis of Cas9-induced events following a single DSB. Using FISH probes, we pointed out the high genomic instability at the DNA break point highlighted by megabase–scale deletion or gain. Array-CGH confirmed that terminal truncation of the targeted chromosome can occur and showed it started exactly at the targeted locus. At short term, it will be mandatory to further decipher this risk, in particular, for evaluating biosafety of clinical trials that are already underway. To deal this issue, we developed an alternative approach excluding the use of a DSB. For that, we used the Cas9D10A-nickase that induce a single-strand break (SSB), and co-supplied the cells with a single-strand desoxynucleotide (ssODN). We achieved high rate of homology-directed repair HDR without InDels. Furthermore, this approach also allows avoiding terminal truncation, offering a safer way to perform gene editing.

P575

Engineering monocyte lineage-specific glucocerebrosidase expression in human hematopoietic stem cells using genome editing: A universal strategy for genetic correction in Gaucher disease

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Gaucher disease is a lysosomal enzyme deficiency caused by mutations in the GBA gene. For its most common form (type 1), the disease manifestations are largely due to pathological organ infiltration by diseased macrophages. Chronic, life-long administration of either recombinant enzyme or orally-available substrate reduction therapy are the two currently available therapies for this disease. An alternative treatment approach is to engineer the patient's own hematopoietic system to restore glucocerebrosidase expression, thereby replacing the affected monocyte/macrophage compartment and potentially constituting a one-time therapy for this disease. Towards this goal, we established an efficient ex vivo genome editing approach using CRISPR/Cas9 to target monocyte/macrophage-specific glucocerebrosidase expression cassettes to the CCR5 safe harbor locus in human CD34+ hematopoietic stem and progenitor cells. The modified cells are capable of generating human glucocerebroside-expressing macrophages in vivo and in vitro and maintain long-term repopulation and multi-lineage differentiation potential in serial transplantation studies. The safe harbor-approach using a lineage-specific promoter constitutes a universal correction strategy for all pathological mutations in this disease and circumvents potential detrimental effects of ectopic expression in the stem cell compartment.

P577

Base editors-mediated generation of point mutations in IPS cells

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CRISPR base editing enables prespecified single-base conversions in genomic sequences with unprecedented accuracy and efficiency without generating DNA double strand breaks. In this study, we used cytosine and adenine base editors (CBEs and ABEs) for the generation of specific point mutations in human induced pluripotent stem cells (hiPSC). We show that point mutations can be efficiently generated in genes by transfection of BE-mRNA and specific sgRNAs. As a proof of concept, we generated ten patient-independent human iPSC lines with different recurrent mutations in the TP53 gene. Our approach gives an unprecedented opportunity to generate isogenic hiPSC lines with patient-specific mutations. This strategy does not only allow better genomic and pathophysiology resolution but also removes the necessity to search for suitable patients with specific mutations and reduces the efforts to obtain approvals from ethics committees.

P578

High-capacity adenoviral vectors encoding full-length dystrophin and CRISPR-Cas9 nucleases for targeted correction of myogenic cells from Duchenne muscular dystrophy patients

ABSTRACT WITHDRAWN

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P579

Generation of cellular models to study gene therapy strategies for Pompe disease

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Pompe disease (PD) is a rare disorder caused by mutations in the lysosomal acid alpha-glucosidase enzyme (GAA) gene that lead to accumulation of glycogen in multiple tissues. The replacement enzyme therapy is the only therapeutic option but is effective only for a subset of Pompe patients and partially effective. As an alternative, gene therapy (GT) could offer a definitive cure by restoring the normal expression of the GAA gene. The purpose of this project was to generate murine GAA-KO Sol8 cell lines to study efficacy and safety of GT strategies for PD. Using CRISPR/Cas9 system, we have generated Sol8 cell lines lacking the first ATG in exon 2. However, all these clones showed a GAA band of approximately 70kDa (that could correspond to the processed form), similar in size to that found in Sol8 cells. This data suggested a potential use of an alternative ''ATG'' 3' of the canonical start codon. However, since this truncated GAA miss the leader peptide, we found lower GAA activity, indicating that these ATGko-GAA-cells are a suitable model for patients harboring homologous mutations. Interestingly, we found two potential ATGs in the mGAA gene that could explain the 70kD protein and the remaining GAA activity observed in ATGko-GAA-cells; one at the end of exon 2 and another at exon 5. The latest is also a hot spot for mutations in Pompe patients with a severe phenotype. We are now developing new cellular models lacking completely all GAA protein and activity by targeting these two ATGs.

P580

Efficient generation of knock-out mice by in vitro and in vivo electroporation of CRISPR/Cas9 system to model rare metabolic inherited diseases and cancer predisposition

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Knock-out (KO) mice for essential genes provide valuable disease models in which new therapeutic approaches could be tested. New genome editing tools allow the generation of precise mutations resembling human mutations and in shorter periods of time. Moreover, improved protocols of zygote electroporation of nucleases avoid the main technical barriers for this procedure such as isolation and microinjection of embryos. In addition, a recent method of delivering the genome editing components directly into fertilized eggs within oviducts in situ, also avoids the surgical transfer of embryos. We have generated new mouse models deficient in genes involved in metabolic rare diseases such as Primary Hyperoxaluria Type 1 (PH1, Agxt1 gene) and Pyruvate Kinase Deficiency (PKD, Pklr gene), and in a tumor suppressor gene, because existing animal models are not completely suitable for testing the advanced therapies or develop embryo lethality. We designed pairs of specific sgRNAs targeting exon 1 to generate precise and controlled deletions causing premature stop codons that lead to absence of protein in all three genes. Ribonucleoproteins were delivered by in vitro electroporation of embryos and subsequent transfer or in vivo oviduct electroporation of fertilized eggs. Similar results were achieved in all genes. More than 90% born animals were edited following in vitro electroporation of embryos and up to 30% edition was observed after in vivo delivery. High variability of edited variants were generated and after variant segregation, KO mice strains were established. Disease hallmarks have been verified in the three developed models.

P581

Targeted gene insertion for the treatment of X-linked agammaglobulinemia (XLA)

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X-linked agammaglobulinemia is an inherited immunodeficiency caused by mutations in the gene encoding the Bruton Tyrosine kinase (BTK) that impair B cell development and lead to the absence of mature B lymphocytes in peripheral blood and lymphoid tissues. Consequently, XLA patients suffer from recurrent and often life threatening bacterial infections, partially ameliorated by a life-long immunoglobulin therapy. Although gene therapy for XLA using lentiviral vectors has shown promising results in murine models, a gene editing approach could overcome the issues related to deregulated expression of BTK and potential genotoxicity. Our strategy is to use a CRISPR/Cas9 system coupled with an adeno-associated virus type 6 (AAV6) vector as donor template, to integrate a codon optimized BTK cDNA (coBTK) into its own locus in hematopoietic stem cells (HSCs), in order to restore BTK physiological expression. To test our approach, we have delivered a Cas9-gRNA ribonucleoprotein complex (RNP) targeting the first coding exon of BTK using different AAV6 donor constructs into BTK-deficient DG75 B-cells. Knock-in of a coBTK cDNA containing either a strong synthetic Kozak sequence or additional BTK regulatory regions restored levels of BTK protein comparable to those found in wild-type cells. When testing our gene editing tools in HSCs, we were able to achieve editing frequencies of 80% and targeted gene integration of a PGK-GFP reporter cassette in the BTK locus in up to 45% of the cells. The engraftment potential of edited HSCs is currently being tested in xenograft models.

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P582

Gene therapy by correction of point mutations in the endogenous locus of mammalian cells using Repair-PolyPurine Reverse Hoogsteen hairpins

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The correction of single point mutations in the DNA is one of the strategies to fight against monogenic diseases. PolyPurine Reverse Hoogsteen (PPRH) hairpins are single-stranded DNA molecules formed by two polypurine mirror repeat domains, linked by a pentathymidine loop, and bound through intramolecular reverse Hoogsteen bonds. These molecules can bind by Watson-Crick bonds to their specific polypyrimidine target in the dsDNA. Then, a Repair-PPRH consists of a PPRH hairpin core bearing an extension sequence at one end, which is homologous to the DNA sequence to be repaired but containing the wild type nucleotide instead of the mutation. We attempted to repair point mutations in the adenosyl phosphoribosyl transferase (aprt) gene. We corrected nonsense mutations caused by single substitutions using different Repair-PPRHs. The correction of the mutations was confirmed by DNA sequencing, mRNA expression and enzymatic activity. We also performed Whole-Genome Sequencing analysis comparing the aprt mutant (S23) and the repaired cells. This analysis showed no off-target effects due to the treatment with the Repair-PPRH. We also explored the possible mechanism that accomplished the gene-repair. First, we performed gel-shift assays to demonstrate the binding properties of the repair-PPRH. Moreover, we showed the formation of a D-loop structure upon the binding of the PPRH hairpin core to the target dsDNA. We hypothesized that the formation of this structure triggers a homologous recombination event that finally introduces the corrected nucleotide. In summary, we demonstrate that Repair-PPRHs can achieve the permanent correction of single-point mutations in the endogenous locus of mammalian cells.

P583

Nanoparticle delivery of CRISPR/Cas9 for treatment of cystic fibrosis by homology independent targeted integration

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We are developing a gene editing therapy for Cystic Fibrosis (CF), a genetic disorder affecting around 1 in 2,500 babies born in Europe. Our goal is to integrate the whole CFTR cDNA at the AAVS1 safe-harbour locus by nanoparticle-mediated delivery of CRISPR/Cas9 and donor template. Homology directed repair (HDR) is often selected for correction of genomic mutations, however this approach will not work for non-mitotic epithelial cells where, due to protracted G0/G1 phasing, non-homologous end joining (NHEJ) is the primary repair mechanism. We are therefore investigating a novel NHEJ-based approach to Cas9-mediated transgene knock-in, known as homology independent targeted integration (HITI), which allows for precise integration of a chosen sequence by cutting both the genome and donor sequence. As proof of concept, a GFP expression cassette was targeted to the AAVS1 locus in HEK293T cells. First, a HITI donor template bearing a splice acceptor and promoter-less GFP reporter gene flanked by 20-nt AAVS1-specific sgRNA target sequences was constructed. Cells were then co-transfected using receptor-targeted lipid/peptide nanoparticles (RTNs) containing pre-formed Cas9/sgRNA ribonucleoprotein (RNP) complexes and donor template. Upon nuclear entry, the AAVS1-sgRNA-flanked reporter cassette and the endogenous AAVS1 target gene are concomitantly cleaved by Cas9, promoting NHEJ-mediated transgene insertion at the site of the Cas9-induced chromosomal DSB. HITI enabled GFP expression in 14% of cells, as quantified by flow cytometry. Site-specific donor integration was validated by end-point PCR. Thus we have demonstrated HITI-mediated gene insertion at potentially therapeutic levels, which we are currently evaluating for CFTR insertion into primary CF cells.

P584

Defining safety margins of therapeutic genome editing by understanding mechanisms and liabilities of CRISPR-based molecular entities

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CRISPR has redefined the concept of gene therapy, but despite its technological advancement, safety evaluation of CRISPR-based therapies remain fundamental for its translation to the clinics. As of today, CRISPR safety is focused on genotoxicity (off-targets, on-target translocations and chromosomal rearrangements), Cas9-induced immunogenicity and DNA double-strand break (DSB) mediated p53 activation. Biochemical and molecular assays are being developed to identify and characterize these side effects. However, a deep assessment and understanding of how the unwanted outcomes are linked to different CRISPR entities is still unknown. Moreover, defining the therapeutic index of CRISPR-based therapeutics is fundamental to identify the biologic modality (plasmid, mRNA or protein), its concentration and their consequent impact on the cell fitness. Thereofre, we develop a tailored multiparametric cellular assay to identify the key toxicokinetic-toxicodynamic (TK/TD) events generated by different CRISPR editing machineries using a single guide RNA targeting EMX1 or FANCF genes. In parallel, we also developed a proteomic analysis to assess cellular perturbation after treatment with CRISPR molecules. Both approaches have the potential to help translating genome editing in vivo in human, increasing the confidence of a smooth transition from bench to clinic.

P586

Development of cellular models to study efficiency and safety of gene repair in Wiskott-Aldrich Syndrome

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Wiskott-Aldrich Syndrome (WAS) is a primary immunodeficiency that is a good target for gene therapy (GT) due to competitive advantage of WAS-expressing cells. Several GT clinical trials are ongoing using lentiviral vectors (LVs) for gene addition into hematopoietic stem cells (HSCs). However, although highly efficient and with a good safety profile, LVs do integrate semi-randomly in active sites, generating potential genotoxicity concerns and a poor physiological expression pattern of the WAS protein. The ideal therapeutic strategy for WAS GT would be the correction of the mutations that cause this disease by GE. This strategy must provide the entrance of the tools (nucleases and donor DNA) efficiently and safely. Our hypothesis is that next generation gene editing tools will allow in a near future the genetic correction of genetic disorders such as WAS. We have generated two reporter cellular models (K562 GF-WAS-P y K562 SEWAS84) which allow us to compare the efficiency and safety of homologous directed recombination (HDR) of different delivery systems and different donors configurations using CRISPR/Cas9 targeting WAS sequences. Our results showed that the insertion of the CRISPR/Cas9 target sequence into the donor DNA enhance HDR efficiency without affecting specificity. In addition, we have showed that the level of off-target integrations depends on the copy number of the target locus. We have finally compared different delivery systems for WAS GE in hematopoietic stem cells showing that nucleofection of ribonucleoprein complex (Cas9/gRNA) is the more efficient system achieving up to 90% cutting efficiency.

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P587

A CRISPR/Cas9 edition protocol for human myoblasts to generate disease models

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CRISPR/Cas9 gene edition offers many therapeutic and research possibilities for neuromuscular disorders. However, a difficulty shared by research groups is applying CRISPR/Cas9 editing methods to cultured myoblasts: while these methods are easily applicable to cell lines, myoblasts prove to be much more difficult to work with, which precludes the efficient use of this technology as a research tool. We have set up working protocol to generate edited human myoblasts with this technology. Our protocol, involves the use of specific transfection reagents, tagged plasmids and FACS sorting of the transfected cultures to achieve gene edition in myoblast cultures derived from single colonies. We have now used this new protocol to generate immortalized myoblast models carrying the exon 52 deletion, a larger 45–55 deletion, and an endogenous overexpression of utrophin. We have confirmed this by sequencing and we have characterised protein expression in our edited cultures by in-cell western blotting. We expect that our experience optimising the CRISPR/Cas9 protocol in myoblast may be of use to other researchers interested in this technique, and will also be happy to share our edited cultures with those interested in using them as a research tool.

P588

Cell cycle determines efficiency of homology directed repair depending on the integrity of nuclear envelope

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CRISPR/cas9 is widely applied for gene manipulation using non-homologous end joining or homology directed repair (HDR). Single strand oligodeoxynucleotide (ssODN) has been used as homologous template and developed high efficiencies than dsDNA mediated homologous recombination. Even though there is a lot of work to improve the efficiency of single strand template repair (SSTR), it is still a lack of work on the correlation between cell cycle and SSTR efficiency. In this study, we focused on the cell cycle to improve SSTR efficiency, and found that mitotic phase, especially meta- and meta-phase, was the optimal timing due to the absence of the nuclear envelope. The Cas9 protein with nucleus localizing signal (NLS) readily migrated to the nucleus but the nucleotide template was blocked from nuclear transport until mitosis stage. This causes non-homologous end joining prior to the arrival of the homology template. In further experiment to overcome the nuclear envelope, NLS linked ssODN enhanced SSTR efficiency by more than 4 folds over control. In conclusion, HDR efficiency relies on the formation of DSB by Cas9 and the simultaneous access of the donor templates. To this end, it is necessary to consider the cell cycle or efficiently deliver the donor template to the nucleus.

P589

Cell cycle dependent activation of a pro-inflammatory transcriptional program in Hematopoietic Stem and Progenitor cells (HSPCs) in response to DNA damage

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Ex-vivo gene correction strategies exploit the co-delivery of DNA double strand break (DSB) by artificial nucleases and corrective DNA templates via adenoviral means in cycling HSPC. We recently discovered that cycling HSPCs activate a p53-mediated DNA damage response upon editing that negatively impacts on their engraftment capacity upon transplantation (Schiroli, Conti et al. Cell Stem Cell, 2019). Here, we study the impact of single and multiple DSB and DNA templates on HSPC functionality in relation to their cell cycle state. We first induced DSB by irradiation in quiescent or cycling HSPC and discovered that cells, actively cycling at the moment of damage, displayed a higher amount of physical DNA damage as detected by comet assay and DI-PLA. Nevertheless, we found that, HSPCs proficiency in DNA repair was comparable and independent from their cell cycle state. However, HSPC damaged when cycling showed reduced clonogenic capacity and a greater upregulation of pro-inflammatory cytokines upon DSB compared to not-cycling counterparts. This was accompanied by an increased accumulation of micronuclei, cytosolic DAPI-positive chromatin fragments. This functional impairment was also observed when editing (DSB+AAV6-mediated

delivery of DNA donor template) was performed in cycling HSPCs in comparison with not-cycling cells or upon multiple DSBs by artificial nucleases of lower specificity. Altogether, our findings indicate that cell cycle state may influence HSPC response to DSBs ranging from transient to more prolonged proliferation arrest up to the activation of an inflammatory program.

P590

Gene modification of HEK293A cells using CRISPR-Cas9 SAM technology for the transcriptional activation of dysferlin gene

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Dysferlinopathy is an inherited in an autosomal recessive manner disorder caused by mutations in dysferlin gene. Dysferlin plays a clue role in sarcolemma repair. Synthesis of defective protein leads to disruption of repair processes in muscle tissue and its atrophy. Variety of researches are aimed at this hereditary disease treatment, yet dysferlinopathy remains incurable. Part of the main reason for the low effectiveness of treatment methods is the absence of a model that most accurately reproduces the disease pathological processes. Animal-based models are widely used, but due to significant genetic differences, they are not able to fully reproduce all the features of the humans' disease course. A promising way to treat dysferlinopathy is currently gene therapy and model systems based on human cell lines expressing dysferlin, which are of interest for testing new gene-therapeutic drugs. The aim of our work was to create a test system using CRISPR-Cas9 SAM technology in vitro for the development and screening of drugs for dysferlinopathy treatment. Transcription activation of DYSF gene mRNA in HEK293A cells was carried out by using produced recombinant lentiviruses encoding transcription factors MS2-P65-HSF1, dCas9-VP64 and DYSF specific guide RNA. The cells were selected with zeocin, blasticidin, hygromycin antibiotics for each lentivirus respectively to obtain a pure population of transduced cells. Dysferlin protein expression was confirmed by Western blot analysis. The resulting CRISPR-Cas9 SAM – transcriptionally activated HEK293A cell line can be used in further studies of RNA and DNA editing therapeutic approach and drug screening for dysferlinopathy treatment.

P591

Novel cell-based assays for the assessment of potential toxic effects of CRISPR/Cas9 genome editing

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Programmable DNA-modifying enzymes, based on the CRISPR/Cas9 system, provide precise and facile molecular mechanisms for editing genomes of single cells, tissues, organs, and whole organisms. Although the CRISPR/Cas system was discovered more than 30 years ago, only the elucidation of its mode of action in the early 2010s led to the development of very broad genome editing application. However, fast translation into clinical testing has raised some concerns. Indeed, before broad clinical use, deeper insights into the toxicity profile of large-scale application of designer nucleases in general and CRISPR/Cas9, in particular, will be required. Whereas frequencies and locations of off-target cutting have been broadly addressed, little is known about their potential biological consequences. In our research, we aim to establish a novel, sensitive in-vitro assay that will allow addressing the potential of the long-term toxicity of designer nucleases, particularly off-target toxicities, with high predictive value. Our assay is based on genetically marked indicator cells and flow-cytometry as well as digital-PCR based read-outs. We show that it can be adapted to multiple cell types, incl. primary cells such as human foreskin fibroblasts (NUFF) and mesenchymal stromal cells (MSC) and allows detection of growth-promoting off-targets at frequencies below 1%. We suppose that our method might become highly useful in the expanding field of clinical genome editing.

P592

Designer RNA binding protein based on PPR protein, as a new modality for targeted therapy

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New technologies have recently emerged that enable targeted editing of genomes in diverse systems, by using ZF finger and TALE protein, and CRISPR/CAS9 system. These advances are driving new approaches to many areas including therapeutics, agriculture, biotechnology and studies of genome structure and function. In contract, versatile, programmable RNA binding module that enables manipulation of a single specific RNA in the living cell, is still immature, although the significance of complexed alternative splicing and huge amount of ncRNA has been suggested. The PPR (pentatricopeptide repeat) motif-containing protein, that organizes a large family in plants, is a sequence-specific RNA or DNA binding protein involving in multiple aspects of organelle RNA/DNA metabolisms including RNA stability, processing, RNA editing, and transcription. Natural PPR proteins consist of a tandem array of PPR motifs (degenerated 35 amino acids) in 2 to 27 repeats. Recently, we cracked the RNA/DNA recognition code: one PPR motif corresponds to one nucleotide, and amino acid variances at three particular positions confer the nucleotide specificity with programmable manner. In this congress, we would introduce mainly about designer RNA binding protein based on PPR protein and the medical use. The custom PPR protein is able to design to be targeting 15 to 20 nt RNA sequence with properly work ratio of more than 80%. We show PPR-based application in animal cells, including in vivo RNA imaging, regulation of translation and

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splicing of a specific RNA target molecule, via conjugation of functional domain to the designer PPR protein.

P593

From fiction to science – clinical potentials of gene editing

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In recent years, gene therapies and their application for clinical medicine have been standing in the spotlight of drug development. Especially since the discovery of the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeat/ CRISPR-associated nuclease 9) system and the recognition of its great potential in both experimental research as well as in the clinical setting, the interest in such gene editing technologies is on the rise. They have revolutionized biological research and also hold great potential for the future of gene therapy development. CAR T cells are likely one of the biggest success stories of gene therapies to date and they have been used to successfully treat relapsed/refractory large B cell lymphoma. Using gene editing techniques that are able to perform specific and targeted manipulations of the genome, researchers were able to significantly improve the CAR T cell technology, both in terms of expected safety and efficacy. Indeed, the next generation of CAR T cells, allogeneic CAR T cells and CAR T cells with enhanced functionality, are already in clinical trials, looking to cross the finish line. Based on real-life case studies and a review of ongoing pre-clinical and clinical studies, this contribution will address how gene editing technologies are already transforming the gene therapy landscape. It will also discuss how advancements of the gene editing toolkit can have an implication on diseases that are difficult or impossible to tackle with current treatment approaches.

P594

Aldevron's CRISPR-associated nucleases for gene editing; tools to support discovery and therapeutic programs

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Gene editing using CRISPR-associated nucleases, including SpCas9, offers a novel approach to treat many diseases. SpCas9 now is being evaluated in multiple preclinical and clinical programs. While many vendors offer research-grade SpCas9, these nucleases are not applicable to clinical trials due to construct design and quality grade. Aldevron offers SpCas9 and AsCpf1 nucleases optimized for development and clinical applications. In addition to wild-type SpCas9, Aldevron offers SpyFi™ Cas9 which supports gene editing with significantly reduced off-target effects. Aldevron's gene editing proteins are supported by a robust, scalable manufacturing protocol and an extensive quality package. These proteins are available in a range of quality grades from research grade to GMP, allowing for a seamless transition from research applications to the clinic.

P595

Superoxide dismutase 3 augments survival rate of mesenchymal stem cells in the nutritionally stressed condition

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Mesenchymal stem cells (MSCs) have been extensively studied and used in regenerative medicine for cell-based therapies. However, the poor survival rate of MSCs limits its therapeutic application and nutrient starvation-induced early death within the first few days of transplantation is one of the undesirable factors limiting its efficacy. Therefore, it is quite important to manipulate MSCs before engraftment so that they can resist the stress imposed by the starvation. Reductions of oxidative stress and autophagy have been found to prolong the life span of MSCs. In this study, we explore the role of superoxide dismutase 3 (SOD3) in regulating MSCs survival under serum-starved condition. Here, we found that overexpression of SOD3 in MSCs exhibited enhanced survival rates with reduced reactive oxygen species levels than normal MSCs under serum-starved condition. In addition, overexpression of SOD3 in MSCs attenuated starvation-induced apoptosis with enhanced autophagy. Moreover, we have demonstrated that SOD3 protects MSCs against the detrimental effect of serum starvation through modulation of AMP-activated protein kinase/sirtulin 1, extracellular signal-regulated kinase activation, and promoted translocation of Forkhead box O3a to the nucleus. These studies highlight an important role of SOD3 in regulating the survival of MSCs under starvation-induced cell death and SOD3 overexpressed MSCs can be a better potential therapeutic agent with enhanced survival rate than normal MSCs in cell-based therapy.

P600

Application of combined gene and cell therapy within an implantable therapeutic device for the treatment of severe haemophilia A

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Hemophilia A (HA) is a rare bleeding disorder caused by absence or dysfunction of FVIII protein. New regenerative medicine approaches to treat HA require insights into cell compartments capable of producing FVIII. We and others previously demonstrated that FVIII is produced specifically in endothelial cells. The aim of our work is to develop a novel ex vivo cell-based therapy using a medical device (Cell Pouch™, Sernova) leading to an improvement in patient quality of life. We isolated blood outgrowth endothelial cells (BOECs) from

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healthy and patients' blood. HA BOECs were transduced with a lentiviral vector carrying the B domain deleted form of FVIII under the Vascular Endothelial Cadherin promoter (LV-VEC.hFVIII) and were characterized for endothelial phenotype and for the number of integrated LV copies/cell ($\sim$3). We observed that FVIII was expressed by 80% of LV-VEC.hFVIII transduced cells and was efficiently secreted in the supernatant. Ten million LV-VEC.hFVIII-BOECs were transplanted intraperitoneally in association with cytodex® 3 microcarrier beads in NOD/SCID gamma-null HA mice (NSG-HA) (n = 6). BOECs survived and secreted FVIII at therapeutic levels (12%) up to 18 weeks and ameliorate the bleeding phenotype of HA mice. Finally, LV-transduced HA BOECs were transplanted into a prevascularized subcutaneous, scalable medical device (Cell Pouch™), optimized for sustained secretion of therapeutic FVIII in NSG-HA mice, showing BOEC engrafted and activity up to 16 weeks post-transplant. These results pave the way for future human clinical testing in HA patients by transplantation of GMP produced autologous gene corrected BOECs with no sign of tumorigenicity within this device.

P601

Correcting bleeding disorders using blood clotting factors produced *in vivo* by shielded engineered allogeneic cells

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1: Sigilon Therapeutics

Current haemophilia therapies require frequent protein infusions yet are unable to address long-term complications due to prolonged trough periods with suboptimal factor levels. To overcome these drawbacks, alternative modalities such as gene and cell therapies are being investigated. Spheres made with AfibromerTM biomaterials shield engineered human cells from immune rejection while preventing the fibrotic foreign body response around the spheres, enabling sustained, therapeutic factor levels. To evaluate whether sustained delivery of blood clotting factors by implantation of spheres containing engineered human cells producing hFVIII, hFIX or hFVII is dose adjustable and durable, various sphere doses were administered intraperitoneally (IP) to murine wild-type and knockout disease models. Factor production was evaluated via a combination of plasma protein levels, factor activity and bleeding (activity) assays. Administration to wild-type mice resulted in sustained plasma levels of blood clotting factors. Studies of FVIII-producing spheres in haemophilia A knockout-mice resulted in dose-dependent levels of functional hFVIII in plasma, with a corresponding correction of bleeding time and blood loss in a tail bleeding model. Taken together, these data demonstrate that administration of spheres made with Afibromer™ biomaterials shielding engineered human cells results in sustained factor production and efficacious correction of the bleeding phenotype in murine preclinical models. The sustained factor levels achieved after a single IP implantation create a viable alternative to traditional factor infusion or investigational gene therapies, with several important advantages. We aim to pursue first clinical studies in Haemophilia A patients and to develop Shielded Living TherapeuticsTM products for other chronic bleeding disorders.

P602

Combination of exosomes and near-infrared responsive gold nanoparticles: new selective and specific therapeutic vehicle

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Exosomes are extracellular vesicles (50 -150 nm of diameter) considered key elements for the intercellular communication. Although they are proposed to be ideal vehicles for the targeting of novel therapies, very little is known about the selectiveness and specificity of the transference processes involving exosomes released from different cells. PEGylated Hollow gold nanoparticles (PEG-HGNs) are near-infrared (NIR) responsive nanoparticles (NPs) which are able to generate localized heat by the use of NIR light leading to cell death when applying optical hyperthermia. In this study, we demonstrate the selectivity of in vitro exosomal transfer between certain cell types and how this phenomenon can be exploited to develop new specific vectors for advanced therapies. Firstly, PEG-HGNs were successfully incorporated in the exosome biogenesis pathway of placental stem cells (MSCs) and they were released as PEG-HGNs-loaded exosomes (PEG-HGNs_MSCs_EXOs). Exosomes were characterized by confocal microscopy, western blot, nanosight, zeta potential and electronic microscopy. Afterwards, time lapse microscopy and atomic emission spectroscopy demonstrated the selective transfer of the secreted exosomes only to the cell type of origin when studying different cell types including cancer, metastatic, stem or immunological cells. Finally, the preferential uptake to selectively induce cell death by light-induced hyperthermia was demonstrated. This work highlights the potential of exosomes as advanced therapeutic vectors and provides a better understanding to design selective therapies for different diseases.

P603

Lentiviral vectors pseudotyped with murine syncytins efficiently transduce B cells *in vitro* and *in vivo*

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Our laboratory is interested in the use of lentiviral vectors (LV) for in vivo gene delivery and explores various envelope pseudotypes for this purpose. Syncytins are fusogenic glycoproteins produced from endogenous retroviral sequences integrated in the genome of vertebrates 25–45 millions years ago. Syncytins are involved in many biological processes involving cell fusion. We report the possibility to pseudotype LV with murine syncytins A or B (LV-SynA and LV-SynB) and demonstrate that these particles are infectious in the presence of Vectofusin-1, a transduction adjuvant peptide, achieving titers of 1.4 ± 1.2 E + 07 IG/mL (n = 14). LV-SynA or LV-SynB enable high levels of transduction of B cells in vitro as shown with A20IIA murine B lymphoma cells and with murine spleen primary CD19+ B220+CD3-B cells (respectively $88 \pm 10\%$ and $89 \pm 4\%$ transgene positive cells). Both immature and mature murine bone marrow B cell subsets are efficiently transduced.

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In vivo, a single intravenous injection to mice of LV-SynA encoding luciferase (5E+05 IG/mouse) allows detectable gene transfer in the spleen and B cell transduction was confirmed by the measure of vector integration in sorted CD19+ cells three weeks post-injection. This study reports the efficient pseudotyping of LV with murine syncytins, and reveals the strong tropism of syncytins for B cells. Ly6e, the receptor of syncytin-A was confirmed to be expressed on A20IIA cells as well as on CD19+ spleen cells suggesting that LV-SynA utilizes this receptor for B cell entry. Studies are ongoing to explore possible therapeutic applications of syncytin-LV in B cell-mediated immunotherapy.

P604

DNA immunization against tumor epitopes using a versatile delivery platform that exploits inherent mechanisms of antiviral defense

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Cancer vaccines have many advantages such as exquisite specificity, low toxicity, and the potential for a durable treatment effect. However, the limited immunogenicity of tumor antigens makes them challenging to develop and novel immunization strategies are required for a proper activation of T cells. We hypothesized that plasmids encoding engineered viral proteins could efficiently stimulate the immune system which is able to detect and eliminate certain viral threats. We showed that, when electroporated in the muscle of mice, a plasmid coding for the vesicular stomatitis virus glycoprotein (VSV-G) induced locally increased levels of IL-6, IL-12 and CCL2 suggesting its ability to trigger innate immunity. We then demonstrated that the VSV-G sequence can be engineered to incorporate various foreign T-cell epitopes. When inserted in permissive sites, both MHC-I and MHC-II ovalbumin-restricted epitopes were adequately processed and induced the proliferation of specific CD8+ and CD4+ T cells, respectively. When these two epitopes were co-delivered, antigen specific in vivo killing assay showed 65% of target cell killing and a significant therapeutic effect against B16F10-OVA tumor was observed. The therapeutic efficacy was further reinforced by the co-administration of anti-PD1 and anti-CTLA4 antibodies. Several engineered VSV-G sequences were constructed to target various tumor epitopes and they significantly delayed tumor growth of B16F10 melanoma, P815 mastocytoma and CT26 colon carcinoma, demonstrating the versatility of the delivery platform. Plasmids encoding modified VSV-G sequences have the many advantages of DNA vaccines that are simple to construct, easy to produce and they thus hold promise for future clinical developments.

P605

Engineered B cells as a universal platform for the treatment of enzymopathies

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Enzymopathies are a disturbance of enzyme function, including genetic deficiency or defect in specific enzymes. Current treatment methods are insufficient and rely on precarious hematopoietic stem cell transplant (HSCT) and costly lifelong enzyme replacement therapy (ERT). An alternative approach would be to modify a patients more malleable and accessible cells, such as lymphocytes, to express large quantities of enzyme and re-infuse these cells into the patient; therapeutic enzyme can then be excreted from engineered cells in vivo and cross correct endogenous cells. B cells are ideal for this application as they become long lived and inherently have the metabolic activity to generate large quantities of protein (i.e. antibody). To this end, we established CRISPR/Cas9 methods for gene knock-in (>60%) and knockout (>95%) in human B cells. Subsequently, we have applied this platform for the treatment of Mucopolysaccharidosis type I (MPS I). MPS I is an lysosomal disease caused by deficiency of alpha-L-iduronidase (IDUA) enzyme. In order to engineer B cells capable expressing ultra-physiological levels of IDUA, we developed an approach to simultaneously knockout the endogenous BCR and introduce a IDUA overexpression cassette at the heavy chain locus. By eliminating the cells ability to produce BCR, and subsequent antibody, it will likely remove a significant metabolic burden, allowing for enhanced production of enzyme. We have engineering human B cells with our IDUA encoding vector in this manner and begun treating a mouse model of MPS I on a NOD/SCID/Il2rγ background by transplantation of engineered B cells. Results will be presented.

P606

Exploring combinatorial relief of multiple innate immune blocks for efficient gene engineering of quiescent human hematopoietic stem cells

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Quiescent human hematopoietic stem and progenitor cells (HSPC) remain highly refractory to genetic manipulation potentially due to the expression of antiviral innate immune factors. The HIV-1 restriction factor SAMHD1 interferes with reverse transcription in non-dividing, quiescent cells such as macrophages but its role in HSPC remains unclear. To address the role of SAMHD1 in human HSPC, we used lentiviral vectors (LV) that directly incorporate the SIV accessory protein Vpx, which degrades SAMHD1, directly into vector particles. While Vpx-incorporation gave only a modest benefit in HSPC prestimulated with growth-promoting cytokines, a significant advantage was observed when transducing freshly isolated, quiescent HSPC. Vpx was additive with Cyclosporin H (CsH), a compound we have shown to overcome an innate block to vector entry in HSPC. Our combinatorial Vpx-CsH strategy yielded up to 60% of gene marking in unstimulated HSPC in vitro. CsH enhanced also targeted genome editing in quiescent HSPC although overall efficiencies remained low. As expected, unstimulated HSPC engrafted more respect to their stimulated counterparts in vivo despite lower cell input. Importantly, up to 90% of gene marking was achieved in vivo for quiescent HSPC transduced in presence of CsH alone. However, although no toxicity was observed ex vivo, Vpx-exposed HSPC showed lower engraftment and gene marking levels upon transplantation,

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indicating that Vpx and possibly SAMHD1 degradation can affect their fitness. Overall, our results indicate that multiple innate immune barriers restrict gene transfer into quiescent HSPC and pave the way for the development of genetic engineering strategies directly in unstimulated HSPC.

P607

Therapeutic approach for multiple sclerosis using a bioelectronic cell implant

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Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS, affecting more than 2 million people worldwide. MS is characterized by demyelinating lesions of nerve fibers in the brain and spinal cord. The most common form is relapsing-remitting MS, characterized by unpredictable attacks that may or may not leave permanent deficits followed by periods of remission. To date, the treatments available prevent flare-ups in recurrent forms but do not cure. Indeed, beta interferon injection (IFNβ) several times per week remains a therapy of choice for the treatment of MS. However, these repeated injections are associated with inflammatory reactions at the injection site. It is therefore essential to develop innovative methods for taking medication. Here, we evaluated a new approach based on optogenetics implants to control IFNβ protein delivery in MS patients. This optogenerapy approach is based on subcutaneous implantation of genetically engineered cells encapsulated in a semi-permeable membrane that enables diffusion of small molecules. The goal of this study was to validate the biocompatibility of implants and the therapeutic efficacy in EAE model mice. Different tools have been implanted in the laboratory such as surgical procedure in sterile condition as well as the study of cell viability and the evaluation of local immunological effects of implant. The novelty of the approach relies on the control of the secretion of proteins for therapeutic purposes. The therapeutic action associated with a fine dosage of therapy would prevent adverse immune responses such as pain and local irritation at the injection site.

P608

Development of virus-like particles for CRISPR/Cas9 targeted delivery

ABSTRACT WITHDRAWN

P609

Human endogenous retroviral envelope glycoproteins syncytin-1 and syncytin-2 enable effective lentiviral transduction of human primary B cells and dendritic cells

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Human endogenous retrovirus envelope genes such as syncytins may be useful to design well-tolerated tools for gene therapy. Syncytins are placental glycoproteins with fusogenic ability. Here we show that HIV-1-derived lentiviral vectors (LV) can be pseudotyped with human syncytin-1 or syncytin-2, generating stable particles which can be concentrated and become infectious in the presence of Vectofusin-1, a cationic peptide used as transduction additive. However, the cellular tropism of syncytin-pseudotyped vectors is selective. Surprisingly, blood primary B cells but not T cells were efficiently transduced with these vectors without prior activation. All human circulating B cell subsets were transduced including naive and memory B cells. Transduced B cells could be expanded in culture demonstrating stable proviral integration. The vectors also transduced blood CD11c+ HLA-DR+ CD14+/- CD3-CD19- immature dendritic cells. Receptors of syncytin 1 and 2, respectively ASCT2 and MFSD2a, are expressed at higher levels on human primary B cells and myeloid dendritic cells than on T cells, correlating well with the differential transduction observed. Upon intravenous injection into NSG mice engrafted with human blood, syncytins vectors were found to transduce a small proportion of human B cells in

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some mice in preliminary experiments. LV pseudotyped with Syncytin-1 also transduced murine B cells in vitro. Syncytins provide novel opportunities to develop gene therapy and gene engineering applications in B cells and immature dendritic/ myeloid cells. These results also suggest that physiological interactions between syncytins and blood cells may possibly occur and remain to be explored.

P610

Transcriptome analysis of metastatic prostate cells infected by Oncolytic Adenovirus 11p encoded ADP gene (RCAd11pADP) expose important antitumor pathways

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The cellular gene expression in metastatic prostate cells (PC3 cells) infected by RCAd11pADP RCAd11pRFP and Ad11pwt were studied by using sensitive Ampliseq total RNA sequencing method. In total about 4000 genes were identified as differentially expressed sequences more than 2-fold. The antitumor effect of DES was compared between the wt virus and vectors. Significant fold changed genes with down-regulated genes in prostate cancer were observed and involved in TNFα signaling pathway via TN-κB, Kras signaling pathway, epithelial mesenchymal transition (EMT), Androgen response as well as Transforming growth factor beta receptor signaling pathway. Both up and down regulated genes in p53 signaling pathway was observed. 19 genes from PC3 cells treated with various virus vectors were further investigated by using RT2 profiler PCR array, we demonstrated that RCAd11p vector mediated tumor suppression via at least up-regulated 5 genes and down regulated 14 genes. Therapeutically down-regulated oncogenes in prostate cancer cells infected by RCAd11pADP was revealed. Consequently, our findings demonstrated that RCAd11pADP is potent vector candidate for using as antitumor agents for clinical trials.

P611

Development and pre-clinical characterization of an oral solid dosage formulation for gene delivery to intestinal mucosa

ABSTRACT WITHDRAWN

P612

Development of stealth RNA vector (SRV) for innovative gene and cell therapy

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Lentivirus vectors and AAV vectors are successfully used in various gene therapy clinical trials for inducing stable gene expression in tissue cells. On the other hand, these vectors still have various drawbacks as a versatile tool for gene and cell therapy. We have developed defective and persistent Sendai virus vector (SeVdp vector) (Nishimura, et al. 2011) capable of stable gene expression, based on unique mutant Sendai virus strain clone 151. SeVdp vector induces interferon only at low level, and this observation lead us to create ‘‘Stealth RNA vector (SRV)’’, a new generation RNA-based gene delivery/expression platform more suitable for gene and cell therapy. SRV was reconstructed from structure-optimized synthetic RNA for escaping the defense system of host cell against invading pathogen. SRV can be installed with up to 10 cDNA (with total length up to 13,500 nt) on a single RNA genome, and can express them stably and simultaneously, without interfering function of host cells. This characteristic is quite advantageous for expressing proteins with huge size (e.g., coagulation factor VIII and dystrophin), proteins with multiple subunits (e.g., IgG, IgM and bispecific IgG), and proteins requiring co-factors (e.g., lysosomal sulfatases and phenylalanine hydroxyrase). While SRV allows stable gene expression, SRV also can be erased completely from the cells by interfering vector-derived RNA-dependent RNA polymerase. As the DNA-based platforms are difficult to be erased from the cells, this characteristic makes the SRV an ideal and distinguished platform for reprogramming somatic cells with plural transcription factors and for gene editing with improved safety.

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P613

Poly(beta aminoester) nanoparticles encapsulating mTOR siRNA for lung cancer therapy

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Lung cancer is one of the leading causes of death, mainly due to the lack of efficient therapies. Currently, the standard of care is the administration of a chemotherapeutic drug, which, together with the low efficiency in killing tumours, produce high toxic side effect due to the systemic distribution. Therefore, novel treatments are required. It is well known that cancers are produced by spontaneous mutations that provoke an over-expression of genes, such as mTOR. In this context, the use of small interfering RNA (siRNA) able to knock down these oncogenes to revert the disease may be a promising alternative. Specifically, knocking down mTOR expression resulted in tumour cells apoptosis in previous in vitro studies. However, considering the labile character of siRNA and its poor stability in biological conditions, the use of a delivery system, such as polymeric nanoparticles, is a must. Poly (beta aminoester) nanoparticles were previously demonstrated to efficiently encapsulate nucleic acids and enhance transfection efficiency. In the current work, these polymers were used for the encapsulation of mTOR siRNA. The formation of small nanoparticles (<200nm), with slight polydispersity (PDI <0.3), cationic surface charge and high entrapment efficiency was demonstrated. These nanoparticles were capable of gene knockdown in lung cancer cell models, thus producing a specific cytotoxicity in these diseased cells while being biocompatible in contact with healthy cells. Summarizing, we have designed a novel therapeutic approach for lung cancer patients based on polymeric nanoparticles encapsulating siRNA targeting oncogenes that showed promising results in first proof-of-concept studies.

P614

Towards optimising lentiviral vectors for gene therapy through structure informed genome modification

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Lentiviral vectors (LVs) are being successfully used as therapeutic agents in a series of clinical applications. HIV-1 derived LVs consist of 4 independent plasmids. Here, we focus on the transfer vector that contains the therapeutic gene and the cis-acting elements that drive its expression including the packaging signal(psi). LVs carry two copies of transfer vector RNA via a process known as dimerisation. We aimed to improve the infectivity of vectors via targeting their dimerisation and packaging properties based on the hypothesis that WT HIV-1 regulates genome encapsidation tightly by recognising dimeric RNA. Introduction of mutations aimed to create vectors whose RNA is more likely to adopt the dimeric conformation and therefore be packaged. To evaluate this, we developed a novel RT-qPCR assay to measure their relative packaging efficiencies in a Co-Transfection competitive environment. Biochemical characterisation showed that transduction and packaging efficiencies were highly influenced by the region where the mutations were introduced. Northern blots confirmed that the propensity of mutated vector RNA to dimerise has increased as hypothesised. Finally, we explored by SHAPE the structure of psi in our vector RNAs by studying the influence of regions adjacent to it. Our structural analysis revealed that the presence of gag sequences stabilise the psi element, suggesting their role in supporting a stable RNA conformation that can be packaged and offering a potential explanation for their requirement in the transfer vector plasmid for maintenance of infectious titres. These findings will enable us to design more efficient vectors for a variety of clinical applications.

P615

Safer and more stable iPSC generated using Doggybone DNA vectors

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The application of induced pluripotent stem cell (iPSC)-derived cells in clinical trials is in its infancy but the potential is vast. A key asset of iPSCs is the ability to apply autologous cell therapies, but to date most current or approved clinical trials are using fully characterised allogeneic or non-allogeneic cell banks. Until now, all approved clinical trials worldwide utilise iPSC generated using EBNA1 expressing plasmids containing the OriP sequence to maintain a self-replicating episome. These vectors are amplified in bacterial hosts, containing bacterial DNA motifs recognised by the transfected cell's innate defence responses. Moreover, the continued forced expression of the Epstein-Barr virus EBNA1 protein is known to alter gene expression as well as elevate oxidative stress and DNA damage. All of these have potentially significant implications for the safe clinical use of iPSC generated using OriP/EBNA1 plasmids. We describe efficient iPSC reprogramming by applying equivalent gene sequences transiently expressed from Doggybone DNA (dbDNA) vectors free of OriP/EBNA1 sequences, bacterial motifs and produced in a chemically defined, low endotoxin, cGMP compliant manufacture. In direct comparator experiments with the current state-of-the-art OriP/EBNA1 episomes, dbDNA vectors produced iPSC colonies with the same efficiency but dbDNA-iPSC displayed evidence of greater stability in terms of maintenance of pluripotency. Mechanistic evaluations showed that the persistence of OriP/EBNA1 episomes resulted in elevated STAT1/IFN signalling. This in turn resulted in increased spontaneous differentiation in OriP/EBNA1-iPSC. We propose a potential that utilising dbDNA vectors presents a safer and more stable approach to iPSC development.

P616

Development of antitumor immunity inducing RNA drug and a novel pyro-drive jet injector

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Inactivated Sendai virus (hemmaglutinating virus of Japan) envelope (HVJ-E) stimulates anticancer immunity through the recognition of viral RNA genome fragments. We analyzed RNA fragments to be required for anti-cancer effects of HVJ-E. DI

(defective interfering) particle of Sendai virus Cantell strain was focused on because the viral fractions containing more DI particle showed stronger anti-cancer effects than other strains. The DI particles include incomplete RNA genome (544 base) with stem region of pan-fried shaped in secondary RNA structure. In this study, we revealed that the DI RNA genome rather than the standard whole genomic RNA plays a predominant role in the induction of IFN-b and proapoptotic proteins expressed in cancer cells. Furthermore, we developed much smaller RNA fragment using the DI particle RNA as a template, and named stem-loop RNA (sl-RNA). sl-RNA has 25-base double strand stem part and 25-base single strand loop part in the secondary RNA fragment structure, which can be chemically synthesized. Moreover, to deliver the RNA to tumor tissue efficiently, we developed pyro-drive jet injector (PJI). The PJI is a novel injector system capable of injection depth adjustment, and high-speed fluid ejected from PJI penetrates skin and disperses the sl-RNA into targeted tumor tissue. After injecting sl-RNA to B16F10 (mouse melanoma) tumor by PJI three times, the tumor growth was strongly suppressed. Several chemokines secretions were increased, and macrophage infiltrations and macrophage polarization to M1 (anti-tumorigenic) were observed in the sl-RNA injected tumor sections. These findings provide a novel nucleic acid medicine for the cancer treatment.

P617

Enabling efficient transduction of mesenchymal stromal cells by HAdV-5-based vectors

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Viral vectors based on human Adenovirus type 5 (HAdV-5, shortly Ad5) belong to the most commonly used vectors in gene therapy research. Particularly as oncolytic viruses for cancer therapy, they have been extensively studied over many years both preclinically and clinically. Although there have been only little safety issues, clinical success so far has been limited, one likely reason being cellular and non-cellular barriers preventing efficient vector access to tumour tissue after systemic administration. To address this hurdle, mesenchymal stromal cells (MSCs), which show a natural ability to migrate to tumours, have been used in several studies. This strategy aims to use MSCs, as carrier cells to hide the viral particles from sequestration mechanisms, increase the viral load due to intracellular virus replication, and transport them to the tumour site where newly produced particles are set free. However, MSCs are hardly transduced by commonly used Ad5 vectors. In this study, we therefore aimed to improve transduction of MSCs with Ad5 vectors. We evaluated different molecules (chemicals, peptides, proteins) for their potential to enhance transduction of MSCs. Moreover, since vector transduction of MSCs was greatly improved in the presence of positively charged molecules, we generated a new charge-modified Ad vector that demonstrated significantly improved access to MSCs and other cell types including tumour cells. Furthermore, we confirmed adequate MSC migration towards tumour cell lines upon transduction in vitro. Given that data, we developed a new superior Ad5 mutant vector for MSC-based oncolytic therapy approaches.

P618

Combined plasmid delivery of angiogenic growth factors: preclinical assessment of different approaches

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Cardiovascular diseases are caused by insufficient blood supply and local tissue ischemia. One of the promising strategies for treatment of these conditions is ''therapeutic angiogenesis'' - gene delivery to express angiogenic growth factors and stimulate natural process of blood vessels formation and growth. Earlier it was shown that introduction of a mixture of plasmids encoding VEGF165 and HGF leads to restoration of blood flow in mouse ischemic limb and efficacy of combined delivery was superior to each plasmid administered alone. Combined approaches by mixing 2 plasmid DNAs have limited prospect in clinical translation so the development of a single vector carrying 2 therapeutic genes is a potential decision. We tested different approaches for co-expression of VEGF165 and HGF genes including development of vectors with internal ribosome entry sites (IRES), vector with bidirectional promoter and vector with two different promoters. To evaluate production of VEGF165 and HGF, HEK293 were transfected and concentrations of grow factors were evaluated by ELISA. Biological activity of HGF and VEGF was evaluated in HUVEC tube-formation assay. Ex vivo analysis of VEGF165 and HGF production by explanted murine skeletal muscles after plasmid injection was performed. All plasmids provide synthesis of VEGF165 and HGF proteins by human cells and stimulates capillary formation in vitro but only vector with two independent promoters provides production of both grow factors in vitro and in vivo in significant amount and equimolar ratio and can be used for gene therapy drug development.

P619

In vivo evaluation of replication competent and defective RNA-virus based episomal vector system

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RNA virus-based Episomal Vector (REVec) is a gene delivery system based on Borna disease virus that causes persistent infection without integrating into host genome. Previously, we reported that REVec achieves stable transduction of several types of stem cells and the cells of the CNS in vitro. Although REVec holds a potential for integration-free long-term gene expression system, it's in vivo tissue tropism has not been examined to date. To further explore downstream applications of REVec, we conducted detailed analysis of the tissue tropism of replication competent and replication defective vectors. To conduct real time in vivo imaging of REVec, firefly Luciferase

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encoding vector and EGFP encoding vector were developed. Neonatal Lewis rats were administered with 1 x 10^3 of replication competent or defective REVec encoding either Luciferase or EGFP, and the vector distribution was monitored using an IVIS Lumina imaging system. At 12 weeks post-administration, animals were sacrificed, and proteins and RNA samples were collected. Protein expression of transgene in different organs was analyzed by Western blot, and genomic RNA of REVec was quantified by qRT-PCR. The replication competent REVec was initially observed in the brain, eye, and spinal cord, and was observed in various other organs including intestine, stomach, caecum, pancreas, heart, and reproductive system at later time point. In contrast, the replication defective REVec was observed exclusively in the brain. In summary, we conducted the first detailed analysis of REVec in vivo, and revealed tissues types that are susceptible to REVec transduction.

P620

Delivery of an endosomolytic CRISPR-Cas9 RNP via receptor-mediated endocytosis for cell-targeted genome editing

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The RNA-guided genome-editing enzyme CRISPR-Cas9 has been successfully employed to target different genetic disorders in model systems. However, selective delivery to specific cells or organs for genome editing in vivo is a substantial barrier to therapeutic translation. To address this unmet need, we have developed a platform for receptor-mediated uptake of the Cas9 endonuclease and guide RNA ribonucleoprotein (RNP) complex as an alternative to currently available methods of in vivo delivery. Molecular targeting and endocytosis of the Cas9 RNP can be induced by the hepatocyte-specific asialoglycoprotein receptor (ASGPr) bisconjugated to the Cas9 protein, demonstrating proof-of-concept for liver targeting. Analogously, tethering Cas9 to a cell-targeting antibody drives specific uptake into T cells. Endosomal escape, a necessary step for cytosolic delivery and subsequent nuclear localization, is facilitated by endosomolytic peptides (ELPs) recruited to the RNP surface in a programmable manner. This platform represents a novel approach for therapeutic delivery that combines the appealing safety profile of Cas9 RNP with the ability to use molecular targeting for tissue specificity.

P621

Effect on alpha-Gal A activity of short-term systemic therapy with a solid lipid nanoparticle based non-viral vector in a knockout mouse of Fabry disease

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Fabry disease (FD) is an X-linked lysosomal storage disorder caused by a deficiency of the lysosomal enzyme, alpha-galactosidase A (alpha-Gal A). The lack of enzymatic activity results in a systemic accumulation of neutral glycosphingolipids. FD is a good candidate to be treated with gene therapy, since it corresponds to a single gene disorder in which moderately low levels of enzyme activity should be sufficient for clinical efficacy and, thanks to cross-correction mechanisms, the transfection of a small number of cells will potentially correct distant cells too. The aim of the study was to evaluate the capacity of a a non-viral vector based on solid lipid nanoparticles containg a GLA plasmid to incresase the levels of alpha-Gal A in plasma and tissues of a knockout (KO) mouse of FD. A single dose of the vector containing 60 μg of plasmid was administired through the tail vein, and after 7 days, the animals were sacrified. Alpha-Gal A activity in plasma, liver, spleen, heart, and kidney was measured by an anzymatic assay. Results were compared with those obtained in wild type and non-treated KO mice. After the administration of the vector in KO mice alpha-Gal A activity increased in plasma and tissues (from 2 to 5 times) in comparison to KO non-treated animals. In plasma, the enzymatic activity reached a value of 4% of that in wild type mice, and 6%, 13%, 12% and 15% in liver, spleen, heart and kidney, respectively.

P622

RAGE-targeting delivery of anti-microRNA-92a antagomir for the treatment of acute lung injury

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Acute lung injury (ALI) is an inflammatory lung disease. Gene therapy for ALI has been investigated to reduce lung inflammation. The receptor of advanced glycation end-products (RAGE) is induced and plays an important role in inflammation response in ALI. RP1 peptide was reported previously to bind RAGE. In this study, the R3V6 peptide linked with the RP1 peptide (RP1R3V6) was synthesized and evaluated as a gene carrier into the lungs of ALI animal models. Since inhibition of microRNA-92a (miR-92a) has been shown markedly reduced lung inflammation response, antagomir miR-92a (AMO92a) was delivered into the lungs using RP1R3V6. In a gel retardation assay, AMO92a and RP1R3V6 formed stable complexes. In vitro cytotoxicity test showed that the AMO92a/RP1R3V6 complex was less toxic, compared with AMO92a/R3V6 or AMO92a/PEI25K. In vivo administration of the AMO92a/RP1R3V6 complex into an LPS-induced ALI model reduced the miR-92a Level efficiently. Furthermore, the AMO92a/RP1R3V6 complex reduced the pro-inflammatory cytokine levels (TNF-a/IL-6/IL-1b) more efficiently than AMO92a/R3V6 or AMO92a/PEI25K. In conclusion, the RP1R3V6 is an efficient carrier of AMO92a into lungs and may be useful for the treatment of ALI.

P623

Microfluidic ynthesis of ionizable lipid nanoparticles containing CRISPR/Cas9 ribonucleoprotein complexes

ABSTRACT WITHDRAWN

P624

Development of novel injector: Pyro-Drive Jet Injector (PJI) application to intradermal DNA vaccination

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DNA vaccine has evolved with time, and even though DNA vaccination could be applied to different fields such as cancer or infectious diseases, the delivery method of DNA vaccine still needs to be improved. To resolve this issue, we investigated the potential of pyro-drive jet injector (PJI). Here, we determined the suitable injection conditions to deliver plasmid DNA to the epidermis or dermis on both rat and mouse models. Under suitable injection conditions, the PJI injection directly delivered Cy3-labeled plasmid into cell nuclei in these regions at higher frequency than by traditional syringe method using 3D microscopic analysis. Additionally, after PJI injection with luciferase expression plasmid, luciferase expression was 7–37 times higher than traditional syringe injection group. Since plasmid injection by PJI was able to successfully induce protein expression, we wanted to know whether PJI is capable as a DNA vaccine device. To investigate this aim, we used ovalbumin (OVA) expression plasmid in an antibody production model. When OVA plasmid was injected by PJI, the resulting OVA antibody was detectable in the rat model. PJI injection stimulated antibody production dose dependently. In contrast, needle syringe method did not result in robust antibody production. In summary, we have not only determined the applicable PJI injection conditions to target the epidermis or dermis of murine skin, but have also shown effective protein expression and antibody production abilities by PJI. PJI is a reliable method of DNA delivery, resulting in robust protein expression and show potential in improving DNA vaccine efficiency.

P625

Suicidal gene therapy of leiomyoma via delivery of herpes thymidine kinase gene by means of $\alpha v\beta 3$ integrin-targeted peptide-based nanoparticles

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Uterine leiomyoma is the most common benign tumor of the female reproductive tract. Precise localization of tumors makes the disease a perfect target for suicidal gene therapy using herpes thymidine kinase (HSV1-TK) gene transfer. We developed novel $\alpha v\beta 3$-integrin-targeted peptide-based carriers for plasmid DNA delivery into $\alpha v\beta 3$-expressing cells, including primary cells derived from patients with leiomyoma. DNA-nanoparticles composed of iRGD ligand-conjugated peptides or ligand-free peptides were studied for their physicochemical properties. The specificity of cell penetration and DNA delivery were demonstrated by ligand competitive DNA uptake and transfection experiments in cells with varying $\alpha v\beta 3$-integrin expression (PANC-1, HeLa, HEK293). Suicidal gene therapy with HSV1-TK gene and subsequent ganciclovir treatment was held for PANC-1 pancreatic cancer cells and primary leiomyoma cells. $\alpha v\beta 3$ surface expression was analyzed on PANC-1, HeLa, HEK293 and leiomyoma cells and was found as 35%, 18%, 3% and 73%, respectively. Proliferative Alamar blue and Trypan blue tests on PANC-1 cells and leiomyoma cells showed a decline of proliferative activity among cells transfected with HSV1-TK gene carrying complexes in comparison with LacZ gene transfected cells. Significant difference was found in the apoptosis level between HSV1-TK gene transfected and control cells. Addition of c(RGDfK) ligand during YOYO-1-labeled DNA uptake analysis blocked ligand-conjugated complexes transport significantly. The study shows that utilization of peptide-based nanoparticles modified with iRGD-ligand is a promising approach to development of targeted DNA delivery system. Developed carriers demonstrated high specificity and transfection efficacy of leiomyoma cells with subsequent successful suicidal gene therapy. This work was supported by RFBR grant 17-04-01463.

P626

Mesenchymal stem cells derived membrane vesicles induced by cytochalasin B demonstrate immunomodulatory properties

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MSCs exhibit immunosuppressive and anti-inflammatory activities which are helpful for the therapy of autoimmune diseases and transplantant rejection. Extracellular vesicles (EVs) are mediators of intercellular communication. EVs demonstrate biological and immunological properties of parental stem cells. Due to safety and applicability for large-scale production EVs are perspective therapeutic tool. We used approach of increased production of membrane vesicles using cytochalasin B. Cytochlasin B-induced membrane vesicles are used as a vector for drugs delivery. Nowadays, biological activities and therapeutic potential of CIMVs are under investigation. (CIMVs) were obtained from mouse (CBA×C57BI/6) adipose stem cells. Molecular composition of MSCs-derived CIMVs was characterized by multiplex analysis. Next, the influence of intravenous injection of CIMVs on immune system was evaluated. Antibody titer in blood serum was evaluated using the hemagglutination test. The reported study was funded by RSF according to the research project № 18-75-00090. Multiplex analysis showed that CIMVs contain growth factors, cytokines and chemokines similar to parental MSCs. We observed that the antibody titer against sheep erythrocytes in the serum of mice that had previously received an intravenous injection of MSCs or CIMVs was 1.5 and 1.7 times less, respectively, than in the serum of native mice. We found that CIMVs contain the same biologically active molecules as the parental cells, and, show immunosuppressive activity similar to MSCs. These properties make them an attractive tool for immunosuppression therapy.

P627

Recombinant plasmids containing picornaviral self-cleaving 2A-peptides and expressing VEGF and FGF2 growth factors induce angiogenesis in vivo

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Background: Multigenic systems, promoting simultaneous expression of several therapeutic genes represent a promising tool for gene therapy. These systems provide the possibility to combine several genes with synergetic potential that increases their therapeutic efficiency. In the present study, we investigated the angiogenic potential in vivo of previously constructed plasmid pVax-VEGF-p2A-DsRed, pVax-FGF2-p2A-DsRed and pVax-VEGF-p2A-FGF2-DsRed, containing picornaviral 2A-peptide self-cleaving sequences and expressing different combinations of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF2) and reporter gene DsRed.

Methods: All procedures were approved by the local animal ethics committee (Protocol 5 27/5/2014, conducted as part of Program of Competitive Growth of Kazan Federal University and supported by Grant NSC-3076.2018.4. Functional activity and angiogenic potency of recombinant plasmids were evaluated after subcutaneous transplantation of genetically modified HEK293T cells in Matrigel to immunodeficient mice BALB/c Nude (age 6–8 weeks).

Results: Using tools of molecular genetics it has been shown that transplanted genetically modified cells expressed recombinant pro-angiogenic factors. Overexpression of VEGF and/or FGF2 resulted in increased tube density and hemoglobin concentration, fibroblast migration which is evidence of pro-angiogenic effect of these plasmids. It is worth noticing that the most intensive pro-angiogenic effect was observed in the group with pVax-VEGF-p2A-FGF2-DsRed administration.

Conclusion: Achieved results have demonstrated that created genetic constructs could be used for further investigations for target gene delivery and induction of therapeutic angiogenesis. The most potent recombinant plasmid combines two pro-angiogenic growth factors with mutual synergistic effect, stimulating formation of functional blood vessels

P628

Effect of intranasal administration of mesenchymal stem cells on the average speed of movement and time of activity and NO level in olfactory bulb of rats after simulation of brain stroke

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In physiological conditions, neuroblasts, which are generated by neural stem cells in olfactory bulbs, migrate to brain areas with intensive formation of new neural networks. After stroke, neuroblasts migrate to the area of neurodestruction. These findings are a compelling argument for further research to develop new treatments by enhancing endogenous neurogenesis in brain injury. We found that intranasal administration of mesenchymal stem cells (MSC) in the acute period after occlusion of the common carotid arteries is accompanied by a more rapid restoration of the approximate motor activity in experimental animals. This process was accompanied by an increase in the number of MSC in olfactory bulb of rats. MSC also partially increased the level of NO in olfactory bulb, reduced after brain stroke

P629

Evaluation of the cationic solid lipid nanoparticles carrying siRNA against EphA2 receptor as non-viral delivery systems

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For gene delivery purposes; cost-efficient, easily producible & scalable non-viral delivery systems are considered as an

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alternative to viral vectors, which may have high immunogenicity and severe side effects. One such system for non-viral delivery is cationic solid lipid nanoparticle (cSLN), which has been used for gene silencing due to numerous advantages such as low toxicity, long stability, enhanced permeability and retention effect and co-delivery of oligonucleotides with lipophilic drugs. Here, we prepared two cSLNs (designated cSLN-Db and cSLN-Dt containing DDAB and DOTMA cationic lipids, respectively) by a hot microemulsion method for siRNA delivery. We characterized these cSLNs in terms of physicochemical properties i.e. complexation with siRNA, nuclease protection, stability and morphology. Subsequently, we evaluated the transfection efficiency, cytotoxicity and gene silencing efficiency of these cSLN:siRNA complexes in PC-3 and DU145 prostate cancer cells in vitro. In both cell lines, the transfection efficiency of cSLN-Db was higher than cSLN-Dt as assayed by green fluorescence positivity using flow cytometry supported by fluorescence microscopy. The transfection efficiency of cSLN-Db was equivalent (in DU145) and even superior (in PC-3) to commercial transfection reagent Dharmafect-2. We observed that cSLN-Db:siEphA2 complex, targeting the EphA2 receptor, successfully silenced EphA2 at both mRNA and protein levels in PC-3, but not in DU145. However, silencing EphA2 didn't make a significant change in cell viability. To our knowledge, this is the first study investigating cSLNs for siEphA2 delivery. Further studies of cSLN-Db:siEphA2 complex are warranted as it may provide an alternative to the clinical drug candidate, siEphA2-loaded liposome (EPHARNA).

P630

Identification of cellular targets for DNA delivery from recombinant L. lactis strains *in vivo*

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Use of bacteria as a carrier for oral delivery of plasmid DNA encoding a vaccine antigen has been studied extensively. Food-grade Lactococcus lactis (L. lactis) is one of such carrier candidates. However, low levels of gene expression from a eukaryotic promoter in available L. lactis vectors limited induction of immune responses against antigens encoded by the vectors. Furthermore, the mechanisms of plasmid DNA transfer from L. lactis to host cells remain unclear. In this study, to increase the gene expression efficiency, we developed a novel expression plasmid, pLEC, by cloning replication region (repD/E) and a selection marker (erm) into a eukaryotic expression plasmid pFN21, which has CMV promoter/enhancer, intron, and SV40 poly A signal. Luciferase gene expression in Caco-2 co-cultured with L. lactis-carrying pLEC: Nanoluc, pLEC-encording Nanoluc, was greatly increased as compared with that of L. lactis-carrying pCMV253:Nanoluc, a plasmid we used previously, suggesting that pLEC is a promising plasmid for the L. lactis DNA delivery system. Then, we investigated the cellular target for L. lactis DNA delivery in vivo. Immunohistochemical analyses of the small intestines from mice orally administrated with L. lactis-carrying pLEC:EGFP demonstrated the presence of GFP-positive cells in the lamina propria and the sub-epithelial dome of Peyer's patches, suggesting that dendritic cells, macrophages, T cells, B cells, or stromal cells received pLEC:EGFP from L. lactis. Cell surface marker analysis of the GFP-positive cells is under way. Our results will be helpful for clarifying the DNA delivery mechanisms of L. lactis in vivo.

P631

Novel non-toxic polymers for overcoming the endosomal barrier

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The capability to escape the endosome is crucial for the delivery of any therapeutics. Cationic delivery agents can be highly efficient at overcoming the endosomal barrier but often show severe toxic side effects. Because of their physicochemical properties, they are prone to interact with cell culture media components, especially serum albumin, which may inhibit their delivery efficiency under serum-containing conditions. Here, a series of novel pH-responsive polyacrylamides with systematically varied pendant-group structure was studied to identify the correlations between basicity and delivery of model compounds, including small molecules, macromolecules and biologicals, into the cytosol. The homopolymers, synthesised in a straightforward procedure, exhibit good compatibility with human erythrocytes and very low cytotoxicity towards different cell lines. The release of fluorescent models probes, such as calcein and labelled dextran (70 kDa), from endosomes into the cytosol was analysed by confocal laser scanning microscopy and quantified by flow cytometry. The delivery efficiency was found to correlate with the degree of protonation of the polymers at endosomal pH. Interestingly, the presence of serum in the cell culture medium seems not to inhibit cellular uptake and release of fluorescent probes from endosomes. In summary, we designed and characterised a new class of hydrophilic homopolymers that appear to be non-toxic for different cell lines but are capable of facilitating endosomal escape in vitro.

P632

Fusion-dependent formation of lipid nanoparticles containing macromolecular payloads

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The success of Onpattro™ (patisiran) clearly demonstrates the utility of lipid nanoparticle (LNP) systems for enabling gene therapies. These systems are composed of ionizable cationic lipids, phospholipid, cholesterol, and polyethylene glycol (PEG)-lipids, and are produced through rapid-mixing of an ethanolic- lipid solution with an acidic aqueous solution followed by dialysis into neutralizing buffer. A detailed understanding of the mechanism of LNP formation

is crucial to improving LNP design. Here we use cryogenic transmission electron microscopy and fluorescence techniques to further demonstrate that LNP are formed through the fusion of precursor, pH-sensitive liposomes into large electron-dense core structures as the pH is neutralized. Next, we show that the fusion process is limited by the accumulation of PEGlipid on the emerging particle. Finally, we show that the fusion-dependent mechanism of formation also applies to LNP containing macromolecular payloads including mRNA, DNA vectors, and gold nanoparticles.

P633

Optimization of a nanoparticle gene delivery system for intravesicular delivery of DNA to bladder mucosa

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Many bladder diseases could benefit from local delivery of therapeutic proteins. Recent success of intravesicularly administered viral-based gene therapy in BCG-refractory superficial bladder cancer (SBC) demonstrates the therapeutic potential of localized gene therapy with immune-modulating agents. However, clinical use of viral vectors is limited by viral immunogenicity, which could diminish efficacy after repeated dosing; complexity and cost of vector production; and logistical complications associated with clinical implementation (e.g. biosafety containment and cold-chain storage). Our aim was to develop a safe and efficient non-viral vector platform for the bladder to overcome these limitations. Our dually-derived oligomeric chitosan (DDX)-based DNA polyplex nanoparticle is designed for gene transfer to mucosal tissues. Formulation optimization for bladder epithelium was done by generating a DDX library comprising various levels of conjugated arginine and hydrophilic polyol and formulating with a DNA plasmid. Transfection efficiency of these formulations in cultured bladder epithelial cells was assessed and ranked. Top performing formulations were confirmed for in vivo activity by intravesicular administration to mouse bladder. Selected polyplex formulations were further modified by adsorption of polyethylene glycol–polyglutamic acid block copolymers to the nanoparticle surface for colloidal stability in urine and improved in vivo potency. Formulations were dried to a stable powder-in-bottle form that is rehydrated prior to administration. We also screened a panel of plasmid backbones containing different promoters and enhancers in vitro and in vivo to identify a strong expression construct for bladder epithelium. In conclusion, we have developed a robust gene delivery system for bladder epithelium ready for preclinical characterization.

P634

Cytokine profile and immunophenotype of cytochalasin B induced membrane vesicles

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Almost all human cells release micro- and nanostructures named extracellular vesicles (EVs) in extracellular environment. EVs transfer mRNA, miRNA, siRNA, mtDNA, DNA, transcription factors, chemokines, cytokines, and growth factors from parental cells to recipient cells. It was found that stem cell-derived EVs retain specific surface markers and molecular composition of parental cells. EVs mediate beneficial effect of parental MSCs and demonstrate ability to stimulate cell proliferation, improve viability and chemotaxis of recipient cells. Since EVs deliver biologically active molecules to target cells and might be a perspective therapeutic tool. Therefore, here we compared immunophenotype and molecular composition of cytochalasin B-induced membrane vesicles (CIMVs) and parental MSCs. The reported study was founded by RSF according to the research project № 18-75-00090. Cytochalasin B blocks polymerization of actin microfilaments of the cytoskeleton that leads to release of large numbers of MVs from cytoplasmic membrane. Flow cytometry analysis revealed that MSC-derived CIMVs (from human/rat/mouse) carry human MSC specific receptors (CD90, CD29, CD44, CD73), rat MSC specific receptors (CD90.1, CD49e, CD29) and mouse MSC specific receptors (CD90.2, CD44, CD49e) respectively. Multiplex analysis confirmed the presence of all studied growth factors (EGF, FGF-2, VEGF), cytokines (G-CSF, GM-CSF, Flt-3L) and chemokines (IP-10, MCP-1) in MSCs derived CIMVs and in parental cells (MSCs from rat and mouse). We found that the immunophenotype and molecular composition of CIMVs similar to MSCs. Therefore, CIMVs are attractive instrument of cell-free therapy.

P635

mRNA synthesis reagents and manufacturing: Research through clinical development

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Advances in messenger RNA (mRNA) technology bring new possibilities for the treatment and prevention of diseases. As a relatively new therapeutic modality, mRNA offers advantages: It can instruct the ribosome to make almost any protein, it functions without entering the cell nucleus, and it degrades without leaving behind unwanted or harmful genetic traces. The clinical use of mRNA was initially limited due to low efficacy and undesirable immunogenicity. These issues have been addressed through optimization and modification of mRNA elements such as the 5' cap, nucleoside structures, poly (A) tail and 5'/3' UTRs. As a result, increasing demand for clinical use is straining manufacturing capacity. To reduce supply chain risk, minimize lead time and ensure the highest quality for our clients, Aldevron manufactures the linear plasmid DNA template and recombinant enzymes used for mRNA synthesis performed by in vitro transcription (IVT). The production of mRNA at Aldevron can begin with the electronic construct sequence or supercoiled plasmid DNA provided by the client. During mRNA synthesis, Cap1 and poly (A) tail structures are added enzymatically or alternatively co-transcriptionally using a cap analog and template-encoded poly (A) tail sequence. Modified bases can also be incorporated dependent on project requirements. Purification is accomplished by column chromatography. A robust process was established by rigorous development. mRNA production is available in a range of quality grades from research grade to GMP and the process is identical between the service grades, allowing for a seamless transition from research applications to the clinic.

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P636

Non-viral *in vivo* mRNA delivery for cancer research, vaccination or gene therapy

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A major challenge to the use of nucleic acids in both Vaccination and Gene therapy is its efficient in vivo delivery to the targeted cells or site of action. Delivery of mRNA in vivo is even more challenging, because mRNA is less stable, and can illicit an immune. Therefore, we developed a non-viral delivery reagent that efficiently encapsulates mRNA to i) protect mRNA from degradation and ii) to prevent triggering of an unwanted immune response. Here we demonstrate that our cationic reagent can be used to directly inject mRNA via local and systemic administration routes to efficiently reach a wide range of tissues (including spleen and lymph nodes). Furthermore, the transient nature of mRNA transfection can be beneficial for a number of other applications, including cellular reprogramming, genome editing (CRISPR/Cas9) and vaccines.

P637

Validation of a relative standard curve RT-qPCR method for the measurement of a therapeutic microRNA in plasma and tissue samples

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MicroRNAs (miRNAs) are small non-coding RNAs that have been identified as key endogenous biomolecules that are able to regulate gene expression at the post-transcriptional level. Misfunction or abnormal expression of miRNAs have been linked to the occurrence or development of various human diseases, including cancers. In recent years, therapeutic treatments focusing on miRNAs as targets have emerged and entered pre-clinical and clinical stage. As with all pharmaceuticals, the safety of these miRNA-targeted therapeutics must be assessed. The safety assessment for biologics includes among others, testing for biodistribution and persistence prior to any clinical testing. The data presented discusses the validation of a RT-qPCR standard curve method for the measurement of a therapeutic miRNA. The method was applied to PK assessment in plasma and biodistribution tissue samples, in a GLP environment to allow its use in pre-clinical and clinical trials.

P638

A fusion peptide with an LPS-binding peptide and a RAGE-binding peptide as a carrier of plasmid DNA with anti-inflammatory effects

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Acute lung injury (ALI) is an inflammatory lung disease caused by sepsis, infection, or ischemia-reperfusion. The receptor for advanced glycation end-products (RAGE) signaling pathway plays an important role in ALI and RAGE is over expressed in the lung epithelial cells of ALI. Lipopolysaccharide (LPS) is typical of pathogen-associated molecular patterns (PAMPs) that is inflammatory. In this study, the HRBP was designed as an anti-inflammatory fusion protein, based on the lipopolysaccharide (LPS)-binding domain and RAGE-binding domain of high mobility group box-1 (HMGB1). Recombinant HRBP was expressed and purified using nickel-affinity chromatography. In LPS-activated RAW264.7 macrophage cells, HRBP reduced the levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). These results imply that HRBP decreases subsequent inflammatory reaction and NF-kB activation that is one of the typical pathways of ALI. It can also imply that HRBP inhibits LPS and RAGE successfully. Due to the positive charges of HRBP, it formed stable complexes with plasmid DNAs (pDNAs). In vitro transfection into the L2 lung epithelial cells, HRBP complex with pDNA showed higher transfection efficiency than naked pDNA. Therefore, HRBP may be a gene carrier with anti-inflammatory effects and useful for the treatment of ALI.

P639

Development of nano-sized non-viral DNA delivery system for gene therapy purposes

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Gene therapy is an intracellular delivery of genomic materials into specific cells for the treatment of genetic and acquired disorders. In order to have an efficient therapeutic effect, nucleic acids should be delivered stably into the cells. Delivery sytems are essential for efficient delivery of genes. Alhouh viral sytems are widely used for gene delivery, non-viral delivery systems have received significant attention because of their favorable properties, including lack of immunogenicity, low toxicity, cost-effectiveness and potential for tissue specificity. In this study we have developed a vesicular nano-sized, non-viral DNA delivery sytems and evaluated the effect of surfactant HLB on physicochemical properties such as morphology, vesicle size, polydispersity index (PDI) and zeta potential. As a vesicular system, niosomes were prepared by thin film hydration method. Tween 80 and Span 80 were selected as non-ionic surfactants with different HLB values and cholesterol and cationic agent stearylamine were used at the same molar ratios for the preparation of both formulations. LV-RFP plasmid DNA was used as a model gene for evaluation of niosome: DNA complexes as nioplex delivery system. Although, cationic agent stearylamine was used at the same ratio in the formulations, the charges were found different for both niosomes. Tween 80 niosomes were found cationic and Span 80 niosomes were found anionic. The vesicle size of niosomes were measured 348 nm for Tween 80 niosomes and 230 nm for Span 80 niosomes. Our cationic Tween 80 niosomes can be used as non-viral DNA delivery system.

P640

Assessment of toxicity of planar shaped nanomaterials using flow cytometry

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The studying toxicity of graphene oxide (GO) is connected with a growing number of publications on its use as a drug delivery system. However, there is already some evidence of the toxicity of GO for living objects, such as mammalian cells and microorganisms. In this regard, the search for methods and agents that reduce the toxicity of GO is needed. At the same time, approaches that don't require the physical removal of excess graphene from the medium are relevant. Analysis of kaolin and graphene toxicity and apoptosis-inducing potential on the cells was carried out with the flow cytometry method using BD FACS (USA) instrument. The mammalian cells were stained with Dead Cell Apoptosis Kit with Annexin V-FITC and PI. During the analysis, the number of annexin positive cells and PI positive cells was determined. We have shown that GO induces apoptosis in rat skin fibroblasts. Natural nanoclay kaolin didn't exert a toxic effect on cells in the concentration under study. Also, kaolin reduced the toxic effect of graphene on eukaryotic cells. Unique properties of graphene-based materials promise their versatile practical applications, including that in biomedicine. This work is performed according to the Russian Government Program of Competitive Growth of KFU and funded by the subsidy (project 6.7743.2017/6.7) allocated to the KFU, RFBR № 18-53-80067 and the government of the RT grant 18-44-160001. As the use of GO as a vehicle for drug delivery is restrained by its toxicity, combining it with kaolin could help to resolve this problem.

P641

Magnetically modified halloysite nanotubes - nanocontainers for drugs

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Nanotubes of halloysite mineral are non-toxic and biocompatible nanomaterials. Our assessment of the toxicity of this material on a well-known unicellular test object, a microorganism (S. cerevisiae yeast) showed that 90% of the cells retain viability and enzymatic activity. Non-toxic halloysite nanotubes are widely used in medicine, cosmetology, and other areas. Our laboratory is conducting researches on the use of halloysite nanotubes in biological and medical fields. The works are performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and were funded by RFBR according to the research projects № 18-29-11031 and № 18-53-80067. The structure of halloysite nanotubes allows loading them with various medicinal compounds. Containers based on halloysite nanotubes prolonged (long-term) release the drug and have an effective therapeutic effect of the substance on the target cells. Halloysite nanotubes in the natural (native) state do not possess paramagnetic properties. Therefore, in our work, we have given the artificial paramagnetic properties for halloysite nanotubes by the formation of a layer of magnetite on the outer surface of the tubes. Further, nanomaterials with artificial paramagnetic properties by a powerful external magnetic field are delivered and concentrated at the target, for example, around cancer cells. The loading of halloysite nanotubes with medicinal compounds and targeted delivery to cells will be an effective tool in the fight against cancer, as it will provide an acute and prolonged therapeutic effect on target cells.

P642

A COST network to improve the delivery of antisense RNA therapeutics

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A big hurdle for the efficacy of antisense oligonucleotides (ASOs) is their deficient delivery to target tissues but very little is known about the mechanisms by which ASOs are taken up by different tissues and specific cells. The ASO field is fragmented, with researchers in academia and industry working in isolation on specific diseases, focusing on therapeutic effects in target tissues. The European Cooperation in Science and Technology (COST) Association is the longest-running framework supporting trans-national cooperation (www.cost.eu). Approved COST Actions are not restricted to original applicants: they are open to anyone with a legitimate interest to join. Funding is used for workshops, training schools, short scientific missions, and dissemination. We represent a COST Action (Delivery of Antisense RNA ThERapeutics-DARTER, www.antisenserna.eu) that aims to network in the field of nucleic acid therapy delivery to allow RNA-targeting nucleic acid drugs to reach their full potential and become a mainstream therapeutic option. DARTER will act until October 2022 through three working groups with research objectives (delivery strategies, model systems, safety and toxicology) and stakeholder communication working group. The objective is achieving consensus on protocols for assessment of ASO delivery and toxicology, and training new researchers within a cooperative research framework. In addition, we will have a particular focus on sharing negative results: a session on negative results will be included in each workshop and training school. This new network is currently comprised of more than 240 researchers from 27 European countries, plus USA, Canada, and China, and is open to new collaborators.

P643

Selective DNA-damaging activity of curcumin@halloysite nanoformulation

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Chemotherapy is the main method for treatment of malignant neoplasms. The design and fabrication of new drugs which selectively affect tumor cells is essential for the effective therapeutic programs. We analyzed the effects of the natural compound curcumin on cancer and non-malignant cells. The method of DNA-comets is one of the generally accepted techniques for studying of DNA damage and genotoxicity of chemical compounds. Migration of the chromosomal DNA of the cell to the anode from the site of its lysis reflects the degree of DNA damage. We used halloysite nanotubes as nanocontainers for curcumin delivery into cells. Curcumin was loaded into nanotubes using standard protocol. Human skin fibroblasts and human lung carcinoma cells A549 were used as model cells. We have observed the DNA damaging properties of curcumin, and, that is characteristic, curcumin possessed selective activity.

Effects of curcumin led to an increase in DNA-damaging activity (about 3 times) in the case of cancer cells as compared to fibroblasts. We assume that this method of curcumin delivery will help to study in detail the mechanism of its selective DNA damaging effect. The work was done at the expense of subsidies allocated as part of the state support of Kazan (Volga Region) Federal University in order to increase its competitiveness among the world's leading scientific and educational centers, and through funding under the state 16.2822.2017/4.6. Also, the work was partially carried out with the financial support of the Russian Foundation for Basic Research No. 18-53-80067.

P644

A mixed micelle of His-Arg conjugated PAMAM G2 with cholesterol and Glycyrrhizic acid as gene delivery system into the lung cells

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Acute lung injury (ALI) is an inflammatory and tissue injury disease caused by various infection, sepsis, aspiration and multiple trauma leading to respiratory failure. Nowadays, gene therapy is an approach to treat diseases related to ALI. Polyamidoamine (PAMAM) dendrimer surfaces have a positive charge that would be reasonable to expect charge-interactions with the anionic phosphate backbone of DNA. Thus, PAMAM is able to be applied in gene delivery system with their biocompatibility, structural control and functionalizability. In this study, PAMAM, generation two, was conjugated with histidine, arginine and cholesterol. Histidine and arginine increased the gene delivery efficiency into the nucleus by improving endosomal escape and cellular uptake. Cholesterol helps the polymer in micelle formation. In the result, His-Arg conjugated PAMAM G2 with cholesterol (PAMAM-HR-chol) could form a complex with DNA which makes DNA transfection more effective. Glycyrrhizic acid (GA) is the major active constituent of licorice root. In addition, GA has an amphiphilic structure that is expected to form mixed micelle with PAMAM-HR-chol naturally and the pharmacological effects of GA are anti-inflammatory, anti-allergic, and antioxidant. Gel retardation study showed that PAMAM-HR-chol/GA/pDNA formed stable complexes. Moreover, transfection efficiency of this complex was higher than PAMAM-HR-chol/pDNA and PEI25K/pDNA complexes and has less cytotoxicity in L2 cell. Therefore, PAMAM-HR-chol and GA complex with plasmid DNA (pDNA) were used as gene delivery carrier with anti-inflammatory effect that would be effective in gene therapy for ALI diseases.

P645

Effect on the electrical characteristics of spontaneously active neurons of the visceral ganglion of the nervous system of the mollusk Helix lucorum

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Effective methods of treatment of spinal cord injury today do not exist. This is due to the fact that medicines, when administered, face certain problems. Therefore, there is considerable interest in the use of polymer systems as drug delivery systems, in particular methylprednisolone in the spinal cord in trauma. The aim of the work was to assess the effect on the electrical characteristics of spontaneously active neurons of the visceral ganglion of the nervous system of the mollusk Helix lucorum. This work was supported by a grant from the RFBR 18-315-00267. It was shown that under the influence of methylprednisolone, a decrease in the excitability of neurons occurs, accompanied by the activation of Na / K-ATPase. Perhaps, in our case, a decrease in excitability occurred not due to membrane mechanisms, since the membrane is depolarized and does not prevent excitation, but due to the influence of methylprednisolone on intracellular processes. When exposed to the isolated nervous system in a solution containing the L6 micellar complex and methylprednisolone, the threshold for generation of the action potential was significantly increased 30 minutes after the start of the exposure. However, the frequency of generation of neuron action potentials was reduced. It is possible that in the composition of the micellar complex, methylprednisolone enters the neuron and reduces the excitability of the nerve cell by acting on the excitation processes inside the cell, changing the threshold of the action potential, without adverse effects on the state of the neuron membrane.

P646

Encapsulation of E. coli bacteria cells with polyelectrolytes as a method of obtaining microcapsules for targeted drug delivery

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Coating of bacteria using layer-by-layer application of polyelectrolytes provides nanoscale capsules. Polyelectrolyte shells, obtained after the destruction of bacteria, are characterized by catalytic or affinity properties, stability, permeability, compatibility, and controlled release of the internal material from the capsule. The effectiveness of the layer-by-layer deposition of polyelectrolytes was evaluated by zeta potential measurements using a Zetasizer Nano ZS instrument. The work was performed according to the Russian Government Program of Competitive Growth of Kazan Federal University, funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (project 16.2822.2017/4.6) and by RFBR according to the research project № 18-34-20126. Usually, PAH / PSS coatings are used as polyelectrolyte shells. In this study, E. coli cells were encapsulated using PDADMAC / PAA polyelectrolytes. It was found that, depending on the polyelectrolyte, the zeta potential of E. coli cells changed. With a PDADMAC coating, the zeta potential was 19 ± 1.20 mV, with a PAA coating it was -33 ± 0.81 mV, thereby confirming the effectiveness of the coating. Such nanocontainers can be used as a means of delivering DNA and proteins into cells for vaccine production and gene therapy.

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P647

Evaluation of methylprednisolone delivery to nerve tissue using polymer conjugates in spinal cord injury in rats

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Currently, methylprednisolone (MP) is the only official drug with neuroprotective effect, which is recommended for use in the clinic after spinal cord injury. However, the improper distribution and unstable molecular structure of the MP leads to side effects. Therefore, there is interest in the using polymer systems as the drug delivery systems. The aim of the work was to assess the effectiveness of MP delivery to the nerve tissue using polymer conjugates in the acute phase of spinal cord injury after contusion in rats. The work was funded the subsidy allocated to by Kazan Federal University for the state assignment in the sphere of scientific activities №17.9783.2017/8.9. The micellar composition, consisted of methylprednisolone succinate (MPS) and a chemically modified copolymer ethylene oxide and propylene oxide, was elaborated as a polymer delivery system. Transport and hydrolysis of MP were analyzed using tandem mass spectrometry, which showed that using the micellar form of MPS, MP concentration is proportional to the concentration of intravenous infusion. This targeting ability prevents the drug accumulation in non-target tissue and minimizes the side effects. Muscle responses were recorded by epidural stimulation of the spinal cord. Changes in the excitability of spinal centers when using a complex of MPS and polymer correlates with changes in the application of MP, but noted the preservation of the effect of MPS complex more than 6 hours. The results demonstrate the possibility of preserving MPS in combination with the polymer in the spinal cord tissues and expanding the therapeutic window of MP.

P648

Generation of immunologically invisible transgenic porcine pancreatic islet cell clusters after single cell engineering and islet reassembling to support xenograft survival

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Xenotransplantation of transgenic porcine pancreatic islets offers a promising alternative source to circumvent current limitations posed by the scarcity of allogeneic donors. We have investigated the feasibility to generate tissue engineered SLA silenced islet cell clusters (ICC) to decrease xenogeneic immune responses Pancreatic islets single cell suspensions were generated by enzymatic digestion of porcine ICCs. Single cells were silenced for SLA expression by lentiviral vectors encoding for Nanoluciferase as reporter gene and for short hairpin RNAs targeting beta2-microglobulin or class II transactivator, respectively. SLA transcripts were evaluated by real-time PCR and protein levels by flow cytometry and fluorescence microscopy analyses. The effect of SLA silencing was evaluated in human T and NK cell cytotoxicity assays. SLA-silenced pancreatic beta-cells were then reassembled into ICCs in stirred bioreactors. SLA class I silencing reached a level of up to 84% and class II by up to 50% on pancreatic islet cell. Silencing SLA expression did not affect cell viability and the insulin-producing beta-cell phenotype. Xenogeneic T-cell immune responses ($p < 0.05$) as well as antibody-mediated cellular-dependent immune responses ($p < 0.01$) were significantly decreased. Silencing SLA class I expression did not increase susceptibility to NK-cell cytotoxicity. In stirred bioreactors, tissue engineered islets showing the typical 3D-structure and morphology of ICC were assembled from SLA-silenced pancreatic cell suspensions. These data shows the feasibility to generate low immunogenic porcine ICC from transgenic pigs after single cell engineering and post-transduction islet reassembling that might serve as a robust alternative to allogeneic pancreatic islet cell transplantation.

P649

Relationship between oil-degrading bacteria Alcanivorax borkumensis and nematode Turbatrix aceti

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Chemical and physical techniques are more often used for cleaning up oil-contaminated habitats. However, complete degradation is mainly accomplished by oil-degrading bacteria that inhabit these environments. Nematodes can be efficient in oil degradation because of stimulating the reproduction of microorganisms in the oil-contaminated soil. The cooperation between nematodes and oil-degrading bacteria can be exploited for acceleration the processes of bioremediation. In this study, we investigated the relationship between free-living nematodes Turbatrix aceti and oil-degrading bacteria Alcanivorax borkumensis. This work is performed according to the Russian Government Program of Competitive Growth of KFU and funded by the subsidy (6.7743.2017/6.7 and MD-6655.2018.4) allocated to the KFU. Also, the study was supported by RFBR #18-34-00778mol_a. According to the results of nematode chemotaxis assay using the bacteria A. borkumensis (2.2×10^9 CFU) and Escherichia coli (2.2×10^9 CFU) as a control, we found that A. borkumensis bacteria don't repel nematodes (chemotaxis index was -0.2), and nematodes have approximately the same preference for both E. coli (52%) and A. borkumensis (48%). Nematodes were cultivated in three different media: in oil-contaminated nematode growth medium with and without bacteria and in standard medium with bacteria. Using various microscopy techniques, oil was detected along the entire length of the nematode digestive system. We found that nematodes cultured in the absence of bacterial food in oil-contaminated medium passed through all stages of development, although they had low reproductive potential. In oil-contaminated medium supplemented with oil-degrading bacteria the nematodes developed in the same way as in a standard medium with bacterial food.

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P650

Hyperspectral microscopy in the characterization of aluminosilicate halloysite nanocontainers

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Hyperspectral microscopy - an analytical tool for nanomaterials. It is a novel technology that combines hyperspectral imaging with new optics and computer software that provide fast identification of micro and nanomaterials. This method can be used to detect, identify and characterize nanomaterials. In our laboratory, hyperspectral microscopy is used to characterize nano- and micro-sized objects of inorganic nature, in the analysis of biological samples. Using this method of microscopy, we can identify and classify nanomaterials in a sample. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University, funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (project 6.7743.2017/6.7) and was funded by RFBR according to the research projects No. 18-53-80067. Natural halloysite nanotubes can be used as a container for delivering substances to target cells. Hyperspectral microscopy can help to identify and determine the site and localization of such containers in biological samples.

P655

In silico, biochemical and cell-based integrative genomics identifies precise CRISPR/Cas9 targets for human therapeutics

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For CRISPR/Cas9 to be an effective and safe therapeutic drug in the treatment of diseases that would benefit from the reduction of pathogenic proteins, it can be beneficial to induce a high rate of knockout through indel frameshifts at the on-target locus and minimize any off-target editing. We present an integrative genomics approach that combines in silico, biochemical and cell-based genome-wide off-target discovery of 13 CRISPR/Cas9 guides designed to eliminate the expression of a secreted protein. In silico prediction that allows 3 mismatches in the genome and 4 mismatches in coding DNA sequence identified 1483 loci, the biochemical discovery assay SITE-Seq discovered 225 loci, and a cell-based oligo capture assay identified 172 loci. Validation of these off-target discovery profiles was conducted through targeted off-target sequencing on all empirical loci discovered and the top 390 in silico loci. Our targeted off-target sequencing revealed that in silico-based methods had the lowest contribution to validated off-target discovery. The biochemical discovery assay SITE-Seq was the most sensitive and identified validated indels that were missed in the cell-based oligo capture assay. Therefore, an integrative genomics approach that applies in silico CRISPR guide design followed by empirical biochemical off-target discovery and validation through targeted off-target sequencing is useful to identify precise human CRISPR/Cas9 therapeutic candidates.

P656

Regulatory framework for innovation assessment in advanced therapies in Europe

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Regulation (EC) No 1394/2007 establishes a centralized marketing authorization procedure for Advanced Therapy Medicinal Products (ATMPs) when they are intended to be placed on the market or industrially prepared. However, the competent authorities of each Member State (Regulatory Medicines Agencies and/or HTA bodies) are responsible for the reimbursement of each ATMP in its territory, meaning that reimbursement models vary from country to country. Therefore, there is a lack of a harmonized, consistent and transparent evaluation procedure for ATMP pricing and reimbursement. Among the European reimbursement models, there are some key factors that influence on the final reimbursement decision. In all cases therapeutic benefit, patient's benefit and unmet medical needs are taken into consideration. Another relevant aspect, especially in the advanced therapy field, would be the innovative value of the product. In this sense, different Regulatory Agencies have taken different approaches. Here we remark Italian, German, French and Swedish approaches to their innovation assessment

P657

Galectin-3 as a NETosis mediator in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by generation of circulating autoantibodies that target multiple organs. NETosis is a cell death pathway characterized by extrusion of chromatin bound to cytosolic and granular contents called neutrophil extracellular traps (NETs). Accumulating data suggest that aberrant NET degradation play roles in the pathogenesis of SLE. Galectin-3, a β-galactoside-binding animal lectin, is involved in modulation of the neutrophil functions. Whether galectin-3 contributes to the SLE pathogenesis remains unclear. The aim of this study was to investigate if galectin-3 impacts NETosis and contributes to the pathogenesis of SLE. We used clinical samples of SLE patients and a pristane-induced lupus-like mouse model to examine the correlation of glatectin-3 with NETosis. Our results showed that SLE patients expressed higher levels of galectin-3 in the PBMCs than normal individuals. Galectin-3 was detected in NETs of human neutrophils treated with phorbol 12-myristate 13-acetate (PMA) for inducing NETosis. Treatment with lactose reduced NETosis in a dose-dependent manner. In animal studies, we found that pristane-treated C57BL/6 mice exhibited immune complex deposition and had elevated galectin-3 expression in the hemorrhagic lung. Compared to wild-type mice, galectin-3 KO mice had lower levels of proteinuria, reduced pulmonary hemorrhage, decreased inflammatory cell infiltration, and enhanced survival time following pristane treatment. While NETosis were detectable in lipopolysaccharide (LPS)-treated neutrophils of wild-type mice, they were absent in their galactin-3 KO counterparts. Taken together, our results suggest that galectin-3 may be involved in the pathogenesis of SLE through mediating NETosis.

P658

Transformation potential of CRIPSR/Cas9 based genome editing can be assessed by soft agar colony formation assay and growth in low attachment plates

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With the potential use of CRISPR/Cas9 based genome editing for the treatment of genetic human diseases, safety concerns rise, amongst others, from Cas9 mediated cleavage of DNA in undesired regions (off-target editing). Although theoretically rare, off-target cleavages could lead to malignant transformation. While extensive DNA analysis, e.g. next generation sequencing, and in vivo tumorigenicity studies have been proposed by health authorities to assess CRISPR/Cas9 safety, monitoring in vitro the transformation potential of gene editing may offer an attractive additional set of safety information. A known hallmark of transformation is anchorage independent growth, which can be assessed by soft agar colony forming assay (SACF). Recently, a new assay based on growth in low attachment plates (GILA), has been described and suggested as a quicker alternative to a classic soft agar. Here, we use a CRISPR/Cas9 based approach to transform immortalized MCF10A cells and we compare the sensitivity and performances of GILA and a miniaturized digital SACF. As positive controls, genes whose deletion prompts transformation in MCF10A cells were chosen amongst a list of candidates previously shown to induce hyperproliferation in a CRISPR/Cas9 two-dimensional screening. We confirm PTPN12 as strong inducer of transformation in MCF10A cells, and p53 as weak inducer that requires concomitant mutations in other genes to promote transformation. A titration of sgRNAs targeting PTPN12 was used to assess limit of detection for both GILA and mini SACF, which performed similarly.

P659

Biosafety testing of cell and gene therapies; rapid methods and regulatory expectations

ABSTRACT WITHDRAWN

P660

Public Healthcare System as key player in leveraging advanced therapy supply chains

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In the last years, we have witnessed a considerable R&D+i growth in the field of Advanced Therapies Medicinal Products (ATMPs): cell therapy, gene therapy and tissue engineering. Many global leader companies and public research institutions have been investing large amounts of money in what it is considered a highly profitable global market, with prominent commercial opportunities for the treatment of a wide range of diseases. Up to now, a total of 14 ATMPs have been approved by the European Medicines Agency. However, this so-called revolution of healthcare is not a path of roses. Marketed European ATMPs have faced several difficulties reaching the commercialization approval and afterwards; all those approved from the first marketed ATMP in 2009–2013 have been withdrawn. In this regard, the selected business models of these ATMPs have proved to be unrealistic exploitation plans, mostly due to poor supply chain & reimbursement strategies and, subsequently, unsuccessful patient access, which has led to a lack of reimbursement options and total economic failures for the industry. In addition, the number of authorized ATMPs is expected to grow, which could entail a significant burden for Public Healthcare Systems (PHSs). Taking as a paradigm the Spanish Model for Organ Transplantation, we propose here a potential supply chain strategy for commercial ATMPs by harnessing the established operational and administrative structures within PHSs and exploring new public-private partnership opportunities with the industry. Thus, a sustainable approach is envisaged by sharing expertise and resources in order to achieve a cost-effective, patient-centered objective.

P661

Hurdles of environmental risk assessment procedures for advanced therapies medicinal products

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In order to be commercially approved, advanced therapy medicinal products (ATMPs) that are also genetically modified

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organisms (GMOs) need to undergo an environmental risk assessment (ERA), which consists in an analysis of the risks to human health and the environment that the medicinal product may cause due to its release during clinical development or after entering the market. This work aims to review the regulatory issues that need to be taken into consideration for carrying out an ERA procedure, comparing the EU and US. The European regulatory framework for environmental procedures and the dissimilarities in its implementation across the Member States are analysed in more detail, comprising also the implications at a logistical level. In addition, this paper provides a brief insight into the non-clinical and clinical assessments that should be carried out during the development of the product in order to conduct a proper ERA, and thus facilitate its marketing authorisation and post-marketing monitoring. Finally, the need for a European harmonization regarding environmental procedures for ATMPs is discussed.

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P662

ATMPs at the transition from nonclinical to clinical stage: How to smartly mitigate risk and uncertainties

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1: Biopharma Excellence

The transition from nonclinical to clinical stage is a key step in drug development which is associated with risks and uncertainties. For Advanced Therapy Medicinal Products (ATMPs) this step is particularly challenging as nonclinical animal models are often of limited relevance or not available at all. Furthermore, risks and uncertainties arise from the manufacturing process, the surgical procedures associated with the cell collection or the method of administration. Knowing the risk means controlling the risk. We have successfully developed science-driven, tailor made risk mitigation strategies for different first in class ATMPs progressing from nonclinical to clinical stage. Based on real-life case studies, this contribution will address how risk mitigation strategies can be established to smoothly progress to the clinical stage.

P663

Streamlined high performance extraction and quantitation of host cell residual DNA

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1: Thermo Fisher Scientific

Here we report on high performance Host Cell Residual DNA extraction and quantitation using a streamlined and high throughput semi-automated workflow. Sample preparation and extraction is achieved in under two hours using a sample preparation kit based on functionalized magnetic beads and a semi-automated, magnetic particle processing instrument. Sample quantitation is carried out on a real time PCR instrument. Recoveries of greater than 85% are obtained from a standard spiked solution with CV% less than 10% for Chinese Hamster Ovary (CHO) host cell DNA. Excellent linearity is observed for concentration ranges 3 pg/mL to 300,000 pg/mL of CHO residual DNA.

P664

Preserve and recreate: manufacturing and quality development for tissue, gene and cell therapy products

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1: Biopharma Excellence

Cell, gene and tissue engineered therapeutics, collectively known as ATMPs, are highly complex biological entities utilized for patient needs. The versatility of their biological structure has to be supported in vitro by an adequate manufacturing process, carefully designed to maintain clinically relevant biological functions of the final drug. In this respect, ATMP development probably comes closest to the paradigm ''the product is the process''. Fast entry of early ATMP prototypes in the proof-of-concept studies and the use of accelerated regulatory pathways do not allow much head start for the development of optimized manufacturing strategies. Due to the relatively young age of ATMPs in the biopharmaceutical field, not many references are available for the analysis of the specific aspects of ATMP development. What is important to consider early on to successfully navigate the ATMP quality toward patient and regulatory needs? The specifics of ATMP manufacturing processes and quality aspects are analysed in the view of optimal utilization of the classical tools of the development life cycle, such as establishment of critical process parameters, technology transfer and process validation. Key characteristics of biological starting materials and their influence on setting up a robust manufacturing process are discussed. Furthermore, it is analysed how to integrate the variability of starting material into the process validation approach.

P665

Regulatory and nonclinical considerations for translation of gene therapy medicinal products to first-in-human clinical trials

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Gene therapy medicinal products represent a major class of innovative therapies with a high level of structural and mechanistic complexity that substantially differs from most traditional medicinal products. Because of their unique characteristics and associated risks or safety concerns, the regulatory considerations and the nonclinical studies required to support first-in-human clinical trials may represent a bottleneck for development, slowing progress toward early clinical development. Thus, developing a strategic regulatory and nonclinical plan addressing the issues related to the path to the clinic can facilitate translation as well as being attractive for investors. Critical nonclinical issues include the availability of relevant animal models, the design of proof of concept studies, the inclusion of juvenile animals, the suitability of the toxicology program or other safety aspects and represent important scientific challenges. In addition, a clear and optimal regulatory strategy can help optimise product development, improve success, reduce costs and ultimately speed up access to clinical trials and marketing authorisation. Finding the right balance between the timing of data

availability, regulatory requirements and strategic needs for regulatory processes including paediatric investigation plan, scientific advice, or orphan drug designation can have important consequences. Thus, a clear definition of the product and a strategic regulatory development program can save time and avoid unnecessary studies and expenses.

P666

A rapid alternative to culture based mycoplasma detection

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1: Thermo Fisher Scientific

Per regulatory requirements, cell-culture based therapies must be free of mycoplasma. Manufacturers have traditionally outsourced testing to labs that specialize in the 28-day culture-based test method. For manufacturers of gene and cell therapy products, as well as other low-dose and short shelf-life therapeutics, it is not feasible to wait 28 days for test results. Thus, the need for rapid mycoplasma test results has also increased. Real-time PCR based assays provide a viable alternative to the culture based method and provide results in hours while meeting the required sensitivity. Following validation, regulatory filing and review, users across multiple therapeutic modalities have received regulatory acceptance for use of a rapid, real-time PCR based mycoplasma detection assay.

P667

Scalability strategies for cell manufacturing

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Cell-based therapeutic products are being consolidated as a conventional treatment, as highlighted by the recent rise of phase I-III clinical trials, its incorporation into healthcare systems and the increase in commercialization. The AND&TAT coordinates a network of GMP laboratories, belonging to the Andalusian Public Health System, which make it possible to offer these innovative therapies to the population. Currently, most ATMPs used are being manufactured using manual methods (flasks or cell-factories). These time-consuming methodologies, require highly-qualified human resources, imply a scalability bottleneck and will not be able to accommodate future needs. We have evaluated the different available technologies to prepare the System for the expected increase in cell production demand. Automated 2D planar technologies, comparable to 2D-manual methods, could be design with limited surface, appropriate to autologous treatment, or in multiple size modules, being the easier transferring option. Packed-bed system represents the most optimized space alternative due to 3D cell distribution, favoring niche environment but, affecting harvesting efficiency and cell potency. Microcarrier suspension culture platforms require a thorough process of optimization in each scale-up step; moreover, aggregation, shear stress and detachment are critical in those devices. In conclusion, given the heterogeneity of cell types, doses, and disease indications, there is not a ''one-size-fits-all'' standardized manufacturing platform which could be viewed as a solution. Any platform scale will require an exhaustive transfer process from manual manufacturing protocol to automation, and should be chosen according to the specific medicinal product requirements and individual needs.

P668

Nontrivial properties of small heat shock protein IbpA from Acholeplasma laidlawii

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Small heat shock proteins (sHSP) are ubiquitous molecular chaperones preventing the irreversible denaturation of proteins. In the genome of only one free-living Mycoplasma Acholeplasma laidlawii only one gene encoding the protein α-sHSP AlIbpA is present. This work is devoted to the study of the role of N - and C-termini of AlIbpA in oligomerization and chaperone-like activity of protein. We show that, regardless of temperature, AlIbpA forms a heterogeneous mixture of 24-dimensional globules, fibrils and huge protein aggregates. Removal of either 12 or 25 N-terminal amino acids leads to the formation of fibrils and increases the protein's ability to prevent temperature aggregation of insulin, indicating that the N- termini is not involved in chaperone activity, while responsible for the formation of globules. Since E. coli IbpB inhibits the formation of fibrils by IbpA, we assume that the N-terminus AlIbpA behaves as an autoinhibitor and the regulator activity of C-terminus, which complements the lack of IbpB. In turn, the removal of the C- terminus or replacing its LEL motif by SEP disrupts the temperature stability of AlIbpA and completely eliminates chaperone function, while the protein still remains predominantly in the globular state and is able to bind to insulin. Taken together, the data demonstrate nontrivial properties of AlIbpA, where competition between the N - and C-termini for interaction with the α-crystalline domain regulates the transition to a fibrillar or globular forms representing the molecular mechanism of regulation of AlIbpA activity. Research was funded by the Russian Science Foundation (project No. 17-74-20065).

P669

Developing a standard operating procedure for clinical trial advanced therapy investigational medicinal product administration; a single centre experience

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Background: University Collage London's Institute of Child Health in collaboration with Great Ormond Street Hospital (UCL GOS ICH) has delivered autologous Retroviral (RV) and more recently Lentiviral (LV) Gene Modified CD34+ cells for over 15 years. During this time clinical practices have developed and changed and trials have become increasingly complex with the additional layer of commercialisation. In the last five years the UCL GOS ICH gene therapy trials programme

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has rapidly expanded and the amount of advanced therapy investigational medicinal product (ATIMP) delivery has also thus increased. Our team has gained extensive knowledge which we have used this to develop a standard operating procedure (SOP) to standardise the delivery of ATIMPs. During the last 15 years, the team have encountered challenges including changing protocols, collaboration with commercial companies, damaged product bags and multiple good clinical practice audits.

Methods: Our team of three nurses documented every step of the process multiple times, then a different nurse audited and monitored the process multiple times to ensure the SOP reflected current practice.

Results: Various Corrective and Preventative Actions (CAPA) and audit findings have been utilised to facilitate change and used to develop this SOP that will effectively standardise and safely deliver our Gene Therapy autologous Lentiviral Gene Modified CD34+ ATIMPs to the patients.

Conclusions: The authors have developed an SOP that standardises the safe delivery of ATIMPs in the clinical area. It details receipt of ATIMP in the clinical area to the safe administration and subsequent documentation of the administration.

P670

Role of Mitochondria-Associated Membranes in recombinant AAV mitochondrial integration and trafficking

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(r)AAV-1 has been shown to integrate within the mitochondrial genome (mtDNA) in human and mouse skeletal muscle upon intramuscular administration. Recently, the study of the capsid interactome has also shown that the rAAV interacts with proteins within the external and internal mitochondrial membrane. However, the mechanism of rAAV mitochondrial trafficking and integration still remains unexplored. As the mitochondria-associated membranes (MAM) represent the connection sites between the endoplasmatic reticulum and the mitochondria, we explored if they could play a role and we determined whether increased numbers of MAM correlated with higher rAAV mitochondrial integration frequencies. To this aim, we first generated stably transfected HEK293T cells overexpressing proteins known to affect the number of MAM (cyclophilin D, glucose-regulated protein 75 or mitofusin-2) by using lentiviruses encoding the proteins of interest. The cells generated were then infected with a rAAV-2 for the subsequent isolation of total- and mtDNA, followed by LAM-PCR and qPCR on both fractions. Our data show that CypD and Grp75 overexpression resulted in an increased number of mitochondrial IS (mtIS) 11 and 14 IS for CypD and Grp75 versus 8 mtIS in untreated HEK293T (data analysis of MFN2 is in progress). For normalization purposes, we determined the number of IS per mitochondrial genome (mtChr) and CypD and Grp75 yielded 2.2- and 2.8-fold raised mitochondrial integration frequencies ranging between 6.45x10-7 and 8.14x10-7 IS/mtChr. This study will allow finer evaluating the risk of gene therapy approaches targeting mitochondria-rich tissues, as well as exploring rAAV's potential for the treatment of mitochondrial disorders.

P671

Enhanced anti-inflammatory properties of human mesenchymal stromal cells by transient co-expression of CXCR4 and IL-10

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Mesenchymal stromal cells (MSCs) currently constitute the cell type that is more frequently used in advanced therapies due to their unique immunomodulatory properties. However, the therapeutic efficacy of these cells in different disease animal models has only been partially reproduced in human clinical trials. Aiming at improving the therapeutic efficacy of these cells, most probably due to a ''hit and run'' effect, we pursued to enhance their migration and anti-inflammatory properties. For this purpose, we developed a second generation of human adipose tissue derived MSCs by means of the transient expression of CXCR4 and IL10 using monocistronic and bicistronic mRNAs. Modified MSCs maintained the typical immunophenotype and differentiation capacity of unmodified MSCs. Functional in vitro assays showed an increased migration capacity and improved immunosuppression as compared to unmodified MSCs. Moreover, in vivo experiments in a LPS-induced inflamed pad mouse model demonstrated the increased anti-inflammatory potential of CXCR4-IL-10 expressing MSCs when compared to unmodified ones. In addition, a reduction in the leukocytes infiltration in inflamed pads was observed. Biodistribution assays showed an increased presence of CXCR4-IL10-MSCs in inflamed pads as well as a reduction in the number of trapped MSCs in the lungs, revealing an improvement in site-directed migration of modified MSCs. Taken together, our results demonstrate that the transient expression of homing and anti-inflammatory molecules enhances the therapeutic effect of human MSCs in a preclinical inflammatory mouse model.

P672

Long-term protective effects of adipose-derived mesenchymal stromal cell therapy in experimental colitis

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Mesenchymal stromal cells (MSCs) are multipotent cells present in numerous tissues and possess immunomodulatory and regenerative properties leading them to be used in the clinic for treatment of immune-mediated disorders. It is well-known that MSCs exert their therapeutic function through the, so called, 'hit and run' mechanisms and that their persistence in vivo is rather limited. In this study, we investigated whether MSC therapy has an impact in the long-term. To address this point, we studied the long-term effects of adipose-derived MSCs (aMSCs) in a mouse model of inflammatory bowel disease induced by dextran sulphate sodium salt (DSS). We carried out two cycles of 7-day DSS treatment, three months apart. A single dose of intraperitoneal-aMSCs was infused during the 1st 7-day DSS cycle. Colitic mice treated with aMSCs showed a significant reduction in the disease activity index and body weight loss accompanied by increased survival during the 2nd 7-day DSS cycle when compared to untreated DSS-induced colitic mice. Preserved colon morphology, reduced extension of the inflammatory areas and attenuated leukocyte infiltration with respect to the untreated colitic mice were also observed. These findings suggest that cell therapy with aMSCs can induce a stable immune response that confer sustained protection to acute inflammation in the long-term.

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P673

Transcriptomic characterization and gene therapy of balancing dysfunction in Slc26a4-defected mice

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Recessive mutations in the SLC26A4 gene are a common cause of hereditary hearing impairment in humans. To date, several mouse models with defected Slc26a4 gene have been generated. Most of them demonstrated both hearing loss and balancing dysfunction, such as circling behaviors. In contrast to the hearing phenotype that has been exhaustively investigated in these mice, pathogenetic mechanisms underlying the balancing dysfunction remain largely unexplored. In this study, we harvested the inner ear vestibular organs from a Slc26a4-defected mouse model which harbors the common c.919-2A>G mutation in the Asian patients, and performed RNA-seq analyses on the extracts. Transcriptomic characterization and pathway analyses revealed that the neuroactive ligand-receptor interaction, serotonergic synapses, and GABAnergic synapses might play a crucial role in the pathogenesis of balancing dysfunction related to Slc26a4 mutations. We then designed gene therapy for delivering the Slc26a4 gene into the mouse inner ear using the Anc80 virus. Microinjection in newborn mice demonstrated high infection efficiency of Anc80 in the vestibular hair cells, and the expression of pendrin, the protein encoded by Slc26a4, was also confirmed on immunofluorescent studies. The phenotype of balancing dysfunction in the Slc26a4-defected mice improved after gene therapy. Our results provide insights into the molecular pathology of balancing dysfunction related to SLC26A4 mutations, for which gene therapy is likely to become a treatment option in the future.

P674

Safety, feasibility and trends of efficacy of intravenous injection of autologous adipose-derived mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: A phase I-IIa multicenter randomized triple blind placebo controlled trial

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with rapidly progressive disability and gradually or abrupt progressive muscular paralysis and atrophy with a short survival of 2–5 years. Mesenchymal stem cell therapy has been proposed as a promising approach for treatment of ALS. A phase I-IIa, multicenter, controlled, triple-blind clinical trial, randomized in four groups, has been conducted. Forty subjects were randomized into one of the following arms: placebo group, 1 million MSC /kg, 2 million MSC / kg, and 4 million MSC / kg. The manufacturing process of the Investigational Medicinal Product (IMP) was carried out in the Cell Manufacturing Unit of the Regional University Hospital in Malaga (coordinated by AND&TTA). The main objective is to evaluate the safety and trends of efficacy of intravenous administration of 3 doses of autologous mesenchymal stem cells (MSC) from adipose tissue in patients with ALS. The efficacy data are currently being analyzed. Secondary outcome measures were determined by means of changes in the ALSFR-R, in forced vital capacity and in muscle bulk estimated by MRI. No related deaths occurred. A total of 12 adverse reactions were related to the infusion procedure or IMP: 8 infusion site phlebitis, 2 infusion site thrombophlebitis, and 2 deep venous thrombosis. In conclusion, prospective studies with a larger sample and longer-term follow up, are urgently required to assess the clinical benefits of cell-based therapy including improvement in disease progression and quality of life and prolongation of survival in people with ALS.

P675

Bone marrow mesenchymal stem cells accelerate wound healing after hernia repair with polypropylene surgical meshes

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Polypropylene (PP) surgical meshes are commonly used for tissue reinforcement in abdominal hernias, being associated to fibrosis, infections and pain. Here we hypothesized that stem cells could help to reduce these adverse effects when combined with surgical meshes, due to their immunomodulatory potential,. For this study, a swine congenital abdominal hernia model was used. PP meshes were laparoscopically implanted in combination with fibrin glue-vehicled porcine bone marrow-derived mesenchymal stem cells (MSCs) (n=5). Control group received the same treatment without MSCs (n=4). Hernia size was evaluated by ultrasonography before the procedure. After 7 and 30 days, hernia size was measured and meshes were biopsied for histological studies. Inflammatory cells, vessels, fibroblasts counts and histological scores were assessed. A statistically significant decrease of hernia size was observed at 7 days in the cell-treated group compared to day 0. Histological analysis revealed a decrease in polymorphonuclear cells, an increase of giant cell counts and small vessels growth in the cell group at all time points, compared to control group. Additionally, fibroblasts were increased at 7 days in PP meshes combined with MSCs. Therefore, our results suggest that the host response after mesh implantation was altered by MSCs, leading to a premature wound healing. Although costs-benefits still need to be balanced, stem cell therapy coupled to surgical mesh implantation may become a valuable approach for hernia repair.

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Mechanical tuning of adult hippocampal neural stem cells using light-responsive Rho GTPases dictates cell fate

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Adult neural stem cells (NSCs) within the hippocampus have a major impact on the regulation of memory and learning. We have shown that NSCs are sensitive to mechanical stimuli from the extracellular matrix for a short temporal window during differentiation (12–36hs) such that soft substrates promote neurogenesis and stiff matrices inhibit it. We previously found that small GTPases of the Rho subfamily, RhoA and Cdc42, are activated by stiff substrates and are necessary to inhibit neurogenesis. To gain precise control over RhoA/Cdc42, we engineered NSCs to express a system for optogenetic modulation of their activity. We found that a short 18-hour pulse of blue light during the first day of differentiation is sufficient to inhibit neurogenesis in cells grown on soft substrates. Using single-cell microisland arrays, we also found that a short activation pulse of RhoA/Cdc42 not only inhibits neurogenesis but also increases astrogenesis. Moreover, when NSCs were grown in neurospheres encapsulated in 3D hyaluronic-acid soft hydrogels, short activation pulses of RhoA/Cdc42 lead to reduction of neurogenesis, recapitulating the changes seen on 2D. Interestingly, we found that fate decision strongly depends on the frequency of illumination, suggesting that pulsatile information encoded in RhoA/Cdc42 activation modulates cell behavior with high temporal resolution. These results indicate that activation of intracellular tension transduction proteins RhoA/Cdc42 using optogenetics recapitulates the effects of stiff substrates on NSCs' fate. These findings will help unravel how mechanical inputs are integrated to specify NSC fate, which could aid the development of cell replacement therapies for neuroregeneration.

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De novo production of genetically engineered T cells is maintained in humans decades after loss of transplanted hematopoietic stem cells

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In our recent clonal tracking study in humans we raised the intriguing hypothesis that long-term lymphoid progenitors (lt-LP) exist capable of survival independent from hematopoietic stem cells (HSC) supply to the thymus. However, no experimental setting was available to date to prove the existence of such cells. Over a decade after retroviral gene therapy for SCID-X1, our patients have lost engraftment of all engineered blood populations (including B cells and originally infused CD34+ progenitors) except for T and NK cells. If there is no lt-LP, we would predict that only long-living mature memory lymphocytes would be surviving in these individuals. Strikingly, we have detected bona fide vector-positive naïve T cells (Tn), by nature short-living cells, many years after the apparent exhaustion of engineered HSC. We firstly performed a systematic validation of such Tn by extensive immunophenotyping (defining Tn as CD45RA+CD62L+CD95-), IFN-y functional assays, TREC measurement and vector copy number evaluation. Then, by means of longitudinal integration site (IS) tracking on sorted T-cell subpopulations we proved identical vector-marking in multiple T-cell subsets while concomitantly recapturing identical IS within true Tn isolated over a time window of 10.1 years to as long as 14.9 years after loss of engineered HSC. High-throughput TCR profiling and IS collection from NK cells are underway to assess the de novo and multi-lymphoid output of what to all evidence appear to be an active lt-LP population which have outlasted transplanted HSC. These findings could have significant implications for the development of new lymphocyte-based gene and cell therapies.

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ISwap: a bioinformatics tool for index switching detection in vector integration site studies

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Dedicated PCR protocols and next generation sequencing technologies allow to study genomic vector integration sites (IS) in present of hundreds of DNA samples simultaneously. Sequencing reads belonging to different samples can be distinguished by the presence of sample-specific nucleotide sequences (indexes) embedded in the primers used for the DNA amplification. However, during sequencing, free indexed primers may tag other unrelated samples of the pool library, resulting in the incorrect assignment of sequences. This issue is particularly relevant in clonal tracking studies, where the spreading of IS between datasets caused by index switching could result in inflated multilineage potential, number of long-term repopulating stem cells and other issues. We analysed the index switching levels in our data and found that about 8.5% of sequencing reads were false index combinations resulting from frank events of

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index swapping. Focusing on a sample from a cell line with 6 known IS (a positive control present in all sequencing runs), we calculated the swapping events and the relative abundance in other samples and identified specific molecular structures associated to swapping events. We developed probabilistic and logic algorithm that evaluate the associations between the number of unique molecular identifiers linked to each shear site and read abundance, to efficiently identify and eliminate sequences generated by indexing switching. We validated our method using experimental dataset on different sequencing runs of containing our positive control cell line obtained a precision of 0.75 and a recall of 0.95. ISwap is a computational algorithm that efficiently removes index swapping events.

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A novel Bayesian regression framework highlights dependences of clonal dynamics on different vector designs in *in vivo* gene therapy studies

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In Hematopoietic stem cell Gene Therapy (GT) treatments, vector integration sites (IS) are surrogate of clonal identity and allow the assessment of safety and efficacy by describing how the clonal population is composed and evolve during hematopoietic reconstitution. How the genotype of the treated subject and the lentiviral vector (LV) design used may affect the aforementioned readouts is still not well understood. Indeed, we retrieved IS data from different hematopoietic cell linages longitudinally from four groups of mice transplanted with tumor prone Cdkn2a-/- or wild type bone marrow-derived lineage negative cells that were transduced either with a ‘‘neutral’’ LV or with a highly genotoxic LV construct and we measured over time the Shannon Entropy index to quantify the clonal diversity. In literature, the clonal diversity has only been monitored with simple descriptive statistics. In this work, we developed a Bayesian linear regression framework to model clonal dynamics and identify the effects of the different treatments. The Bayesian framework allows us performing model averaging among the most likely candidates. Our proposed framework highlights that the vector significantly impacts the entropy over time and we observed a faster decrease in clonality moving from the neutral condition up to the most genotoxic one with a 90% predictive probability confidence. Furthermore, we are currently investigating the clonal dynamics among the different lineages within each treatment. These findings may support the development of safer and more efficient vector designs.

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Upregulation of mitotic bookmarking transcription factors may induce enhanced proliferation of human stromal cells by human platelet lysate

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Due to abundant growth factors, pooled human platelet lysate (pHPL) is an efficient human alternative for fetal bovine serum (FBS) significantly enhancing stromal cell (STC) proliferation. However, the lack of standardized production hampers the comparability of pHPL-products and the effect of pHPL-based factors on cellular behaviour is still not fully understood. In this study, three different pHPL-preparations for cultivation of STCs from three different sources (bone marrow, white adipose tissue and umbilical cord) were compared with FBS: Standard pHPL-medium, fibrinogen-depleted serum-converted pHPLS- and mechanically fibrinogen-depleted pHPL-medium. Biochemical parameters were comparable in all pHPL preparations. Immunophenotype and in vitro differentiation potential of STCs were independent of the medium used. Although a luminex multiplex assay of pHPL-based media revealed a decrease of distinct growth factors in fibrinogen-depleted pHPL, all pHPL-based culture media significantly enhanced STC propagation and colony forming capacity compared to FBS. The mRNA expression levels of transcription factors SOX2, cMYC and KLF4, also known as mitotic bookmarking factors, were significantly enhanced in STCs cultured in all pHPL- compared to FBS-media. Independent of the preparation mode, pHPL-media supported proliferation and clonogenicity of STCs while maintaining immunophenotype and in vitro differentiation potential. The mRNA expression of SOX2, cMYC and KLF4 was significantly enhanced in pHPL-cultivated STCs. As these factors may enable a quick re-entry to the cell cycle, this may explain the superiority of human platelet factors for efficient STC proliferation compared to FBS, pathing the way for new chemically defined ideal cell culture media for stromal cell based medicinal products.

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Sonication Linker Mediated-PCR (SLiM-PCR), an efficient method for quantitative retrieval of vector integration sites

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We have developed Sonication Linker mediated- PCR (SLiM-PCR), a method for quantitative Integration Site (IS) retrieval. In this method the genomic DNA is fragmented by sonication to avoid the amplification biases produced by the uneven fragmentation with restriction enzymes, then the sonicated DNA is

directly ligated to a Linker cassette containing a Unique Molecular Identifier (UMI). Amplification of vector/genome junctions is achieved by PCR with oligonucleotides complementary to vector and linker cassette sequences. Therefore, each vector IS can be quantified by counting the number of different UMI-tagged fragments containing the same vector/genome junction. We validated this method on DNA samples composed by DNA extracted from two purified cell clones carrying one and six lentiviral vector (LV) integrations in known genomic positions and DNA extracted from a polyclonal LV-marked cell population, mixed at different ratios. The levels of accuracy and reproducibility of abundance estimates of the IS of the 2 purified clones in the different dilutions closely correlated with theoretical estimations (±5%). When compared to other established techniques, SLiM-PCR showed a 5–10 fold greater efficiency in IS retrieval and a higher linear correlation between the number of retrieved and expected IS ($R2 \simeq 0.9$). SLIM-PCR was successfully applied on different vector platforms including gamma-retroviruses, LVs, Sleeping Beauty Transposons and Adeno-associated viruses. Our results show that SLiM-PCR is a powerful method for IS retrieval and accurate clonal abundance estimation and that can be applied to a broad spectrum of gene therapy platforms.

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Tissue-specific interaction of stromal cells and endothelium *in vitro* leads to a distinct pattern of angiogenic response

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Formation of new vessels during angiogenesis requires a complex of coordinated actions by endothelial cells: their migration, proliferation, formation of lumen and collaterals followed remodeling of the vascular network. At the same time angiogenesis in different tissues during wound healing proceeds differently and there is a direct correlation between angiogenic response and fibrosis. The interaction of tissue-specific stroma with endothelium cells can determine blood vessel growth, which, ultimately, determines outcome of healing. Using a model of long-term co-culture of endothelial cells with stromal cells of the skin, adipose tissue or endometrium in cell sheets (CS) that mimic the organ's stroma, a tissue-specific effect of stroma on survival, proliferation, endothelial migration and network remodeling was shown. We found that adipose tissue cells exerted a protective and stabilizing effect on formation of vascular structures formed during the migration of endothelial cells inside the CS. After interaction with stromal cells of the skin, there was a lack of endothelial cell migration and their isolation on the surface of the construct. Co-cultured with endometrial cells endothelium did not adhere and did not form any structures on the surface of the construct. Thus, using a model of co-culture of endothelium cells on the surface of the CP from stromal tissues from different tissues, we visualised all stages of angiogenesis, including retraction of the network, and the tissue-specific course of this process. Study was supported by RFBR Grant #17-04-01452 (MSC and HUVEC isolation) and RSF Grant #19-75-00067 (staining and structural analysis).

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Cell therapy with adipose-derived mesenchymal stromal cells alters the endogenous trafficking of myeloid populations *in vivo*

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Mesenchymal stem cells (MSCs) are multipotent cells that can be obtained from different tissues and nowadays, thanks to their immunomodulatory properties, are of great interest in the clinic as an alternative therapy for treatment of inflammatory-mediated disorders. It is well-known that their persistence in vivo is limited, thus suggesting that their mechanism of action involves induction of early immune responses. The aim of the present study is to define immune responses that occur soon after the infusion of adipose-derived MSCs (aMSCs) in healthy mice and under inflammation. With this purpose, MSCs were infused intraperitoneally (IP) in healthy and in dextran sulphate sodium salt (DSS)-colitic mice. Following 24 hours, healthy mice had a significant increase in Ly6G+CD11b+ granulocytic and Ly6C+CD11b+ monocytic populations in the peritoneum with a parallel decrease of both populations in peripheral blood (PB). DSS-colitic mice showed a significant increase in Ly6G+CD11b+ granulocytes both in the colon and PB, with respect to healthy mice. IP-aMSC treatment increased Ly6G+CD11b+ granulocytic and Ly6C+CD11b+ monocytic populations in the peritoneum accompanied by a decrease of Ly6G+CD11b+ granulocytes in the colon and PB. Additionally, a decreased proportion of F4/80++CD11b++ macrophages was observed in the peritoneum in aMSC-treated colitic mice with respect to non-treated colitic mice. All these results suggest that MSC treatment induces a very rapid movement of existing myeloid populations in normal as well as in DSS-inflamed mice, suggesting that very early innate responses are taking placed upon infusion of aMSCs.

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Good manufacturing production of limbal stem cells in a tissue bank for clinical application

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ABSTRACT WITHDRAWN

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Depletion of PTN inhibits the osteogenic differentiation potential of dental pulp stem cells

ABSTRACT WITHDRAWN

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Efficacy of intracoronary administration of microencapsulated hepatocyte growth factor in a reperfused myocardial infarction swine model

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Microencapsulation as a delivery system for therapeutic agents allows their minimally invasive, safe and effective administration. Our main objective was to compare the safety and effectiveness of an intracoronary (IC) administration of microencapsulated HGF, placebo microspheres, or saline in a myocardial infarction (MI) swine model. Two days after a 90min mid-LAD balloon occlusion, $5x10^6$ HGF loaded-microspheres (HGF, n = 7), $5x10^6$ placebo microspheres (BLANK, n = 7) or 10 mL of saline (CTRL, n = 7) were administered via IC in swine. Safety was determined by TIMI flow, ECG and TnI values before and after the intervention. Cardiac function was evaluated with cMR before injection and at 10 weeks (EF, %MI, EDVi, ESVi, ΔEF, ΔEDVi, ΔESVi). The inflammatory profile was determined by cytokine values on different time-points of the study. 10 weeks after treatment animals were euthanized, hearts were harvested and histopathologically studied. TIMI flow was impaired in two animals from the HGF group and three belonging to the BLANK group after administration. No TnI changes were evidenced. One animal from the BLANK group died during injection. There were no significant differences between groups in any cMR parameter. The HGF group presented the lowest values of pro-inflammatory cytokines. The histopathological study showed lower inflammation and calcification in the HGF group. The IC injection of 5x106 HGF loaded-microspheres 2 days post-MI, is generally safe but may impair coronary flow. Besides, it does not significantly improve cardiac function compared to control. Microencapsulated HGF administration appears to restrict post-MI inflammation and fibrosis, but it does not improve cardiac function.

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The inflammation mechanisms in myocardial infarction are altered after intrapericardial administration of cardiosphere-derived stem cells and their extracellular vesicles

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Myocardial infarction is associated to an inflammatory response which alters the populations of circulating immune cells and their activation. Stem cells and their soluble mediators have been considered as a therapeutic option for the treatment of this pathology. The aim of this study was to evaluate the immunomodulatory effects of Cardiosphere-derived Stem Cells (CDCs) and their extracellular vesicles (EV-CDCs) in a closed-chest model of myocardial infarction. A total of 18 Large-White pigs were randomly divided into three groups: I) Placebo (n = 6), II) CDCs (n = 6) and III) EV-CDCs (n = 6). Intrapericardial administrations were performed at 72 hours post-infarction. Peripheral blood samples were collected before and after model creation and 24 hours after administration. Leukocytes were isolated and their subpopulations were analysed by flow cytometry. The phenotypic analysis of peripheral blood lymphocytes revealed alterations on activation/differentiation status of CD4+ and CD8+ T cells after the treatments, compared to the placebo group. The results also showed an increase of monocytes and activated monocytes after EV-CDCs administration. In conclusion, both treatments promote significant changes in peripheral blood leukocyte subsets

(lymphoid and myeloid). These changes may be used as biomarkers to predict the severity of the infarction and the therapeutic effects of stem cell-based therapies in myocardial infarction. Altogether, our results suggest that intrapericardial administration of CDCs and EV-CDCs may counteract an exacerbated inflammatory response after myocardial infarction.

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Effects of early intrapericardial delivery of microvesicles obtained from heart-derived cells in cardiac function in an experimental myocardial infarction in swine

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The intrapericardial (IP) administration offers an attractive alternative route for regenerative therapy after myocardial infarction (MI). Available data suggest that the use of extracellular vesicles (EV) recapitulates the effects seen using their cells of origin. The aim of this study was to assess the effects of EV obtained from heart-derived cells or placebo administered early after experimental MI in swine. Three days after a 90-min balloon occlusion of the mid-left anterior descending coronary artery, 19 Large-White pigs were randomly allocated to blindly receive EV (quantified as 9.16 mg of total protein per dose) in 5mL saline (n = 9) or the same volume of vehicle (n = 10) via a minithoracotomy. Cardiac MR studies were performed immediately before and 10 weeks after therapy administration, and processed blindly to obtain left ventricular function parameters (Ejection fraction (EF), end diastolic (EDVi) and systolic volumes (ESVi)). No significant differences between groups were found in MR-derived cardiac function parameters at the end of the study, despite a trend towards improved cardiac function in treated animals (LVEF was 33 ± 16% vs 29 ± 7%, EDVi was 92 ± 30mL/m2 vs 98 ± 19mL/m2, ESVi was 66 ± 33mL/m2 vs 71 ± 19mL/m2 and infarct size 9 ± 4% vs 12 ± 3% in EV-treated animals compared to Control, respectively). No pericardial adhesions were evidenced in any case 10 weeks after surgery. While the IP injection of extracellular vesicles obtained from heart-derived cells is safe 3 days after experimental myocardial infarction in swine, it does not appear to have any beneficial effect on cardiac function.

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Intrapericardial administration of Cardiosphere Derived Cells early after experimental myocardial infarction in swine: safe, easy but of limited effectiveness

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The intrapericardial (IP) administration offers an attractive alternative route for regenerative therapy after myocardial infarction (MI). The aim of this study was to assess the effects of $30x10^6$ CDCs or placebo administered 72h after experimental MI in swine. Eighteen Large-White pigs surviving a 90-min balloon occlusion of the mid-left anterior descending coronary artery were randomly allocated to receive $30x10^6$ CDCs in 5mL saline (n = 8) or the same volume of vehicle (n = 8) via a mini-thoracotomy performed 72 hours after MI. Ejection fraction (EF), end diastolic (EDV) and systolic volumes (ESV) were evaluated by MR immediately before and 10 weeks after cell/vehicle injection. All procedures were blinded. The intrapericardial administration was completed successfully in all cases in a surgery time (knife-to-skin) below 30 minutes. No significant differences between groups were found in MR-derived cardiac function parameters at the end of the study, despite a trend towards improved cardiac function in treated animals (LVEF was 34 ± 10% vs 29 ± 7%, EDVi was 86 ± 21mL/m2 vs 100 ± 19mL/m2 and ESVi 59 ± 23mL/m2 vs 72 ± 20mL/m2 in CDC-treated animals compared to Control, respectively). Moreover, infarct size as a % of the left ventricle at 10 weeks was smaller in treated animals, albeit not significantly (9 ± 4% vs 12 ± 3%). No pericardial adhesions were evidenced in any case 10 weeks after surgery. The IP injection of $30x10^6$ CDCs is safe 3 days after experimental myocardial infarction in swine. While it is not significantly better than vehicle administration, the consistent trends towards better cardiac function and its excellent safety profile warrant further investigations.

P690

External quality control program in advanced therapies

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Quality controls used for the manufacture and release of Advanced Therapies Medicinal Products (ATMPs) remain at an early stage of development and, given the intrinsic nature of the specific cell-based preparations, are subject to human factor issues without technical standardization. For this reason, adopting the experience from the clinical diagnostic field, a specific External Quality Control Program has been created with the aim of harmonizing and guiding the manufacturers to develop safe and effective ATMPs. Once each 6 months, manufacturers attached to the program shall receive a rationally-prepared sample, which is virtually identical to the one that the other participants shall receive. By using their routine analytical methods, they will have to perform the quality controls each manufacturer had voluntarily subscribed and upload the results to the program website. Each one of them shall be able to download a final report, that takes into account the confidentiality of the participants, where their particular results are statistically compared to the others' results. The program guarantees the absolute anonymity of both the participants and the individual results. Manufacturers can select which quality controls they would like to perform among the following initially considered: Microbiological control of cell-based preparations (sterility test), Endotoxins, Mycoplasmas detection, Immunophenotyping, Gram and Calcofluor white staining, Thawing yield, in-house Cellular growth promotion and Karyotype. Other test could be included if required.

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A bioluminescence resonance energy transfer based sensor for the precise determination of non-homologous end joining DNA repair events

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Endonuclease based, therapeutic genome editing is a powerful strategy to treat inherited diseases. However, specificity and efficacy analysis is crucial in early development. Therefore, we generated a bioluminescence resonance energy transfer (BRET) based sensor for measuring the activity and specificity of endonucleases in inducing DNA double strand breaks (DSBs) and quantifying DNA repair through non-homologous end joining (NHEJ). The NHEJ sensor consists of a renilla reniformis luciferase (RLuc8) and a GFP2 domain with a shuttle-cloning box inserted for the integration of any given endonuclease target sequence. The luciferase as the energy donor is acting as an internal standard with which the disruption of the reading frame with premature stop codons of the GFP2 acceptor by indel formation can be normalized. This disruption results in a change of the BRET ratio, which corresponds to the endonuclease and NHEJ activity. The sensor, validated with different endonucleases and cell lines in vitro, showed an endonuclease induced reduction of the BRET ratio. The measured output is endonuclease concentration, but not NHEJ sensor concentration dependent. The sensor/endonuclease system shows outstanding specificity and was used to measure the influence of NHEJ pathway protein knockouts on NHEJ DNA repair. Furthermore, mismatch cleavage analysis for Cas9 endonucleases is possible with the sensor. The generated NHEJ sensor offers a useful tool to answer important questions regarding endonuclease activity, and NHEJ activity in a variety of different cell lines in vitro before advancing to in vivo experiments. The sensor may shorten the development of strategies to treat inherited diseases.

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Integrin α6 improves stemness, proliferation and migration in human mesenchymal stem cells

ABSTRACT WITHDRAWN

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Mesenchymal stromal cell secretome as a promising tool for male infertility treatment

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Male infertility remains a substantial medical problem worldwide. Severe disturbance of complex microenvironment maintaining spermatogonial stem cells (SSC), a stem cell niche, may lead to the lack of effective treatment. Among promising therapeutic approaches one can consider recovery and/or mimicking the SSC niche and its components by cell-based therapy. It is known that mesenchymal stromal cells (MSC) might transmit multiple local and distant cues to their microenvironment thereby coordinating homeostasis of stem cell niches. Furthermore, MSC might support stem or niche resident cells in a paracrine manner replacing the damaged components. Thus, novel insights into the role of MSC secretome in SSC niche recovery would promote development of effective therapies for male infertility. To model SSC niche injury we used a two-week model of rat bilateral abdominal cryptorchidism. After descent of testes, we locally injected a combination of human MSC secretome and collagen as a protective depo or MSC themselves to recover spermatogenesis. We observed that the functional structure of injured SSC niche was recovered after the experimental treatment. We showed increase in Sertoli cell number and restrain of Leydig cell population growth alongside with recovery of their secretory function. We further observed substantial increase in numbers of primary and secondary spermatocytes in seminiferous tubules as well as total and moving spermatozoa numbers located in epididymes. Consequently, fertility of treated animals was restored indicating that the application of either MSC or MSC secretome might be promising for male infertility treatment. The study was supported by RSF (№19-75-30007) and RFBR (№18-315-00403).

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Role play by galectin-1 in regulatory CD4+ T cell therapy for modulation of immune responses in experimental colitis

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Regulatory CD4+ T cells are essential for maintaining homeostasis, adequate immune responses and autoimmunity. Due to their capacity to modulate immune responses, adoptive transfer of regulatory CD4+ T cells has appeared as a promising tool in cell therapy protocols in order to control pathogenic immune responses and in autoimmunity. Galectin-1 (GAL1), a glycan-binding protein, has immunomodulatory properties and is expressed by different cell types including regulatory FOXP3+CD4+ T cells. To determine the role play by galectin-1 in regulatory CD4+ T cells, we used a T cell transfer in vivo model of inflammatory bowel disease (IBD) in which prophylactic and therapeutic effects of GAL1-deficient Foxp3+CD25+ regulatory CD4+ T cells (GAL1-/- Tregs) were assessed. GAL1-/- Tregs showed reduced immunomodulatory capacity compared to wild type Tregs, as suggested by the increase number of GAL1-/- Tregs required to modulate intestinal inflammation induced by FOXP3-depleted CD45Rb+CD4+ naïve T cells into Rag-1 mice. In addition, long term survival of those mice treated with GAL1-/- Tregs is compromised compared to mice treated with wild type Tregs. These evidences highlight the crucial role that Gal1 plays in regulatory CD4+ T-cell therapy. These findings bring to light that an adequate expression of galectin-1 must be taken into consideration in cell therapy protocols with regulatory CD4+ T cells in order to achieve a robust and stable protection in the long term.

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P695

Silencing of prothymosin α as a therapeutic strategy for the treatment of polycystic kidney disease

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Polycystic kidney disease (PKD) is characterized by the expansion of fluid-filled cysts in the kidney, which impairs the kidney function and eventually leads to end-stage renal failure. Despite the fact that numerous signaling pathways involved in pathogenesis of PKD have been targeted to prevent expansion of these cysts, no excellent therapeutic strategy has been applied to date. Prothymosin α (ProT) is a nuclear protein that is essential for cell survival and proliferation. We have previously demonstrated that transgenic overexpression of ProT induces the development of PKD; however, the underlying mechanisms remain unclear. In this study, we employed a mouse PKD model that sustains kidney-specific low expression of Pkd1 to study the molecular mechanism underlying the action of ProT on the development of PKD. We show that ProT was aberrantly upregulated in cyst-lining epithelial cells in PKD mice. We developed an in vitro cystogenesis model to demonstrate that lentivirus-mediated knockdown of ProT reduced cyst formation in Pkd1 knockdown MDCK cells. Furthermore, the expression of ProT was accompanied with a marked increase in protein acetylation in PKD, resulting in the activation of downstream STAT3 that is well-documented to play a pathogenic role in PKD. ProT increased global protein and STAT3 acetylation in PKD mice and patients with PKD. Moreover, knockdown of ProT in Pkd1 low-expressing cells suppressed the activation of STAT3. Collectively, these results indicate that ProT can acetylate STAT3 and thereby activate STAT3 signaling, which contributes to the pathogenesis of PKD. Therefore, ProT is a potential novel therapeutic target for PKD.

P696

Quantitative analysis of spliced X-box binding protein 1 (XBP1) using universal primer for quantitative RT-PCR

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X-box binding protein 1 (XBP1) mRNA processing plays a crucial role in the unfolded protein response (UPR), which is activated in response to endoplasmic reticulum (ER) stress. Upon accumulation of the UPR-converted XBP1 mRNA splicing from an unspliced (u) XBP1 (inactive) isoform to the spliced (s) XBP1 (active) isoform, inositol-requiring enzyme 1 α (IRE1α) removes a 26-nucleotide intron from uXBP1 mRNA. Recent studies have reported the assessment of ER stress by examining the ratio of sXBP1 to uXBP1 mRNA (s/uXBP1 ratio) via densitometric analysis of PCR bands relative to increased levels of sXBP1 to uXBP1 using a housekeeping gene for normalization. However, this measurement is visualized by gel electrophoresis, making it very difficult to quantify differences between the two XBP1 bands and complicating data interpretation. Therefore, we designed sXBP1 specific primer sets for real-time PCR. We also designed universal real-time PCR primer sets capable of amplifying a portion of each u/sXBP1 mRNA that is highly conserved in eukaryotes, including humans, monkeys, cows, pigs, and mice. Therefore, we provide a more convenient and easily approachable quantitative real-time PCR method that can be used in various research fields to assess ER stress

P697

AdrA as a potential immunomodulatory candidate for STING-mediated anti-viral therapy

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Several bacterial di-nucleotide cyclases, including cGAS, and their involvement in STING-mediated immunity have been extensively studied. Here we identified and tested AdrA, a diguanylate cyclase, derived from the gram-negative bacterium Salmonella enteritidis, as a potential adjuvant for STING-mediated anti-viral therapy. Delivery of AdrAwt or its inactive version AdrAmut occurred by an adenovirus (Ad) vector. Wt dendritic cells (DC) infected with Ad-AdrAwt but not mut in vitro showed increased upregulation of activation markers and produced large amounts of IL-6, IL12, MCP-1 and type I IFN. For DC derived from STING-deficient (gt) mice no activation was detected. The potential of AdrA to modulate the antiviral response was addressed in a hepatitis B transgenic

(HBVtg) and in AAV-HBV mouse models. A significant reduction of viremia in serum of Ad-AdrAwt-treated mice compared to Ad-AdrAmut was observed. The viral load in the liver at sacrifice were in line with this finding. To further investigate the role of STING and the subsequent activation of the IFN pathway, the experiment was repeated in AAV-HBV administered Wt, IFNAR k.o. and gt mice. Interestingly, albeit less pronounced than in wt mice, in IFNAR k.o. mice a reduction of serum viremia was achieved; however, no differences on hepatic HBV-RNA levels. No effect was observed in gt animals. These results indicate that while STING is indispensable in the anti-viral involvement of AdrA, type I IFN is not the sole effector molecule reducing viremia.

P698

Effect of salt and sugar on the storage stability of liquid and freeze-dried adeno-associated virus formulations

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Adeno-associated virus (AAV)-based vectors are of increasing interest in gene therapy due to their broad tropism, high transduction efficiency and good safety profile. Despite scientific progress our understanding of some biological and technological aspects of AAVs is still limited, for example with AAV drug substance formulations. AAVs in a liquid formulation tend to aggregate and thus are stored frozen at -80°C. Literature shows that aggregation in the liquid state can be reduced with increasing salt concentrations with more than 150 mM. Freeze-drying is currently explored as approach to increase storage stability, but high salt concentrations lower the glass transition temperature (Tg') and make it necessary to add a sugar as lyo- and cryoprotectant. Here, we freeze-dried AAVs (1x1012 vg/ml) formulated in 15 mM potassium phosphate buffer with potassium chloride (15 300 mM) and sucrose (150 300 mM). The samples were analysed by DLS, SEC, qPCR and nanoDSF. Prior to freeze drying all samples showed single AAV particles. The Tm measurement showed that increased osmolality shifted Tm to higher temperature. After freeze-drying and subsequent storage of 8 weeks (4°C and 25°C), the combinations of salt and sucrose showed the best results in all analytics since samples with salt only performed worst in the freeze-drying process. The liquid formulation in 1x PBS (4°C for 8 weeks) showed no loss of AAV titer in the qPCR, whereas freeze-drying resulted in a loss of 0.5 – 1 log at the moment. Therefore, freeze-drying AAVs is promising and optimisation is ongoing.

P699

Reference values of hematological and biochemical parameters in young-adult cynomolgus monkey (Macaca fascicularis) and rhesus monkeys (Macaca mulatta) nesthetized with ketamine hydrochloride

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Nonhuman primate models are valuable in biomedical research. However, reference data for clinical pathology parameters in cynomolgus and rhesus monkeys are limited. In the present study, we established hematologic and biochemical reference intervals for healthy cynomolgus and rhesus monkeys anesthetized with ketamine hydrochloride. A total of 142 cynomolgus monkeys (28 males and 114 females) and 42 rhesus monkeys (22 males and 20 females) were selected and analyzed in order to examine reference intervals of 20 hematological and 16 biochemical parameters. The effects of sex were also investigated. Reference intervals for hematological and biochemical parameters were separately established by species (cynomolgus and rhesus) and sex (male and female). No sex-related differences were determined in erythrocyte-related parameters for cynomolgus and rhesus monkey housed in indoor laboratory conditions. Alkaline phosphatase and gamma glutamyltransferase were significantly lower in females than males in both cynomolgus and rhesus monkeys aged 48–96 months. The reference values for hematological and biochemical parameters established herein might provide valuable information for researchers using cynomolgus and rhesus monkeys in experimental conditions for biomedical studies.

P700

Antha – a powerful software solution for the automated laboratory of the future

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There are many challenges facing the commercialization of cell and gene therapy products, such as the continuous need for innovation of manufacturing processes and for development of robust assays for product characterization. At Synthace, our goal is to enable scientists to deliver solutions to these and other complex biological problems in a fast, flexible, reproducible and cost-effective manner. We achieve this through Antha – a software platform and language, built to harness the benefits of automation in an equipment agnostic manner for the design, simulation, execution and analysis of complex experimental workflows. This improves throughput, scalability, data integrity and experimental walk away time, while overcoming common automation barriers such as low-level languages and vendor software. Oxford Biomedica has employed Antha to perform automated multifactorial (DoE) workflows in order to improve the efficiency and robustness of their in-house lentiviral vector production. A 3–10 fold increase in vector titre was achieved with an 81% reduction in pure error, while providing up to 40 hours time saving on experimental planning and execution. With Cambridge Consultants, we developed a flexible qPCR workflow which has increased throughput in the laboratory by 50% and reduced hands on time for the operator by over 80%, while ensuring reliability and reproducibility of the protocol. In collaboration with our industrial partners, Antha has been demonstrated to hold substantial value for cell and gene therapy applications. We are actively enhancing our capabilities in analytical execution, data aggregation for bioprocessing, as well as expanding the range of available execution platforms.

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P701

Creation of technology for manufacturing composite titanium polymer implants

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One of decisive factors for successful implantation of individualized implant is selection of effective method for designing and manufacturing implant from biocompatible material that fully corresponds to defect. Separately, individualized implants made of composite materials should be noted. New material is created through combination of dissimilar substances. Properties of new material are quantitatively and qualitatively different from properties of each components. The goal of the work was creation technology for manufacturing composite titanium polymer implants. To create implants, the latest technologies in the field of additive production and deposition of functional titanium coatings were applied. The basis consisted of hexagonal (4 mm side) cellular polymer structure. The structure obtained by sPro60HD. Titanium deposition on structures obtained was carried out by magnetron method. Pressure in chamber is $p=0{,}02$Pa. The flow rate of the plasma-forming Ar gas was $q=0.0005$g/s. Cathode $I=1.2$A; $U=800$V. Sputtering time 10 minutes. The thickness of applied layer was determined by DektakXT profilometer. The coating thickness was 65,93 nm. A sample fragment was implanted into rat to depth of muscular layer in region of spinal column. Duration of experimental studies: 10 days. As a result, it was found that when compared with intact tissue, increase in collagen fibers number was found in experimental group. Number of immune system cells (neutrophils, basophilic mononuclear cells) stayed same. Under conditions studied, it can be stated that Polyamide-12 coated with titanium layer was inert for 10 days. The titanium polymer implant is biocompatible and don't cause inflammatory response.

P702

Leaders in cryogenic RFID technology

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1: Cell Therapy Catapult

Cryogatt Systems Ltd is a high technology company specialising in the development of electronic radio frequency identification (RFID) based labelling, track, trace and audit systems, operating at cryogenic temperatures down to -196°C. It operates in the biomedical market with particular sector emphasis on human fertility, medical research, seed/crop research and Cell/Gene Therapy. A large range of RFID labelled vessels are available to accommodate the market sectors – for example: straws, vials, visotubes, goblets, canisters and canes for the fertility sector; vials, boxes and racks for the medical research sector; crystal vials and cryobags for the Cell/Gene Therapy sector.

P703

Low-level laser enhances gingival wound healing through promoting migration of human gingival mesenchymal stem cells via ROS/JNK/NF-κB/MMP-1 pathway

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Low-level laser has been shown to improve wound healing by promoting mesenchymal stem cell migration and proliferation. However, it remains unknow whether 808nm diode laser influences human gingival mesenchymal stem cells (HGMSCs) and which dose it works well. In this study, we found that low-level laser could promote migration of HGMSCs but not proliferation. Laser could activate mitochondrial ROS, lead to elevate levels of JNK and IKB phosphorylation in HGMSCs, and further activate NF-κB as p65 elevated in nuclear. Taken together, our results indicated that 0.5w low-level diode laser could promote cell migration via ROS/JNK/NF-κB Pathway. These data also provided the evidences to improve the MSC-based therapy for wound healing.

P704

Electromyographic analysis of the injured limb

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According to some authors, fractures frequency of the forearm from 11% to 53% of the total number of fractures of long tubular bones. Despite significant advances in medicine, the percentage of unsatisfactory outcomes very high. The development of post-traumatic complications due both damage to the articular cartilage at fracture, and degenerative changes in capsule-ligamentous apparatus of the joints as a result of prolonged immobilization. Comparative analysis of total electromyography (SA-EMG) of patients with forearm fractures was carried out. Patients are conditionally divided into 3 groups. First experimental group consisted of 9 patients performing dynamic exercises. In the rehabilitation of the second experimental group, consisting of 12 patients, static exercises were used. Patients of the third experimental group, which included 11 patients, performed complex of dynamic and static exercises throughout the rehabilitation. Examinations were carried out at post-immobilization and recovery stages of rehabilitation from the moment of injury and surgical treatment (osteosynthesis with plate and screws), after removal of plaster immobilization and at various stages of rehabilitation with a week interval. The analysis of five-week course of regenerative events after fractures treatment of the forearm allows to conclude, the complex application of static and dynamic exercise in the rehabilitation process allows to achieve considerable growth rate of total EMG, and thus early improvement of physical characteristics and level of general patient health. According to results of separate dynamic and static exercises, preference is given to latter.