Changing the Face of Modern Medicine: Stem Cell and Gene Therapy

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Abstracts

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Invited Speakers

INV001

General considerations for setting up a clinical trial

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Clinical trials are part of clinical research and at the heart of all medical advances. With the help of clinical trials scientists, physicians and the pharmaceutical industry explore whether a medical strategy, treatment, or device is safe and effective for humans. Because of their importance in the development process of a new drug or treatment, clinical trials must be planned and conducted following national rules and regulations. This provides public assurance that the rights, safety and wellbeing of research participants are protected and that research data collected and reported from these studies are reliable and reproducible. This presentation focuses on key aspects that sponsors and investigators should consider when setting up early phase clinical trials with a cellular gene therapy. It focuses on Good Clinical Practice requirements and other guidance available specific to advanced therapies.

INV002

Quality requirements for GLP tox testing and GCLP clinical testing

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1: GLP SR-TIGET San Raffaele – Telethon Institute for Gene Therapy –GLP Test Facility 2: TCL Tiget Clinical Lab, SR-TIGET 3: SR-TIGET 4: Vita Salute San Raffaele University

The ATMPs are complex biological medicinal products. A safety evaluation of data generated on this product category produced in compliance with the principles of good laboratory practice (GLP) and in a GLP certified test facility has to be conducted before starting human clinical trials and for registration purposes. Being ATMP a complex and very dish-omogeneous category of medicine, the non-clinical studies have to be designed on a case by case basis, so that they can provide representative data that address regulatory requirements while assuring scientific integrity and reliability. The quality systems based on GLP and Good Clinical Laboratory Practice (GCLP) principles ensure reproducibility, study reconstruction and data integrity through adequate documentation of study conduct and archiving of data. Laboratories testing human biological samples collected in Good Clinical Practice (GCP) clinical trials are expected to work according to GLP principles. The GCLP guidelines, combining GLP and GCP principles, provide guidance on the quality system to be used when running testing on this sample category. GCLP guidelines may be adopted by regulators during the GCP inspections. Based on the experience of the San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), the key elements of the GLP and GCLP quality systems to be considered (e.g. planning of study, data recording, training of personnel, method validation, etc), challenges and role of Quality Assurance (QA) in setting up a GLP testing facility and a Clinical Lab in the academia environment will be presented.

INV003

Pharmacovigilance in advanced therapy medicinal products (ATMPs): managing greater complexities

M Anelli[1]

1: ProductLife Group

In its website EMA states: ''All relevant legislation and guidelines regarding pharmacovigilance in the European Union (EU) are applicable to advanced therapy medicinal products (ATMPs).'' ATMPs, however, present peculiar challenges and require different approaches to traditional products not only in their pharmaceutical and clinical development, but also in the management and follow-up of post-approval activities. These drugs are defined in EC Regulation 1394/2007 as ''complex products'', which is reflected in the overall product lifecycle. As such, EMA recently released a draft revised guideline on safety and efficacy follow-up and risk management of ATMPs. Once the revision period is completed, the document will replace the current 10-year-old guideline. This presentation will focus on the main similarities and differences between ATMPs and traditional products, with a particular focus on the need for: 1- a risk-based approach 2- timely interactions with EMA to detect (and mitigate) risks 3- long-term follow up of seriously ill patient population where Adverse Events are frequent 4- post-approval studies. A few real-life examples will be shared during the presentation.

INV004

Registry platform for gene and cell therapy: the EBMT approach

C Bonini[1]

1: SR-TIGET, Milan

No abstract available

INV005

Cell manufacturing facility at Lausanne university hospital, GMP rules into a public institution: we do it

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1: Lausanne University Hospital

Regenerative medicine is a challenging task for clinical application into public institution since the manufacturing of ATMP has to be performed under GMP conditions and due to the limited volume that have to be produced.. Lausanne University Hospital implemented a Cell Production Center (CPC) that is now accredited by Swissmedic (authorization n°507482). Due to regulations issues, this implementation has to take in account GMP guidelines for a small unit of 14 collaborators in a context of huge public institution of more than 11'000 collaborators. The workflows of activities such as reception of raw materials, quality control, batch of production, release of final product into a public institution have to be described including directives such as for purchase of material and equipment, personal and financial management. This implementation has also to take in account the requirement of production of therapeutic product, clinical applications, academic research and conflict of interests. The public purchase of specific equipement was also a challenge since at the CPC we develop a concept of biocontainment with module of production Isocell Pro class A into class D clean rooms. The main conclusion is that we have to be pragmatic and step by step define a strategy that always maintain the benefit, security and safety for the patients. In summary, to adapt a small GMP unit into a huge public institution is possible if all partners share their experiences and work together to a general win-win development of their respective quality systems.

INV006

Scaling GMP AAV production

X Swanson[1]

1: Lonza

No abstract available

INV007

Scaling GMP lentiviral vector production

J E Miskin[1]

1: Oxford BioMedica

Gene and cell therapies, or Advanced Therapies, currently attract significant interest from the wider biotech/Pharma and investment communities. For the past 20 years, Oxford BioMedica (OXB) has been a pioneer in the development of products based on lentiviral vectors, with the company being responsible for several firsts in clinical studies based on these vectors. OXB is using this broad CMC, clinical and regulatory experience and know-how to facilitate product development based on lentiviral vector technology, both for company pipeline products and those of our strategic partners. The presentation will outline the different and parallel strategies being adopted by OXB to develop a manufacturing capability to generate vector material with the suitable quality attributes, at the appropriate capacity and scale, together with acceptable cost of goods. There will be an emphasis on the need for continued innovation to enable realisation of the full clinical benefit of these exciting and potentially curative products in patients. Developments in process improvement, scale-up and automation will be discussed, as will the capacity planning strategy to provide security of GMP supply.

INV008

Establishing gene therapy as a medicine and a business

R Philip[1]

1: Spark Therapeutics

No abstract available

INV009

Update on new ATMP guidelines for GMP production: focus on the risk based approach

I Reischl[1 2 3]

1: Federal Office for Safety in Health Care 2: Austrian Agency for Health and Food Safety 3: Institute Surveillance

The GMP for ATMP guideline has been developed as a standalone document applicable to the entire life-cycle of ATMPs. The intent was to ensure a safe product for the intended use while providing sufficient flexibility to facilitate development. The guideline builds on the established GMP framework and adresses specific aspects of this product group. Key aspects of the document will be highlighted in the presentation.

INV010

Risk management planning in the development of advanced therapies (ATMPs)

M Schüssler-Lenz[1]

1: EMA Committee for Advanced Therapies (CAT) 2: Paul Ehrlich Institute

No abstract available

INV011

Market access for ATMP in Europe

M Gabaldo[1]

1: Telethon Institute of Gene Therapy (HSR-TIGET)

Advance therapies represent a significant emerging field in which new treatment opportunities are offered to patients. We may be at the beginning of a new era of curative regenerative therapies, but their inherent nature may create barriers to adoption. More than 700 companies are working on new gene, cell and tissue engineering therapies that have the potential for profound and durable responses in patients with a diverse array of serious and costly conditions, many of which lack current treatments. After years of research and development, advance

therapies are now becoming a commercial reality with several products approved by European Medicines Agency. However, regulatory approval does not mean that these products are available to patients or are paid for by European health systems. In European countries, novel therapies, irrespective of the nature or mechanism of action, have to be evaluated through a formal Health Technology Assessment process, which is followed by (or includes) price negotiations. European patients and citizens need access to safe, effective and affordable medicines while the health care system should be financially sustainable, and innovation should be encouraged. This is perhaps the key challenge for the national competent authorities and public payers as pharmaceutical pricing and reimbursement remains the competence of EU Member States and ATMP developers are hardly convincing health systems that they should invest into highly expensive products with the expectation that long-term patient benefits justify the cost. While the typical ''*traditional*'' approach to medicine development has been a sequential, step-wise progression from clinical trials, through regulatory assessment and approval to reimbursement discussions and agreement on patient access, this model may not be optimal when trying to achieve timely regulatory approval and access to new medicines like advance therapies, particularly for patients with significant unmet medical need. Consequently, through the engagement of various stakeholders, the goal has begun to shift towards a more integrated approach to development to meet the overall target of patient access to new treatments. In the light of increasing financial pressure while further new high-priced medicines are expected to come to the market, new approaches to achieve the above-mentioned objectives might be required. There are a number of proposed alternative reimbursement and financing models to address the potential uncertainty and economic disincentives that may be associated with curative therapies.

INV013

Gene and cell therapy a brief update

H Büning[1]

1: Hannover Medical School

No abstract available

INV014

Novel lentiviral pseudotypes for natural killer based cancer immunotherapies and 'nanoblades' for efficient gene editing in T, B, IPS cells and blood stem cells

E Verhoeyen[1 2]

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Natural killer cells (NKs), with their intrinsic ability to recognize and kill tumor cells represent an interesting tool for immunotherapy, even in an allogenic setting. However, NKs are quite resistant to transduction with classical VSVG pseudotyped lentivectors (LVs). We hypothesized that alternative lentiviral pseudotypes might result in more efficient transduction. The use of BaEV-LVs outperformed other pseudotypes with a transduction rate mean of 30% in freshly isolated NKs and 87% in expanded NKs, even at low vector doses. BaEV-LV transduction efficacy could be attributed to the BaEV receptors' expression patterns. NKs were transduced with BaEV-LVs encoding chimeric antigen receptors (CAR) against CD22 or GD2. Sorted and re-expanded transduced NKs kept their CAR-expression and retained their cytotoxic functions against cancer cells. In summary, these BaEV-LVs represent a NK-transduction technique that will allow the development of efficient NK-based immunotherapies such as CAR-NK cells or enhanced NKs. We have also recently designed ''Nanoblades'', a new technology that will deliver a genomic cleaving agent into cells. These are genetically modified Murine Leukemia Virus (MLV) or HIV derived virus like particle (VLP), in which the viral structural protein Gag has been fused to the Cas9. Nanoblades are extremely efficient in entry and delivery of their Cas9/sgRNA ribonucleoproteins cargo into human T, B and hematopoietic stem cells and IPS cells. Thus, the nanoblades shows high flexibility for different targets including primary immune cells of murine and human origin and therefore have important prospects for basic and clinical translation in the area of gene therapy.

INV015

Insights into adenovirus and AAV vectorology

K Benihoud[1] H Büning[2]

1: CNRS UMR 8203 Vectorology and antitumor therapeutics, Villejuif, France; University Paris-Sud, Faculté des Sciences d'Orsay, Orsay, Franc 2: Institute for Experimental Hematology, Hannover Medical School, Hannover, Germany

Vectors derived from adenoviruses (Ad) and adeno-associated viruses (AAV) are two of the most commonly applied vector systems in preclinical studies and clinical trials. Here, we will provide insights on the main characteristics of both kinds of vectors highlighting their similarities and differences. A special emphasis will be put on capsid genetic engineering in order to control vector tropism or to avoid interaction with the immune system. In addition, the main applications of these vectors in correction of genetic diseases, tumor treatment and vaccination will be discussed.

INV016

Manufacturing and quality control of viral vectors

E Ayuso[1]

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The market approval of adeno-associated virus (AAV)-based gene therapy products and CAR-T therapies in Europe and USA constitutes an evidence that the field is progressing from proof-of-concept studies toward clinical development. Concurrent with this increasing success and application, regulatory agencies have enhanced the stringency of their requirements concerning the quality and purity of these novel drugs. Manufacturing of viral vectors at high quantities and fulfilling current good manufacturing practices (GMP) is still a challenge, and several technological platforms are competing for this niche. Here, I will describe the most commonly used upstream (production) and downstream (purification) methods to produce viral vectors and available options to scale up these processes for industrial applications, using AAV vectors as a practical example. The safety and efficacy profiles of viral-based

drugs not only depend on the purification steps that ensure an efficient removal of product- and process-derived impurities, but also on the development of robust and precise assays for the detection and quantification of these impurities. In fact, even purified vector stocks contain impurities such as DNA, detergents, antibiotics or proteins. In this presentation, I will also discuss the most common analytical tests used for the characterization of the safety, efficacy, purity and identity of drug products as well as the development of next generation methodologies for advanced quality controls.

INV018

A brief overview on iPSC and embryonic stem cell technology

M Puceat[1]

1: INSERM U1251, Marseille

Human embryonic stem cells have been derived in 1998 in J Thomson's laboratory (Wisconsin University, USA) and has become since then a faithful model of organ development and a source of cells for cell therapy of degenerative diseases. Their differentiation can be directed towards any cell lineage by inductive signals provided by morphogens. One example of how to direct their fate toward a cardiomyocyte will be discussed In 2006, Pr Yamanaka laboratory (Kyoto University, Japan)reprogrammed somatic cells into induced pluripotent stem cells (IPSC) using pluripotency transcription factors. They now represent good models of diseases in a dish to better understand them and to test therapeutic drugs. I will show one example of how to uncover a therapeutic target from iPSC derived from cells of a patient with a cardiac valve disease in a study using single cell-sequencing technology.

INV019

Dressing viruses in tumor's clothing: welcome to the cloning-free oncolytic vaccine era

V Cerullo

1: University of Helsinki

How can we fully personalize cancer immunotherapies? Few years back we finally realized that oncolytic viruses have a dual mode of action; changing this paradigm has led us to use oncolytic viruses as vaccines (oncolytic vaccines). Oncolytic vaccines are oncolytic viruses that are able to elicit a strong immune response that is mainly directed towards the virus (virus immunodominance) but as side effect they also elicit a tumor-specific immune response. This concept has enormously enhance d the field and has led to multibillion commercial deal in the field of oncolytic vaccine. However, many challenges have emerged along the way that have limited the full capabilities of oncolytic vaccines. These include, changing the immunodominance away from the viral and towards the tumours. Also, developing and changing viral platforms faster, due to rapid tumour changes. To face these new challenges we have developed different viral platforms based on enveloped and non-enveloped viruses to dress the viruses with tumor specific antigens. The techniques we have developed are rapid and precise and have allowed us to redirect immune response away from the virus and towards the tumor. We have also developed novel techniques for fast tumor-antigen and neo-antigens discovery directly looking at the tumor MHC-I ligandome as well as a rapid algorithm to study immunogenicity of tumor-specific MHC-I restricted peptides. Taken together our pipeline allow us to look at tumor-specific antigen directly from a byopsi, discriminate and study the specificity and the immunogenicity of the tumor peptides and finally mount these peptides directly on the surface of our favorite oncolytic virus.

INV020

Lentiviral vectors: the first 20 years

L Naldini[1]

1: SR-TIGET, Milan

No abstract available

INV021

Cancer stem cells and organoids development: towards a better understanding of the biology behind organoids

D Fessart[1]

1: Inserm

Three-dimensional organoid culture models are powerful models increasingly used to recapitulate the complex nature of organs. However, there are no reports on the genetic basis of stem cell determinants in organoids. More specifically, no biological determinant that enriches stem cell markers to form organoids is known so far. Adult stem cells are rare, immature cells capable of self-renewal and the generation of many differentiated cell types. Organoids are thought to arise from adult stem cells and therefore should be capable of self-renewal and differentiation. Consistent with this idea, primary cells enriched for known stem cell markers are more efficient at forming organoids than the general cell population. Our research focuses on the use of 3D model for studying tissue morphogenesis and tumorigenesis. We present recent results chosen to illustrate key concepts on how we can enrich 1) the stem cells pool and 2) the self-renewal potential of human organoid-derived stem cells. Thus we will highlight the importance of the factors regulating stem-cells derived organoids, and also hESC self-renewal and pluripotency. Striking parallels can be found between stem cells and cancer cells: tumors may often originate from the transformation of stem cells, similar signaling pathways may regulate self-renewal in stem cells and cancer cells, and cancer cells may include 'cancer stem cells' - rare cells with indefinite potential for self-renewal that drive tumorigenesis. The connections between stem cells and cancer are thus important to provide insight into the origins of cancer and will ultimately yield new approaches to fight this disease.

INV022

Overview of CNS gene therapy

N Déglon[1]

1: Lausanne University Hospital

No abstract available.

INV023

Gene therapy for Alzheimer's disease

R G Crystal[1]

1: Weill Cornell Medical College

The APOE gene is a common risk factor for Alzheimer's disease, with APOE4 homozygotes at 15-fold increased risk and the APOE2 gene decreasing risk. Preclinical studies with Alzheimer's mouse models have demonstrated that adeno-associated virus AAVrh.10-mediated gene transfer of the APOE2 gene markedly decreases the amount of CNS amyloid-beta peptide accumulation in APOE4-expressing mice. Studies in non-human primates have shown that AAVrh.10-mediated intracisternal delivery of the human APOE2 coding sequence safely and effectively distributes APOE2, DNA, mRNA and protein throughout the brain, with persistent expression of APOE2 in the cerebrospinal fluid. Based on these studies, with the support of the Alzheimer's Drug Discovery Foundation, we are moving forward with a clinical trial of AAVrh.10hAPOE2 CNS therapy in APOE4 homozygotes with early onset Alzheimer's.

INV024

Gene therapy for Huntington's disease

N Cartier[1]

1: University Paris Sud

No abstract available

INV025

Primary immuno-deficiency, GT vs. alternative strategies how do we move into the 'real' clinical reality

A Thrasher[1]

1: University College London

No abstract available

INV026

Gene therapy in bone marrow failure syndromes

J Bueren[1]

1: CIEMAT, Madrid

No abstract available

INV027

Hematopoietic stem cell gene therapy

O Negre[1]

1: bluebird bio

Gene therapy using autologous hematopoietic stem cells is a one-time treatment option that aims to provide a long-term therapeutic benefit to patients suffering from genetic diseases involving blood cells, such as certain hematologic disorders, primary immunodeficiencies and congenital metabolic diseases. Here, we will focus on β-thalassemia and sickle cell disease, two inherited red blood cell disorders caused by mutations in the gene encoding for the β subunit of hemoglobin, that can result in severe morbidity and early mortality. While migration is changing the global distribution of β-thalassemia and sickle cell disease, both are considered rare diseases in most of Europe and the US. Allogeneic hematopoietic stem cell transplantation is currently the only available curative treatment for these disorders, however its use is limited to a subset of appropriate patients who have a matched sibling donor. Additionally, allogeneic stem cell transplantation is associated with risks such as graft failure and graft-versus-host-disease. Gene therapy offers a potentially transformative option to patients with sickle cell disease and β-thalassemia and thereby provides an opportunity to address a significant unmet need for these patients. LentiGlobin is an investigational gene therapy that introduces a functional copy of the β-globin gene into the genome of a patient's own hematopoietic stem cells with the goal to correct red blood cell defects associated with β-thalassemia or sickle cell disease and consequently reduce or eliminate disease symptoms. Here, we will: review the preclinical development of LentiGlobin gene therapy, provide a summary of clinical outcomes in patients with β-thalassemia or sickle cell disease and highlight ongoing efforts to support a potential marketing authorization.

INV028

Targeted neurotechnologies enabling walking after paralysis

G Courtine[1 2]

1: Center for Neuroprosthetics and Brain Mind Institute, School of Life Sciences, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland 2: Department of Neurosurgery, Lausanne University Hospital (CHUV), Lausanne, Switzerland

The World Health Organization (WHO) estimates that as many as 500'000 people suffer from a spinal cord injury each year, with dramatic consequences for the quality of life of affected individuals. Over the past 15 years, we have developed a multipronged intervention that reestablished voluntary control of paralyzed legs in animal models of spinal cord injury. This intervention acts over two time-windows. Immediately, electrical and chemical stimulations applied to the lumbar spinal cord reawaken the neuronal networks below the injury that coordinate leg movements. In the long term, will-powered training regimens enabled by these electrochemical stimulations and cutting-edge robotic assistance promote neuroplasticity of residual connections – restoring walking without stimulation in animal models of paralysis. We are currently testing these therapeutic concepts in humans who have sustained a spinal cord injury more than 4 years ago. During this talk, I will describe these technological and therapeutic developments in animal models, before sharing preliminary results in humans.

INV029

Retroelements, their polydactyl controllers and the specificity of human biology

D Trono[1]

1: Ecole Polytechnique Fédérale de Lausanne (EPFL), School of Life Sciences, Lausanne, Switzerland

Retroelements have served as a basis to develop some of the most efficient vectors available today for the delivery of therapeutic genes. Retroelements are also major contributors to our genetic make-up since endogenous retroviruses and other retrotransposons may account for more than two-thirds of our genome and exert profound influences on its architecture and expression. They are subjected to epigenetic control through sequence-specific recognition by KRAB-containing zinc finger proteins (KZFPs), which constitute the largest group of transcription factors encoded by higher vertebrates and act by recruiting inducers of heterochromatin and other modulators. Owing to their continuous spread during evolution, endogenous retroelements (EREs) exhibit great degrees of lineage-specificity, and their KZFP controllers reciprocally display very limited levels of inter-species orthology. I will present data demonstrating that EREs and KZFPs partner up to establish human-restricted regulatory networks, some subjected to evolutionary and environmental constraints, others leading to phenotypic speciation, yet all conferring unique characteristics to human biology.

INV030

Elucidating early disease mechanisms in ALS using stem cells

E Hedlund[1]

1: Karolinska Institutet

Motor neurons are highly polarised cells that send axons across large distances in the body. In the fatal disease amyotrophic lateral sclerosis (ALS) these neurons selectively degenerate, with the distal axon and their specialised synapses with muscle, the neuromuscular junctions (NMJs), being the primary pathological targets. Due to the large distance between the motor neuron soma and the NMJ, proper functioning of this dynamic structure requires local control of cellular events, which are likely affected in ALS. To model early degenerative events occurring in distal motor axons and NMJs in ALS, we specify stem cells harboring ALS-causative mutations into motor neurons and muscle, and culture these in microfluidic devices that separate axons from their somas. Co-culture also allows proper connection of motor axons to muscle, and emergence of NMJ structures. In this system we apply Axon-seq, a method with improved sensitivity and accuracy in whole-transcriptome sequencing of axons, combined with single cell RNA sequencing of somas. Our analysis uncovers a clear dysregulation of multiple transcripts, including Nrp1, Dbn1, and Nek1, in response to ALS-causing mutations, and identifies novel therapeutic targets to maintain neural connectivity in disease.

INV031

A mouse model of a human cholestatic liver disease reveals extent and therapeutic potential of mammalian transdifferentiation

H Willenbring[1]

1: UCSF

Transdifferentiation is a physiological backup mechanism and alternative to stem-cell-mediated organ regeneration. In adult mammals, findings of transdifferentiation have been limited to replenishing lost cells in preexisting structures, i.e., in the presence of a fully developed scaffold and niche. Our research shows that hepatocytes in the adult liver can transdifferentiate to build a structure that failed to form in development—the biliary system in mice that mimic the human cholestatic liver disease Alagille syndrome. The bile ducts forming the new biliary system are fully functional—as evidenced by reversal of cholestasis, liver injury and liver fibrosis—and persist after the cholestatic liver injury is reversed. These results reveal extent and therapeutic potential of mammalian transdifferentiation.

INV032

Genome engineered T cell immunotherapies for leukaemia

W Qasim[1]

1: UCL Institute of Child Health

T-cells engineered to express chimeric antigen receptors (CAR) are being widely investigated, in particular for the treatment of B cell malignancies. The first licensed products are now available and most studies have applied integrating vectors to modify autologous T cells. Incorporation of additional genome editing steps to disrupt gene expression has enabled the production of universal CAR- T cell banks from healthy, non-HLA matched donors, designed to be used in an 'off-the-shelf' manner. Clinical applications of Transcription activator-like effector nucleases (TALEN) modified cells are being tested in multicentre studies, and data is accumulating on strategies that can enable allogeneic T cells to overcome HLA barriers. Further iterations are advancing CRISPR/Cas9 systems towards human trials with manufacturing, regulatory and technical hurdles being addressed. An ability to efficiently disrupt specific T cell molecules at high efficiency has also allowed CAR-T cells to be fashioned against T cell and myeloid malignancies, and multiplexing options are offering ever sophisticated possibilities. The application of base editing by CRISPR guided deamination to introduce stop codons has further refined these approaches and is likely to improve safety profiles.

INV033

VSV-IFNb-NIS, an armed and trackable oncolytic vesicular stomatitis virus

K Peng[1]

1: Mayo Clinic

Vesicular Stomatitis Virus (VSV) is a rapidly replicating virus with low seroprevalence in the general population. Thus, intravenous therapy of this virus for the treatment of metastatic cancer is highly feasible. VSV-IFNβ-NIS (Voyager-V1™) is an oncolytic VSV encoding human interferon beta and NIS sodium iodide symporter for imaging the pharmacokinetics of viral replication in tumors. The virus has activity against a broad spectrum of tumor types, and is currently being evaluated for intratumoral or intravenous administration into patients with solid tumors, hematological malignancies and endometrial cancer. We will discuss results from the nonclinical studies using VSV-IFN-NIS, and early results from exploratory correlative studies from the clinical trial.

INV034

Gene therapy for choroideremia: what have we learned from the clinical trials to date

I M MacDonald[1]

1: University of Alberta

Choroideremia is a progressive X-linked retinopathy that results in blindness and although untreatable, recent published trials suggest that ocular gene therapy might be a promising approach. The objective of these Phase I trials has been to determine safety and then enable broader studies with focused outcome measures. Six Canadian subjects affected by choroideremia received subretinal delivery of an AAV2-REP1 vector and peri-operative oral steroid to manage anticipated inflammation. Subjects were followed for 2 years with anatomical and functional tests. One subject had a 15 letter improvement in visual acuity (VA) in his treated eye by three months which was sustained after two years, whereas one patient who experienced retinal inflammation had a reduction of 8 letters. The VA changes in these two patients correlated to their subjective quality of vision. Preservation of the retinal ellipsoid zone, an anatomic measure, was greatest in the treated eye of the patient with the 15 letter gain. One patient had an improvement in VA in his untreated eye which reached 15 letters by 2 years and was assumed to be a learning effect. In all other patients, changes seen at 2 years in treated eyes were similar to their unoperated fellow eyes. Patients with variable VA readings can be detected by a natural history study performed prior to enrollment in order to judge the effect of intervention. Fundus autofluorescence area, an anatomic measure, proved to be a reliable indicator of change and will be an important biomarker in future trials of ocular gene therapy.

INV035

CYP46A1-gene therapy alleviates spinocerebellar ataxia in mouse models

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Spinocerebellar ataxias (SCAs), including Machado-Joseph disease (MJD), the most prevalent ataxia worldwide, are neurodegenerative disorders for which no therapies are available. Deregulation in cholesterol metabolism impairs neuronal function. Brain cholesterol is almost exclusively synthesized in situ and cannot cross the blood-brain-barrier. To be evacuated from the brain therefore maintaining cholesterol homeostasis, superfluous cholesterol is converted into 24S-hydroxycholesterol (24-OHChol), step mediated by the neuronal cholesterol 24-hydroxylase enzyme (CYP46A1). We first demonstrate that CYP46A1 levels are decreased in cerebellar extracts from MJD patients and MJD transgenic mice. Next, we show that overexpression of AAVrh10-CYP46A1 in a lentiviral-based mouse model of MJD conferred neuroprotection by reducing mutant human ataxin-3 accumulation, associated to the preservation of neuronal markers. This was confirmed in adult MJD transgenic mice, in which cerebellar AAVrh10-CYP46A1 overexpression induced a noteworthy alleviation of motor behavior impairments correlated with mitigation of MJD-associated neuropathology, in particular, decrease of misfolded protein, reduction of Purkinje cell loss and of cerebellar atrophy. Then, *in vivo*, in a wild-type circumstance, knocking-down CYP46A1 levels in the mouse brain, via AAV-mediated delivery of a discriminatory shCYP46A1, impaired cholesterol metabolism, induced strong neurodegeneration, that was translated into motor deficits. Remarkably, we demonstrate *in vitro* and *in vivo* that CYP46A1 activates autophagy decreasing mutant ATXN3 deposition. Therefore, our results suggest CYP46A1 as a relevant therapeutic target for SCAs and related neurodegenerative disorders.

INV036

Macrophage therapy for liver disease- preclinical and clinical

S Forbes[1]

1: The University of Edinburgh

Macrophages have important effects in liver injury, regeneration and repair. In the recovery from chronic injury, macrophages in the liver are important in helping the regression of liver fibrosis and stimulation of the regenerative response. Mouse studies show that the injection of bone marrow derived macrophages can promote fibrolysis and liver regeneration indicating their potential as a clinical cell therapy. Leukapheresis is safe in cirrhotic patients and ex-vivo monocyte-derived macrophages from cirrhotic patients have a phenotype comparable to those derived from healthy volunteers. We have therefore sought to develop monocyte-derived macrophages as a clinical cell therapy. We have performed a phase 1 study of autologous monocyte derived macrophage therapy in patients with cirrhosis. This single-arm, dose-escalation trial enrolled patients with liver cirrhosis and a Model for End Stage Liver Disease (MELD) score of 10-16 inclusive. Autologous CD14+ monocytes were collected via leukapheresis and differentiated into mature macrophages under GMP conditions. 9 patients in groups of 3 received an infusion of 1×10^7, 1×10^8 and up to 1×10^9 cells respectively. Subjects were assessed up to 360 days after infusion. Results will be presented for this study. Based upon this data a phase 2 randomised controlled trial is underway to evaluate the efficacy of autologous macrophage infusions compared to standard clinical care.

INV037

***In vivo* gene editing in tissues and tissue stem cells**

A Wagers[1]

1: Harvard University

Effective functioning of the body's tissues and organs depends upon the maintenance of proper cell numbers (homeostasis) and replacement of damaged cells after injury (repair), both processes that require proper functioning of tissue stem cells. My laboratory

is working to advance strategies for genome editing of therapeutically relevant stem cell populations, using experimentally engineered programmable nucleases. Application of this system in a mouse model of Duchenne Muscular Dystrophy (DMD) has shown simultaneous gene targeting in multiple organs of therapeutic interest, with restoration of the mutated Dystrophin protein reading frame, recovery of muscle function, and establishment of a pool of modified muscle stem cells capable of participating in subsequent muscle regenerative events. Further adaptation of this approach suggests the capacity to target multiple, distinct stem cell populations in different anatomical niches, providing a new experimental alternative to conventional transgenic/knockout mouse models and *ex vivo* transduction approaches that should allow for high throughput interrogation of gene functions in stem cells within their native niches. Taken together, this work provides novel avenues for experimentally manipulating stem cell function and suggests new strategies for therapeutic intervention to achieve functional recovery of disease-relevant gene products and promote endogenous repair activity across organ systems.

INV038

Emerging therapies for neurodegenerative diseases

B Davidson[1 2]

1: University of Pennsylvania 2: The Children's Hospital of Philadelphia

Gene therapies represent an emerging modality for CNS therapies for both recessively and dominantly inherited disorders causing neurodegeneration. The late infantile neuronal lipofuscinoses are an example of a fatal, recessively inherited childhood onset disease due to a deficiency in the lysosomal enzyme tripeptidyl peptidase I (TPP1), a non-membrane bound lysosomal hydrolase. Because these enzymes can be processed through the secretary pathway, genetic correction of a small percentage of cells can provide sufficient protein for enzymatic correction of many cells. We will show the utility of genetically correcting distinct cell types in the brain for disease therapy. Once corrected, these cells serve as enzyme secretion depots, providing widespread correction of neuropathology and clinical symptoms in small and large animal model of TPP1 deficiency, with scalability to NHPs. Huntington's disease is among the dominantly inherited diseases due to polyglutamine repeat expansion in huntingtin. HD presents the challenge of removing a toxic, gain of function product, in contrast to gene replacement. Here, we show a novel allele selective CrispR/Cas9 approach to reduce expression of huntingtin in human cells and in mice models of HD. Together these preclinical programs exemplify the challenges and progress in the development of gene therapy for inherited brain diseases.

INV039

Gene therapy in haemophilia: from vision to reality

W Y Wong[1]

1: BioMarin Pharmaceutical

The therapeutic path for haemophilia has had its share of successes and tragedies; from cryoprecipitate to plasma derived factor replacement, to recombinant and extended half-life factor products and now to gene therapy. The ability to abrogate bleeding events long term by achieving clinically meaningful FVIII or FIX activity levels is particularly attractive in haemophilia, and has been demonstrated in ongoing clinical trials. Current data from clinical trials have been highly encouraging. The altruism of those pioneering trial participants must be harvested and shared for the benefit of the entire community. Factors relevant to success include appropriate regulatory approval pathways, vector development, manufacturing scale-up, and control of potential and observed toxicities. The acceptable risk and safety profile seen to date, as well as sustained efficacy with clinically relevant FVIII and FIX activity levels and measurable clinical outcomes such as bleeding rates, joint health and quality of life, hold great promise to be life-changing. We will review data from clinical trials in both haemophilia A and B; and examine the knowns, challenges and unknowns as well as evolving areas. Hemophilia is the first setting whereby gene therapy can fundamentally abrogate an important genetic disease that affects significant number of individuals.

INV040

Identifying extrinsic and intrinsic drivers of variation in cell behaviour in human iPS cell lines

F M Watt[1]

1: King's College London

Large cohorts of human induced pluripotent stem cells (iPSCs) from healthy donors are a potentially powerful tool for investigating the relationship between genetic variants and cellular phenotypes. The Human Induced Pluripotent Stem Cells Initiative (HipSci), funded by the Wellcome Trust and the Medical Research Council, has generated and characterised over 700 open access hiPSC lines derived from 301 healthy donors. Quantitative assays of cell morphology and proliferation have demonstrated a donor contribution of 8-23% to the observed variation between lines. We have now integrated high content imaging, gene expression and DNA sequence datasets from over 100 of the lines to explore the genetic basis of the inter-individual variability in cell behaviour. By applying a dimensionality reduction approach, Probabilistic Estimation of Expression Residuals (PEER), we have extracted factors that capture the effects of intrinsic (genetic) and extrinsic (environmental) conditions. We have identified genes that correlate in expression with intrinsic and extrinsic PEER factors and mapped outlier cell behaviour to expression of genes containing rare deleterious SNVs. Our study establishes a strategy for determining the genetic basis of inter-individual variability in cell behaviour.

INV041

Reducing noise and bias from studies of disease-implicated genetic variation through massively-mosaic stem cell systems

J Mitchell[1] J Nemesh[1] S Ghosh[1] C Mello[1] S McCaroll[1] K Eggan[1]

1: Harvard University, Cambridge, MA

Human pluripotent stem cells including induced pluripotent stem (iPS) cells and human embryonic stem cells have emerged as powerful tools for understanding how inherited genetic variants shape disease propensity and as preclinical model systems for the

development of candidate therapeutics. However, like many biological systems, the genetic insights they can provide suffer from many forms of experimental noise. We will report on two methods that we have developed to combat these losses in signal. Both take advantage of ensemble cultures of stem cells produced from as many as 100 individuals. These ''massively mosaic stem cell systems'' have allowed us to greatly simplify the process of stratifying individuals to their likely response to a drug and have enabled detection of the effects of many common genetic variants on transcription of nearby genes. Ongoing work with these methods promising to provide fresh insights into how common genetic variation shapes risk for diseases such as Schizophrenia.

INV042

No title available

ABSTRACT WITHDRAWN

INV043

Characterization of AAV44.9

J A Chiorini[1] G Di Pasquale[1] R J Chandler[2] E Y Choi[3] B Hubbard[2] S Afione[1] M Khalaj[1] C Zheng[1] B Grewe[1] S G Kaler[3] C P Venditti[2]

1: National Institute of Dental and Craniofacial Research 2: National Human Genome Research Institute 3: National Institute of Child Health and Human Development.

Recent isolation of novel AAV serotypes has lead to significant advances in our understanding of parvovirus biology and vector development for gene therapy by identifying vectors with unique cell tropism and increased efficiency of gene transfer to target cells. AAV44.9 is a natural isolate originally found as a contaminate of laboratory stock of SV15 adenovirus. Its sequence homology places it between clades E and F similar to Rh.8. little is know about the biology of these intermediate viruses. Recent studies have suggested that AAV44.9 is a promising candidate for photoreceptor- targeted gene therapies. However, its broader biodistribution and cell tropism is not clear. To better understand its activity in other organs and general biodistribution mice were transduced by a variety of routes of transduction. Intracerebroventricular injection showed transduction of the cortex, olfactory bulb, cerebellum, choroid plexus and brain stem similar to AAV9. Like AAV9, AAV44.9 also shows transduction of hepatocytes and is able to rescue a lethal model of Methylmalonic acid deficiency in new born mice. Data on the role of cell surface bind and glycan requirements will be present.

INV044

Small but increasingly mighty - Latest advances in AAV biology and vector optimization

D Grimm[1]

1: BioQuant; University of Heidelberg

Gene delivery vectors based on natural or synthetic Adeno-associated viruses (AAV) have taken center stage in human gene therapy, yet it is clear that the current vector generation requires improvements in potency, immunoreactivity and specificity, to fully realize AAV's clinical potential and applicability. Luckily, we have recently seen a wealth of exciting reports that enhance our understanding of fundamental AAV biology and that concurrently expand our opportunities for AAV capsid design and evolution. This presentation will highlight a selection of latest advances that particularly promise to propel the AAV vector field forward, such as novel insights into the role of AAP for particle assembly as well as innovative strategies for high-throughput screening of AAV capsid libraries, including DNA/RNA barcoding. These and other examples that will be discussed not only showcase the current momentum in the AAV community but also provide every reason to believe that the lasting obstacles hampering a wider application of AAV gene therapy can soon be overcome.

INV045

Introduction to the Committee for Advanced Therapies (CAT) and its tasks in the evaluation of advanced therapies

M Schüssler-Lenz[1 2]

1: EMA Committee for Advanced Therapies (CAT) 2: Paul Ehrlich Institute

No abstract available

INV046

EU regulatory aspects of CAR-T cells

M Timón[1]

1: AEMPS

No abstract available

INV047

EU regulatory aspects of rAAV vectors

J H Ovelgönne[1]

1: Medicines Agency, Netherlands

No abstract available

INV048

EU regulatory aspects of genome editing

M Renner[1]

1: Paul Ehrlich Institute

No abstract available

INV049

CARs and TRUCKs: next generation adoptive cell therapy

H Abken[1]

1: University Hospital Regensburg

No abstract available

INV050

Dissecting cancer biology with iPS cell technology

Y Yamada[1]

1: University of Tokyo

Cancer arises through the accumulations of both genetic and epigenetic alterations. Although the causal role of genetic mutations on cancer development has been established *in vivo*, similar evidence for epigenetic alterations is still limited. Cellular reprogramming technology can be used to actively modify the epigenome without affecting the genomic information. In this symposium, I will introduce our recent studies that utilized reprogramming technology for dissecting cancer epigenome and manipulating cancer epigenome. The faithful shutdown of the somatic program occurs in the early stage of reprogramming. Here, we examined the effect of *in vivo* reprogramming on Kras-induced cancer development. We show that the transient expression of reprogramming factors in pancreatic acinar cells results in the transient repression of acinar cell enhancers, which are similarly observed in pancreatitis. We next demonstrate that Kras and p53 mutations are insufficient to induce ERK signaling in the pancreas. Notably, the transient expression of reprogramming factors in Kras mutant mice is sufficient to induce the robust and persistent activation of ERK signaling in acinar cells and rapid formation of pancreatic ductal adenocarcinoma. In contrast, the forced expression of acinar cell-related transcription factors inhibits the pancreatitis-induced activation of ERK signaling and development of precancerous lesions in Kras-mutated acinar cells. These results underscore a crucial role of dedifferentiation-associated epigenetic regulations in the initiation of pancreatic cancers.

INV051

Exosomal PD-L1 as an immune-modulator in cancer

R Blelloch[1]

1: University of California San Francisco

Exosomes are small packages of cellular membrane that travel throughout the body enabling long distance communication between cells. Cancer-derived exosomes can act at a distance to promote primary tumor growth and metastases. How exosomes achieve this outcome remains unclear. At this meeting, I will present data showing that cancer cells secrete the immune-checkpoint protein PD-L1 in the form of exosomes. This exosomal PD-L1 is able to suppress the anti-tumor immune response enabling disease progression. As such, exosomal PD-L1 represents a previously under-appreciated regulator of cancer progression.

INV052

Molecular elucidation and engineering of stem cell fate decisions

D Schaffer[1]

1: University of California, Berkeley

As the field progressively learns more about the mechanisms that control stem cell behavior – namely the hallmark properties of self-renewal and differentiation – deeper insights have been gained into their potential roles and applications in organismal development, adult homeostasis, and regenerative medicine. The niches in which stem cells reside present them with a spectrum of signals to control their behavior, and it has become increasingly evident that not only local biochemical signals, but also biophysical features of the environment that modulate the presentation of this biochemical information, impact cell behavior. For example, spatial and temporal variation in the presentation of cues is important information that modulates fate decisions and tissue structure. Furthermore, the tissue matrix has variable bulk mechanical properties and surface topographical features depending on how its assembled, both of which modulate cell behavior. We have created several technology platforms to investigate the biophysical regulation of stem cell behavior, in particular to understand and control the differentiation of adult neural stem cells and human pluripotent stem cells into neurons. First, we are developing and harnessing optogenetics systems to investigate how cellular signaling dynamics impact fate decisions. Second, we have been developing bioactive, synthetic material systems to investigate the effects of cell-matrix and cell-cell interactions on cellular function. Finally, we work towards translating the basic information that emerges from both of these efforts into safe, scaleable, fully defined, robust culture and implantation systems for stem cell based regenerative medicine efforts to treat human disease.

INV053

Making 3D models that matter: engineering skeletal muscle tissue in a dish

P Gilbert[1]

1: University of Toronto

Skeletal muscle tissue is found throughout the human body and allows for actions such as walking, swallowing, and breathing. A skeletal muscle is comprised of bundles of long cylindrical muscle cells that are often attached to bones via tendons and that contract in unison in response to human intent. Numerous genetic and acquired conditions can impact skeletal muscle performance, but the lack of robust culture models to study contractile skeletal muscle tissues outside of the body has made it challenging to study this tissue. Using 3D printing and tissue engineering approaches, we developed methods to grow arrays of tiny human skeletal muscle tissues in a dish. By combining the muscle tissues with post-mitotic motor neurons derived from human pluripotent stem cells, we show that it is possible to study developmental processes and diseases that impact the communication between motor neurons and muscle that cannot be studied using classic two-dimensional co-cultures. Furthermore, by establishing methods to model and study human exercise and skeletal muscle tissue endogenous repair in a dish we uncover novel regulators of skeletal muscle hypertrophy. This body of work hopes to highlight the synergy of biologists and engineers working side-by-side to create robust culture models in order to advance knowledge and improve tissue health.

INV054

Gene therapy of myotubular myopathy: from preclinical studies to a clinical trial

A Buj Bello[1]

1: Genethon

X-linked myotubular myopathy (XLMTM) is a severe congenital disease of skeletal muscle due to loss-of-function mutations in the *MTM1* gene coding for a lipid phosphatase named myotubularin. Patients typically present with generalized muscle weakness and respiratory failure, and most of them die during early infancy. We have developed a gene therapy approach to treat this disease and shown the efficacy of intravenous administration of recombinant serotype 8 adeno-associated viral vectors (rAAV8) expressing myotubularin in mouse and dog models of the disease, leading to long-term correction of the muscle phenotype. These preclinical results supported the initiation of a Phase 1/2 clinical trial in XLMTM patients (ASPIRO study). An overview of the preclinical and preliminary clinical data will be presented.

INV055

Gene therapy to prevent chaotic behaviours in cardiac electrophysiology

S Priori[1]

1: Istituti Clinici Scientifici Maugeri, Pavia

The idea to develop gene therapy to treat electrical dysfunction of the heart is an appealing strategy because of the limited therapeutic options available to manage the most-severe cardiac arrhythmias, such as ventricular tachycardia, ventricular fibrillation, and asystole. However, cardiac genetic manipulation is challenging, given the complex mechanisms underlying arrhythmias. Nevertheless, the growing understanding of the molecular basis of these diseases, and the development of sophisticated vectors and delivery strategies, are providing researchers with adequate means to target specific genes and pathways involved in disorders of heart rhythm. Data from preclinical studies have demonstrated that gene therapy can be successfully used to modify the arrhythmogenic substrate and prevent life-threatening arrhythmias. Therefore, gene therapy might plausibly become a treatment option for patients with difficult-to-manage acquired arrhythmias and for those with inherited arrhythmias.

INV056

Shining light on cardiac tachyarrhythmias

E CA Nyns[1] B O Bingen[1] C I Bart[1] A Kip[1] R H Poelma[2] L Volkers[1] J J Plomp[1] W Jangsangthong[1] M C Engels[1] M J Schalij[1] G Q Zhang[2] A AF de Vries[1] D A Pijnappels[1]

1: Leiden University Medical Center 2: Delft University of Technology

Background: Cardiac tachyarrhthymias are a growing global health problem, e.g. atrial fibrillation (AF) is predicted to affect 18 million Europeans by 2060. Current treatment of these heart rhythm disorders comprises (i) relatively ineffective and/or toxic medication, (ii) ablation resulting in loss of contractile tissue and (iii) delivery of traumatizing and painful high-voltage shocks. This has initiated the search for alternative therapies. The discovery of light-gated ion channels (so-called channelrhodopsins) in algae and their subsequent application to control neural activity via optogenetics tempted us to explore their usefulness for managing cardiac tachyarrhythmias. Methods: Recombinant channelrhodopsin genes were introduced into cultured neonatal rat atrial cardiomyocytes using lentivirus vectors and in adult rat hearts by local/systemic administration of AAV vectors. Subsequently, tachyarrhythmias were induced by rapid electrical stimulation (*in vitro* AF model, *ex vivo* model of ventricular tachyarrhythmias/fibrillation) or by rapid electrical stimulation together with infusion of the action potential duration-shortening drug carbachol (*ex vivo* and *in vivo* AF models). Next, photocurrents of different strength and duration were induced in the genetically modified cardiomyocytes using LED light. Results: In each of the cardiac arrhythmia models, sinus rhythm could be safely, effectively and repetitively restored by a combination of gene therapy and light administration without causing permanent cell/tissue damage or long-term disturbances of the cardiomyocytes' electrical function. Conclusion: Optogenetics allows the heart to produce by itself electrical currents strong enough for arrhythmia termination. These findings raises the prospect of designing and developing novel, effective and painless strategies of cardiac arrhythmia management through biomedical engineering.

INV057

Gene Therapy of Hemoglobinopathies

M Cavazzana

1: Institut Imagine, Paris

No abstract available

INV058

Towards next-generation gene therapy with *ex vivo*-engineered hematopoietic stem and progenitor cells

B Gentner[1]

1: San Raffaele Telethon Insitute for Gene Therapy (HSR-TIGET)

An increasing number of clinical trials is showing the potential of *ex vivo* gene therapy using autologous hematopoietic stem and progenitor cells (HSPC) to cure genetic diseases. The lentiviral vector platform has emerged as the clinically most successful tool to genetically engineer HSPC, to date. Further refinements include the use of transduction enhancers, tailored conditioning regimens, optimization of hematopoietic recovery times and *ex vivo* HSPC expansion, ideally paired with biomarkers that accurately predict patient outcome. We recently introduced a novel transduction protocol based on shortened *ex vivo* culture time and prostaglandin E2. Initial clinical results of this protocol in the context of a phase I/II trial in children affected by Mucopolysaccharidosis type I –Hurler (NCT03488394) will be available. A next step for pipeline improvements (including gene editing) is the implementation of effective *ex vivo* expansion protocols that make use of molecules fostering HSC division without compromising their long-term repopulating potential. We set up a single cell RNA sequencing platform to more reliably identify HSC in culture and gain a mechanistic understanding on the determinants of their expansion, maintenance and loss through differentiation. This will help to design optimal HSC expansion protocol for future gene therapy applications.

INV059

Cardiac development: basis for disease and regeneration

D Srivastava[1 2]

1: Gladstone Institutes 2: University of California, San Francisco

Heart disease is a leading cause of death in adults and children. We, and others, have described complex signaling, transcriptional and translational networks that guide early differentiation of cardiac progenitors and later morphogenetic events during cardiogenesis. By leveraging these networks, we have reprogrammed disease-specific human cells in order to model genetically defined human heart disease in patients carrying mutations in cardiac developmental genes. These studies revealed mechanisms of haploinsufficiency and we now demonstrate the contribution of genetic variants inherited in an oligogenic fashion in congenital heart disease. In some cases, mutations disrupted repression at non-cardiac gene loci and enhanced open chromatin states at endothelial/endocardial promoters within cardiomyocytes, suggesting a regulated genetic switch between these cell types. We also utilized a combination of major cardiac developmental regulatory factors to induce direct reprogramming of resident cardiac fibroblasts into cardiomyocyte-like cells with global gene expression and electrical activity similar to cardiomyocytes, and now have revealed the epigenetic mechanisms underlying the cell fate switch. Most recently, we identified an approach to unlock the cell cycle in adult cardiomyocytes by introducing fetal cyclins and cyclin dependent kinases, and have been able to induce resident, post-mitotic cardiomyocytes to undergo cell division efficiently enough to regenerate damaged myocardium. Knowledge regarding the early steps of cardiac differentiation *in vivo* has led to effective strategies to generate necessary cardiac cell types for disease-modeling and regenerative approaches, and may lead to new strategies for human heart disease.

INV060

Precision reprogramming approaches to cardiovascular and neurologic disease

K Baldwin[1]

1: Scripps Research

Human genomic studies are generating increasingly large lists of variants linked to our differential risks for prevalent diseases such as cardiovascular and neurologic disease, many of which cannot be well studied in non-human model systems. We have discovered a new set of reprogramming methods to engineer induced human neurons that express genes and gene networks linked to various disorders such as autism, schizophrenia and neurodegenerative disease. We have also applied genetically guided genome editing in iPSCs to demystify a large non-coding risk haplotype responsible for 10-15% of coronary artery disease incidence. We will discuss our results and implications of the findings for translating the results of human genetic studies into appropriately designed and targeted therapies.

INV061

Towards a pluripotent-based cell therapy for Parkinson's disease

L Studer[1]

1: Memorial Sloan Kettering Cancer Center, New York

No abstract available

INV062

Repetitive elements and stem cells

J A Erwin[1 2 3]

1: Salk Institute for Biological Studies 2: Johns Hopkins School of Medicine 3: Lieber Institute for Brain Development 4: J. Craig Venter Institute

DNA derived from repetitive elements comprises nearly half of the human genome. Instability of repetitive DNA sequences is associated with cancer and neurodegenerative disorders. Endogenously encoded Long Interspersed Element-1 (LINE-1 or L1) is the most abundant autonomous mobile element in mammals, comprising about 17% of mammalian genomes. L1 elements contain a promoter and two open reading frames that encode for proteins with RNA binding, reverse transcriptase and endonuclease activity. L1 sequences actively ''jump'' around the genome through a copy-and-paste mechanisms, where L1 RNA is transcribed and then inserted and reverse transcribed into a new genomic location. The expression and mobilization of L1 elements is likely a lifelong process, and retrotransposition activity is tightly regulated in all cell types. As neural stem cells differentiate into neurons, L1 is highly upregulated. While it has been hypothesized that aberrant retrotransposon activity could contribute to neurological disorders, regulating factors and functional consequences in brain are largely unknown. I will present the identification of neural specific regulators of somatic L1 retrotransposition and the finding that L1 genomic variation mediates an important environmental risk factor for aberrant neural development causing deficits in sensory gaiting. These findings suggest that properly tuned levels of somatic mosaicism are essential for healthy cognitive function.

INV063

Mechanisms controlling cell fate decisions in human pluripotent stem cells

L Vallier[1]

1: Wellcome Sanger Institute

Understanding the mechanisms controlling cell fate decisions in stem cells is of great interest since tight regulation of differentiation is necessary for embryonic development, organ homeostasis, tissue repair and disease. Human pluripotent stem cells (hPSCs) induced (hIPSCs) or embryonic (hESCs) have an unsurpassed interest to study these mechanisms. Indeed, they can proliferate indefinitely while maintaining the capacity to differentiate into a homogenous population of endoderm, mesoderm and neuroectoderm cells. This process follows a natural path of development and has already been useful to discover mechanisms relevant for the formation of these germ layers *in vivo*. hPSCs are also compatible with genome wide analyses while a diversity of tools is now available to study gene function during their differentiation. Finally, hPSCs can be further differentiated into cell types relevant for disease modelling, drug screening and cell based therapy. Thus, deciphering the mechanisms controlling their differentiation will facilitate the delivery of their clinical promises. Here, we will present our latest investigations concerning the mechanisms orchestrating pluripotency and cell fate decisions in hPSCs. More precisely, we will describe the molecular interplays by which Acitivin/Nodal signalling coordinates the core transcriptional network, epigenome and epitranscriptome characterising hPSCs not only to maintain their pluripotent state but also to allow differentiation. This knowledge

will be essential to develop universal protocols of differentiation for the production of cell types with a clinical interest.

INV064

Orthologous dependoparvovirus molecular fossils may provide a source of novel structural motifs and capsids for rAAV

R Kotin[2] E Hildebrant[2] J Penzes[3] M Agbandje-McKenna[3] R Gifford[4]

1: Generation Bio, Cambridge, MA 2: University of Massachusetts Medical School, Worcester, MA 3: University of Florida, Gainesville, FL 4: MRC-University of Glasgow Centre for Virus Research

Adeno-associated virus vectors (rAAV) capsids are derived primarily from naturally occurring primate viruses and synthetically engineered variants. To increase the diversity of rAAV capsids, several approaches have resulted in several novel vectors. One strategy, capsid shuffling, involves re-assortment of highly conserved AAV capsid open reading frames (ORFs) following fragmentation. Other methods based on synthesis of *cap* ORFs incorporate selectively randomized codons within the virus protein's (VP) nine variable regions (VR I-IX). The theoretical combinatorial libraries would be too large to synthesize completely, and efficiently screening the synthesized-capsid vectors poses challenges due to the representation of any single capsid in the library, competition for receptors, etc. Rationally designed vectors produced individually or in a pool, are an alternative to randomized capsid libraries that circumvent the dilutive effects of the vast combinatorial libraries, but provide relatively few capsids. An additional method to obtaining novel capsids involves the mining of potential host genome sequence databases for endogenous AAV sequences. Dependoparvovirus signatures identified in whole genome sequences deposited in GenBank using DIGS (https://giffordlabcvr.github.io/DIGS-tool/) indicate that historically a diversity of host species have been infected with AAV that entered the germline to become integrated in the host genome as endogenous viral elements (EVEs). Indels within these EVEs indicate that the virus sequences elements are no longer intact and of indeterminate age. We expanded *in silico* analysis of EVE sequences beyond these reference genomes using DNA from tissue specimens and cell lines of related sister taxa, which allowed us to determine EVE positive and negative lineages. Conservation of the EVE within phylogenetic taxon demonstrate that a single proto-species was infected with an AAV-like particle. Using standardized dating methods, we estimate that in one order, the ancient virus was circulating approximately 77 to 55 million years ago (MYA). These AAV ''fossils'' are distinct sequences relative to traditional AAV gene therapy vector sequences with capsid sequences inferred from the orthologous species EVEs that appear to include novel motifs not represented within AAV species that may be informative for new capsid libraries.

INV065

Gene therapy for DMD: from bench to bedside

J Samulski[1] X Xiao[1]

1: Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

This presentation will detail a 25-yr effort on gene therapy for Duchenne muscular dystrophy (DMD). Duchenne muscular dystrophy (dys) is a single gene defect that is X-linked and primarily only affects males. This gene codes for a mRNA that is over 14kb in size and therefore too large for many of the viral vectors more specifically for AAV packaging capacity of 5 kb. To circumvent this concern, mini dys transgene cassettes were designed to fit in the AAV packaging constraints and tested in MDX mouse model for efficacy. Following design of functional transgene cassettes, an effort was focused on AAV capsids that would provide global transgene transduction without toxicity. Phase 1 studies of direct muscle injection were carried out in 2006 to validate the safety of this transgene cassette. These studies were further extended with Phase 1 safety trials evaluating ''limb infusion'' studies completed in 2009. Finally monkey and canine studies refined the vector design to an novel clinical vector carrying 1) muscle specific promoter, 2) optimized mini dys transgene, 3) packaged in an AAV serotype and chimeric capsid (AAV 9 and AAV 2i8) for systemic delivery. IND enabling studies followed by FDA approved tox biodistribution experiments resulted in approval for transitioning into Phase1/2 clinical trials that have commenced this spring. The supporting studies and early clinical observations will be presented as a case study of developing ''bench to bedside'' gene therapy.

INV066

Starting from the end: analytics driving the manufacturing process of viral vectors

E Ayuso[1]

1: INSERM UMR1089, University of Nantes, Nantes University Hospital

Recent successes in the gene therapy field boosted the interest in the use of viral vectors for clinical trials and, consequently, the global demand has almost collapsed manufacturing facilities worldwide. Concurrent with this, regulatory agencies encourage the scientific community to develop novel analytical tools to ensure the quality and purity of these drugs. Several technological platforms are being used to produce viral vectors at large scale but the quality attributes of such products can be only compared by robust analytical methods. Our laboratory has been deeply involved in the harmonization of adeno-associated viral vector (AAV) characterization through the international reference standard efforts and has developed novel protocols for accurate titration of these vectors. Moreover, recent analytical tools based on next generation sequencing technologies, allowed us to identify and quantify residual DNA species in vectors produced in mammalian and insect cells. Importantly, the identification of these sequences led us to modify the manufacturing process and, in particular, introduce changes in the starting materials. More recently, we have detected the presence of miRNA in the final product (i.e. after purification) of AAV vectors. Although the consequences of this finding are still under evaluation, it seems reasonable that specific purification steps should be implemented to remove these contaminants in the future. While many efforts are focused on the optimizations of upstream methods to increase vector yields by using large volumes (>200L), our examples also showed that it is crucial to push vector analytics forward to drive innovation in manufacturing processes and vector design, and ultimately, improve the purity and safety of these novel drugs.

INV067

New insights in CRISPR/Cas specificity, DNA repair dynamics and DNA repair outcomes in gene edited human hematopoietic stem cells

T Cathomen[1]

1: University Medical Center Freiburg

Therapeutic genome editing with designer nucleases has shown great success in the last few years, leading to their application in several clinical trials. Despite their great potential, engineered nucleases can lead to genotoxic side effects by introducing mutations or chromosomal aberrations through activity at unintended sites, so called off-target sites. Computational prediction tools and recently developed genome-wide methods to identify off-target activity or chromosomal rearrangements unveiled these risks but have some unresolved limitations, such as low sensitivity or failure to detect particular forms of chromosomal aberrations. Novel genome-wide assays that we have established in our lab allowed us to detect off-target activity as well as the resulting chromosomal aberration, including large deletions, inversions and translocations, with unparalleled sensitivity in clinically relevant human cells. Moreover, validation of the next generation sequencing results with digital PCR enabled new insights in the DNA repair kinetics of CRISPR/Cas induced double strand breaks in these cells. In summary, we have developed highly sensitive assays that allow us to expand our understanding of the specificity of engineered nucleases and the DNA repair dynamics and to detect hitherto unidentifiable chromosomal aberrations in gene edited human stem cells.

INV068

Delivering on the therapeutic potential of CRISPR/cas9: development of an LNP-mediated genome editing therapeutic for the treatment of ATTR

Y Chang[1]

1: Intellia Therapeutics

Development of CRISPR/Cas9 as a genome editing tool has triggered intense interest in how to apply this technology to revolutionize medical care and treatment. The development of clinically viable delivery methods, however, presents one of the greatest challenges in its therapeutic application. Hereditary transthyretin amyloidosis (hATTR) is an autosomal dominant progressive disease caused by accumulation of amyloid deposits of misfolded transthyretin (TTR) protein in multiple tissues, including the heart, nerves and gastrointestinal tract. Most circulating TTR protein is produced by and secreted from the liver, and reduction of hepatic TTR protein production is a clinical approach to ATTR disease management. Here we report the development of a lipid nanoparticle (LNP) encapsulating CRISPR/Cas9 components targeting human TTR that enables significant editing of the TTR gene across multiple species, including mice, rats and non-human primates (NHPs). Following a single dose of LNP-delivered CRISPR/Cas9 in both mice and rats, we achieved editing levels that resulted in >97% reduction in circulating serum protein, which was sustained for at least 12 months in mice. Additionally, in a humanized mouse model of hATTR that expresses the V30M mutant form of the human TTR protein, we demonstrated rescue of amyloid deposition in multiple tissues after a single dose of LNP containing the CRISPR/Cas9 components. Concurrently, in NHPs we achieved a therapeutically meaningful level of TTR protein reduction that correlated with robust and significant whole liver editing following single and multiple injections of LNPs. These results were enabled, in part, by favorable safety aspects of our delivery system: the transient nature of the CRISPR/Cas9 components and the biodegradable ionizable lipid component of the LNPs. Consequently, the Spy Cas9 mRNA, sgRNA and ionizable lipid were quickly cleared from circulation, with plasma and liver half-lives of 23 hours and 17 hours, respectively, in NHPs. These findings demonstrate the potential of *in vivo* CRISPR/Cas9 genome editing and suggest that future therapies based on this platform may enable next-generation, curative treatment paradigms for chronic genetic diseases such as ATTR.

INV069

***In vivo* CRISPR gene editing with no detectable genome-wide off-target mutations**

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CRISPR/Cas nucleases hold substantial promise for gene editing-based human therapeutics but identification of unwanted off-target mutations is critically important for clinical translation. To date, a well-validated method that can robustly identify *in vivo* off-targets has not been described, leaving it uncertain whether and how frequently these mutations occur. In this talk, I will describe the development and validation of the Verification of *In vivo* Off-targets (**VIVO**) method, which can robustly and sensitively identify genome-wide CRISPR/Cas nuclease off-target effects *in vivo*. With a guide RNA (**gRNA**) deliberately designed to be promiscuous, we used VIVO to show that CRISPR/Cas nucleases are capable of inducing substantial off-target mutations in mice livers *in vivo*. More importantly, we show that appropriately designed gRNAs (designed to be more orthogonal to the mouse genome) can induce efficient *in vivo* editing in mice livers with no detectable off-target mutations as judged by VIVO. Our results delineate a general strategy for defining and quantifying gene-editing nuclease off-target effects in the context of a whole organism, thereby providing a useful method that can foster development of *in vivo* gene editing therapeutic strategies.

INV070

Universal donor stem cells

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No abstract available

INV071

The human retina and its organoids at single cell resolution

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No abstract available

INV072

Differential regulation of Oct4 targets facilitates reacquisition of pluripotency

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Ectopic transcription factor expression enables reprogramming of somatic cells to pluripotency, albeit with low efficiency. Despite steady progress in the field, the exact molecular mechanisms that coordinate this remarkable transition still remain largely elusive. We will present results that characterize the final steps of pluripotency induction. Specifically, we identify a set of OCT4 bound cis-regulatory elements that are dynamically regulated in differentiation and appear central to facilitating reprogramming. Interestingly, these regions retain a distinct epigenetic signature during *in vitro* and *in vivo* differentiation that may allow them to act as primary targets of ectopically induced factors during somatic cell reprogramming.

INV073

Cell therapy for Parkinson's disease with induced pluripotent stem cells

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The innovation of induced pluripotent stem cells (iPSCs) and previous embryonic stem cell (ESC) technologies are drawing attention to their application for regenerative medicine. Parkinson's disease is one of the most promising target diseases based on the history of fetal nigral transplantation in clinics. Although pharmacological treatments for PD, such as L-dopa, show good response in the early phase, patient outcomes over the long term are unsatisfactory. As an additional treatment, cell therapy with aborted fetal tissues has been performed since 1980's. The limited supply of donor source and the unstable quality of the cells prevent this therapy with fetal tissue from becoming standard. The technology of iPSCs offers a limitless and more advantageous donor source than aborted tissue. One of the advantages is possibility of preparing immunologically compatible donor cells from self-derived or allogeneic iPSCs. We are preparing a clinical trial that involves the transplantation of dopamine neural progenitors differentiated from iPSCs. We have successfully established a protocol for donor induction with clinically compatible grade and have transplanted these neurons into PD models of mice, rats, and cynomolgus monkeys as preclinical studies. The presentation will include the recent results of our research.

INV074

MLLT3 governs human hematopoietic stem cell self-renewal

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Development of methods for *ex vivo* expansion of hematopoietic stem cells (HSC) would greatly improve transplantation therapies. To achieve this, we need better understanding of HSC self-renewal mechanisms and why this machinery fails in culture. Transcriptional profiling of human HSC and their differentiated and cultured progeny identified MLLT3/AF9 as a critical HSC regulator enriched in human HSC in all sources, but downregulated in culture. MLLT3 knockdown disrupted the expansion and engraftment of human hematopoietic stem/progenitor cells (HSPC). Conversely, sustaining physiological MLLT3 levels in cultured HSPC using lentiviral vectors expanded HSPC with superior capacity for engraftment (14-30 fold higher than controls) that was maintained over 6 months and persisted upon secondary transplantation. Only MLLT3 expressing cells repopulated bone marrow HSPC compartment and differentiated lineages in peripheral organs. Limiting dilution analysis verified 20-fold increase in repopulating units among MLLT3-expressing HSPC during 10-day culture. Importantly, MLLT3 did not reprogram progenitors to HSC or cause malignant transformation. MLLT3 localized to promoters of active genes, directly maintaining key HSC regulators such as HLF and MECOM, while indirectly suppressing abnormal activation of immune response and apoptosis genes in cultured HSPC. MLLT3 thus represents a novel HSC maintenance factor that may help expand HSC for therapeutic applications.

INV075

Treatment of lysosomal storage diseases (MPS-IIIA and IIIB) by intravenous administration of AAV vectors

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Lysosomal storage disorders (LSDs) are rare inborn errors of metabolism, characterized by the accumulation of undigested or partially digested macromolecules, which ultimately results in cellular dysfunction and clinical abnormalities. Organomegaly, connective-tissue and ocular pathology, and central nervous system dysfunction may result, with different degrees of central nervous system versus systemic involvement, depending on the disease. AAV vectors are ideal for the treatment of LSDs because their relatively easy manipulation and production in big titers, as well as its proven safety records and long-term expression in the transduced tissues. AAV9 is particularly suitable for LSDs with an important compromise of the Central Nervous

System (such as MPS-III or Neuronal Ceroid Lipofuscinosis 1 and 3) because of its capacity to cross the Blood Brain Barrier and the possibility to be administered by intravenous route. This presentation will review available clinical data from ongoing clinical trials with AAV9 for Sanfilippo Syndrome IIIA and IIIB.

INV076

Intracerebral gene therapy: neurosurgical point of view

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Among all the accesses for brain gene therapy, the direct multiple intracerebral injections are the most challenging. We will report the process from the preclinical studies to the phase I/II clinical trials for MPS IIIA, MPS IIIB and MLD. Advantages and risks will be discussed and final results will be described.

INV077

Ex vivo generated lymphoid progenitors for immune reconstitution in the context of allogeneic transplantation and gene therapy

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The thymic microenvironment (including cytokines and a cell-cell interaction mediated by Delta-4 (DL-4) ligand and its receptor Notch1) supports and influences T-cell commitment. An*in vitro*feeder-free cell culture based on Notch ligand DL-4 has been developed in our laboratory leading to the generation of T-cells progenitors from both human cord blood and adult $CD34^+$ hematopoietic stem and progenitor cells (HSPCs)[1,2]. Since TNFα is constitutively synthesized in the thymus, we explored its role during human early T-cell development. TNFα accelerated early T-cell differentiation and greatly increased the number of generated $CD34^-CD7^+CD5^-$ T-cell precursors after 7 days in the *in vitro* DL-4 culture. TNFα-induced $CD7^+$ T-cell precursors expressed early T-cell commitment markers and had T-cell differentiation potential *in vitro*and *in vivo*when transplanted to NOD/SCID/$\gamma c^{-/-}$ mice. From day 4 to day 7, in DL-4 culture with TNFα, there was a higher rate of proliferative cells. A higher percentage of $CD7^+$ T-cells progenitors generated after 7 days of culture were in S/G2/M phases after exposure to TNFα. These results suggested that TNFα might regulate cell cycle by promoting T-cell progenitors entry into the cell cycle. We have translated our culture system into a clinical grade protocol, which will be used to improve T-cell recovery after haploidentical hematopoietic stem cell transplantation in SCID patients. Our culture system was also successfully combined to gene therapy allowing the production of gene-corrected T-cell precursors from SCID-X1 patient's HSPCs. Our work provides a promising approach to improve T-cell recovery after in human.

INV078

The use of autologous hematopoietic cell transplantation in the treatment of multiple sclerosis

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Autologous haematopoietic cell transplantation (AHCT) is an evolving treatment avenue in multiple sclerosis (MS), which is highly effective in controlling disease activity and improving disability. However, this treatment is associated with intrinsic toxicities and risks compared with conventional therapies. Currently published data suggest that AHCT's use is associated with significant reduction in MS disease activity and marked improvement in disability when used in patients with highly active relapsing remitting disease. Its long term safety and efficacy have not been fully evaluated but as increasing clinical trial data are published, its use is likely to grow. Further randomised controlled studies are needed to compare AHCT with standard disease modifying therapies and to optimise transplant regimens. Mechanistic studies may provide potential markers for response and a better understanding of disease pathogenesis.

INV079

Exploring and engineering the cell-material interface for regenerative medicine and mechanobiology

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Understanding how cells interact with surfaces is key to a host of applications in biomedical science from regenerative medicine and drug delivery to biosensors (1-5). This talk will highlight both exciting advances in engineered biomaterials and in state of the art imaging approaches to enable exploration and engineering of the cell-material interface for regenerative medicine and mechanobiology. 1) T. von Erlach, S. Bertazzo, M. A. Wozniak, C.-M. Horejs, S. A. Maynard, S. Attwood, B. K. Robinson, H. Autefage, C. Kallepitis, A. De Rio Hernandez, C. S. Chen, S. Goldoni, M. M. Stevens#. ''Cell geometry dependent changes in plasma membrane order direct stem cell signalling and fate.'' Nature Materials. 2018. 17: 237-242; 2) N. Reznikov, M. Bilton, L. Lari, M. M. Stevens#, R. Kröger. ''Fractal-like hierarchical organization of bone begins at the nanoscale.'' Science. 2018. 360 (6388): eaao2189; 3) S. Bertazzo, E. Gentleman, K. L. Cloyd, A. H. Chester, M. H. Yacoub and M. M. Stevens#. ''Nano-analytical electron microscopy reveals fundamental insights into human cardiovascular tissue calcification.'' Nature Materials. 2013. 12: 576-583; 4) C. Chiappini, E. De

Rosa, J. O. Martinez, X. W. Liu, J. Steele, M. M. Stevens#, E. Tasciotti#. ''Biodegradable silicon nanoneedles deliveringnucleic acids intracellulary induce localized *in vivo* neovascularization.'' Nature Materials. 2015. 14: 532-539; 5) P. D. Howes, R. Chandrawati, M. M. Stevens#. ''Colloidal nanoparticles as advanced biological sensors.'' Science. 2014. 346: 53-63

INV080

Engineering stem cell-derived human neural tissues to study brain development and disease

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No abstract available

INV081

From cells to organs: exploiting the organ niche for interspecies organogenesis

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Hematopoietic stem cells (HSCs) have provided a number of conceptual ideas and models. Among them is the concept of the ''niche'', a special bone-marrow microenvironment that by exchanging cues regulates stem-cell fate. The HSC niche also plays an important role in HSC transplantation. Successful engraftment of donor HSCs critically depends on myeloablative pretreatment to empty the niche, such as high-dose irradiation or chemotherapy. The concept of the stem-cell niche was further extended to generation of organs. We postulated that an ''organ niche'' exists in a developing animal and that this niche was empty when development of an organ is genetically disabled. This organ niche should be compensated developmentally by blastocyst complementation using wild-type pluripotent stem cells (PSCs). We provided proof of principle of organogenesis from xenogeneic PSCs in an embryo unable to form a specific organ, demonstrating in mouse the generation of functionally normal rat pancreas by injecting rat PSCs into *Pdx1-/-* (pancreatogenesis-disabled) mouse embryos. Furthermore, we succeeded in generating functional mouse pancreas by injecting mouse PSCs into Pdx1-/- rat embryos. When 100 islets obtained from these mouse pancreas generated in rats were transplanted to streptozotocin-induced mouse, blood glucose levels of recipient mice were normalized over a year without immunosuppression. If the principle of interspecific blastocyst complementation holds between human and large animals like pigs or sheep, we may be able to generate functional human iPSC-derived pancreata for the study of organogenesis, disease and therapeutic modelings and ultimately to use as donor organs.

INV082

Translating human development to new therapies with pluripotent stem cells

G Keller[1]

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Human pluripotent stem cells (hPSCs) represent a novel cell source for modeling human cardiovascular development and disease *in vitro* and for developing new therapies to replace or regenerate heart tissue damaged by age or disease. To study and treat diseases that affect specific regions of the heart, it is essential to be able to generate different cardiomyocyte subtypes from hPSCs. In this study, we used a developmental biology-guided approach to identify the key signaling pathways that promote the efficient differentiation of ventricular and atrial cardiomyocytes, with the goal of generating populations highly enriched in each of these cardiac subtypes. We found that retinoic acid (RA) signaling at the mesoderm stage of development is essential for specifying the human atrial lineage. Additionally, we show that atrial and ventricular cardiomyocytes develop from distinct mesodermal subpopulations that can be identified by the expression of retinaldehyde dehydrogenase 2 (RALDH2), an enzyme involved in RA synthesis and glycophorin A (CD235a) respectively. The RALDH2+ atrial mesoderm, but not the CD235a+ ventricular mesoderm responds to retinol (RA precursor) to generate atrial cardiomyocytes demonstrating that this lineage is specified through an autocrine signaling loop during human heart development. Further molecular and functional analyses have shown that the generation of optimal atrial and ventricular cardiomyocyte populations depends on appropriate mesoderm specification. With these developmental insights, we are now able to generate populations highly enriched in specific cardiomyocyte subtypes appropriate for cell-based therapy and disease modeling applications.

INV083

Molecular mechanisms of cellular reprogramming

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The generation of induced pluripotent stem cells (iPSCs) by overexpression of Oct4, Sox2, Klf4 and c-Myc, transformed our classical views of the cellular epigenetic landscape and highlighted transcription factors as powerful tools for cell conversions and tissue engineering. Following this seminal work, several other cell types have also been generated by master transcription factor (TF)-mediated transdifferentiation. However, the molecular mechanisms underlying the diverse cellular identity changes are not well understood. Through the investigation of reprogramming mechanisms, we recently revealed that over-expression of constitutive active Smad3 boosted not only iPSC generation, but also 3 other master TF-mediated conversions, from B cells to macrophages, myoblasts to adipocytes, and human fibroblasts to neurons. This has demonstrated that there are common mechanisms underlying different master TF-mediated cell conversions. To illuminate reprogramming mechanisms further, we have performed CRISPR/Cas9-mediated genome-wide knockout screening during MEF reprogramming with a lentiviral gRNA library containing 90,000 gRNAs. This screening provided us with 9 novel reprogramming roadblock genes as well as 12 genes essential for iPSC generation but not for ES cell self-renewal or MEF proliferation. Excitingly, many of those essential genes enhanced reprogramming when overexpressed together with Yamanaka factors. This data set will be a valuable resource for better understanding of molecular mechanisms of iPSC generation, and which could be influential in achieving any other cell conversions more efficiently and faithfully.

INV084

Unique control of naïve pluripotency in human stem cells and the germline

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Pluripotency is the ability to differentiate into every cell type in the body including cells of the germline. The spectrum of human pluripotency begins with naïve cells, which are the pluripotent cell types found in human pre-implantation embryos, and primed cells corresponding to pluripotent cells of the post-implantation blastocyst just prior to gastrulation and lineage specification. Given the challenges of working with human embryos, modeling the different states of human pluripotency and early lineage specification is achieved using human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs). In this study we performed Assay for Transposase Accessible Chromatin followed by Sequencing (ATAC-Seq) to identify regions of open chromatin unique to naïve and primed hESCs as well as lineage specified human germline cells called primordial germ cells (PGCs) and PGC-like cells (PGCLCs) *in vitro*. We discovered thousands of open chromatin peaks that are unique to each cell type, and identified the transcription factor motifs enriched within each region. One highly significant candidate motif found in the open chromatin of naïve hESCs and germline cells was AP2. In both cases, we determined that this motif is bound by a transcription factor called Transcription Factor AP-2 Gamma (TFAP2C). Using combinations of CRISPR/Cas9, Chromatin Immunoprecipitation, Sequencing and Imaging we show that TFAP2C is required for the establishment of naïve pluripotency by binding a unique set of enhancers including a novel enhancer at the OCT4 locus.

INV085

Reaching beyond the cold chain: formulation design of vaccines to improve potency, enhance distribution and modulate other biological processes

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Vaccine formulation development often identifies molecules with adjuvant properties and others that maintain physical stability of antigen during storage and transport. Several examples illustrating how pharmaceutically acceptable reagents can fulfill both objectives will be discussed. In the first, a target-driven screening method allowed for identification of non-toxic excipients that improved the potency of an Ebola vaccine and which stabilized the vaccine for extended periods of time at ambient temperature. In the second, specific elements of vaccine candidates (engagement of integrins, transgene expression) were found to significantly impact the expression and function of hepatic cytochrome P4503A4 (CYP3A4), responsible for the metabolism of over 60% of drugs currently on the market in several *in vivo* models. Strategies for capitalizing on these effects and for reversing them will be discussed. Taken together, these cases illustrate how a heuristic approach to formulation development can impact the potency and safety profile of vaccines while also extending their shelf life.

INV086

Engineering AAV vectors to evade innate immune and inflammatory responses

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Many new therapeutic modalities including gene therapy rely on nucleic acid-based systems. However, nucleic acids can trigger host immune responses *in vivo* that interfere with the effectiveness of the therapy. For example, studies have shown that the DNA genome of the viral vector adeno-associated virus (AAV) can trigger Toll-like receptor 9 (TLR9), a pattern recognition receptor in immune cells that sets off inflammatory and innate immune defenses after sensing foreign DNA. Interestingly, prior studies showed that Tlr9-/- mice achieved higher and/or more durable levels of transgene expression compared to wild-type mice following AAV gene therapy. Thus, we sought to engineer AAV in a way that would intrinsically avoid triggering TLR9. One strategy for blocking TLR9 activation in cell culture is the administration of specific short single-stranded DNA oligonucleotides (typically 12-25nt) that bind TLR9 with high avidity and antagonize its activation. We hypothesized that these TLR9-inhibitory sequences could retain functional activity when incorporated into a longer strand of DNA, thereby shielding the DNA from TLR9 activation. We engineered various AAV vectors to carry TLR9-inhibitory sequences in untranslated regions. In our *in vivo* experiments in mouse and large animal models, we found that these engineered vectors elicited markedly reduced innate or adaptive immune responses compared to the unmodified vectors, leading to lower toxicity and/or higher transgene expression. Our work highlights an underappreciated source of immunogenicity for AAV gene therapy and offers a versatile, broadly applicable solution. Specifically, incorporating short TLR9-inhibitory sequences within the AAV genome can ''cloak'' the genome, reducing innate immune and inflammatory responses and improving the effectiveness of the therapy. This approach may guide the design of future nucleic acid-based therapeutics.

INV087

Gene therapy for Parkinson disease: implications from a clinical study of AADC deficiency

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In Parkinson disease (PD), cardinal motor symptoms become apparent after striatal dopamine has been reduced to approximately 20% of normal levels. One approach of gene therapy is to restore the local production of dopamine in the striatum by delivering dopamine-synthesizing enzymes. In open-label clinical studies, patients' motor symptoms improved after gene transfer of aromatic L-amino acid decarboxylase (AADC) into the putamen with persistent expression of the transgene. The beneficial effects of AADC gene transfer have also been shown in children with AADC deficiency. The restoration of dopamine synthesis in the putamen provided remarkable improvement in the motor function of all 7 patients across age, genotype, and disease severity. In one patient with a moderate phenotype, cognitive and verbal functions were also improved. Positron emission tomography with 6-[18F]fluoro-L-m-tyrosine, an AADC-specific tracer, showed robust expression in the putamen at two years post gene therapy. The patients

exhibited transient choreic dyskinesia as an adverse event. In both PD and AADC deficiency, dopamine production is assumed to take place in the transduced medium spiny neurons. In PD, it would be advantageous to deliver genes for L-dopa synthesis, such as tyrosine hydroxylase and guanosine triphosphate cyclohydrolase I, in addition to the AADC gene, to reduce motor fluctuations associated with intermittent oral L-dopa intake. Recently, stem cell transplantation has attracted much attention. However, if the primary mechanism underlying recovery in cell therapy is restoration of dopaminergic neurotransmission, gene therapy is a more straightforward approach.

INV088

Expanding AAV transfer capacity in the retina

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As AAV-mediated retinal gene therapy has recently entered the market and is being developed for dozens of different ocular indications, the cargo capacity of AAV vectors restricted to around 5 kb represents a major limitation to their applications for gene therapy of a number of inherited retinal degenerations due to mutations in large genes. Dual AAV vectors each containing one half of a large expression cassette that is reconstituted in target cells upon co-infection by and recombination of dual AAV are effective for some IRDs, however they provide lower transduction levels than single AAV. We have recently tested protein-trans splicing mediated by split inteins as a strategy to expand AAV transfer capacity in the retina. In this platform, delivery of multiple AAV vectors each encoding for one of the fragments of large therapeutic proteins flanked by short split-inteins results in protein trans-splicing and full-length protein reconstitution in the retina of mice, pigs and in human retinal organoids. The levels of large protein reconstitution achieved are therapeutically relevant in mouse models of severe inherited retinal degenerations thus supporting the use of split-inteins-mediated protein trans-splicing in combination with AAV subretinal delivery for gene therapy of inherited blindness due to mutations in large genes.

INV089

Cerebral organoids: modelling human brain development and tumorigenesis in stem cell derived 3D culture

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The human brain is unique in size and complexity, but also the source of some of the most devastating human diseases. While many of these disorders have been successfully studied in model organisms, recent experiments have emphasized unique features that can not easily be modeled in animals. We have therefore developed a 3D organoid culture system derived from human pluripotent stem cells that recapitulates many aspects of human brain development. These cerebral organoids are capable of generating several brain regions including a well-organized cerebral cortex. Furthermore, human cerebral organoids display stem cell properties and progenitor zone organization that show characteristics specific to humans. We have used patient specific iPS cells to model microcephaly, a human neurodevelopmental disorder that has been difficult to recapitulate in mice. This approach reveals premature neuronal differentiation with loss of the microcephaly protein CDK5RAP2, a defect that could explain the disease phenotype. More recently, we have been able to generate organoid based models for human brain cancer and demonstrated their feasibility for drug testing. Our data demonstrate an *in vitro* approach that recapitulates development of even this most complex organ, which can be used to gain insights into disease mechanisms.

INV090

Epigenetic regulation in development, aging and disease

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The development of the iPS cell technology has revolutionized our ability to study development and diseases in defined *in vitro* cell culture systems. The talk will focus on the use of gene editing for the study of epigenetic regulation in development and disease. *1. Monitoring the dynamics of DNA methylation at single cell resolution during development and disease:* DNA methylation is a broadly studied epigenetic modification that is essential for normal mammalian development. Current methods to quantify methylation provide only a static ''snap shot'' of DNA methylation, thus precluding the study of real-time methylation dynamics during cell fate changes. We have established a new approach that enables monitoring loci-specific DNA methylation dynamics at single-cell resolution. *2. Editing DNA methylation in the mammalian genome:* The functional significance of specific methylation events in development and disease remains elusive due to lack of experimental approaches to edit these events. We developed a DNA methylation editing toolbox that fusion of either the catalytic domain of Tet1 or Dnmt3a protein to a catalytic inactive Cas9 (dCas9) to achieve targeted DNA methylation editing with co-expression of target-specific guide RNAs. (i) We first validated this tool by turning on or off two endogenous methylation reporters. (ii) With application of dCas9-Tet1, we observed that active de-methylation can be induced in *BDNF* promoter IV to activate its expression in mouse cortical neurons, and showed (iii) that de-methylation of *MyoD* distal enhancer facilitates reprogramming fibroblasts into myoblasts and subsequent myotube formation. (iv) We show that dCas9-Dnmt3a can open CTCF-mediated chromatin loops by targeted methylation of specified CTCF anchor site thus blocking its binding. Our results established that a modified CRISPR system with dCas9 fused by DNA modification enzymes can be assembled into DNA methylation editing tools to study the functional significance of specific methylation event in the mammalian genome. (v) Finally, we show that these tools can edit DNA methylation in mice, demonstrating their wide utility for functional studies of epigenetic regulation.

INV092

Identification of recurrent genetic variants in hPSCs; a changing landscape

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No abstract available

Selected Oral Presentations

OR001

A novel chimeric antigen receptor CAR-T cell approach eliminates prostate cancer in a mouse tumour model

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Although chimeric antigen receptor (CAR) T cell immunotherapy targeting CD19 has shown remarkable success in patients with hematologic malignancies, the potency of CAR T cells in solid tumors, such as prostate cancer, has been low thus far. Several CARs targeting prostate cancer associated antigens, such as prostate-specific membrane antigen (PSMA), have been described. Although PSMA is abundantly expressed on prostate cancer epithelial cells, the potency of those CARs seems modest both *in vitro* and *in vivo*. One way to overcome moderate potency or unsatisfactory specificity is to develop novel CARs based on highly affine and specific scFv. Here, we report the development of novel 2nd generation CARs based on a scFv derived from the monoclonal antibody 3/F11, which was shown to bind extracellular PSMA with higher affinity and specificity than previously described monoclonals. The generated CAR T cells using retroviral transfer could be expanded massively while keeping T stem cell memory phenotype. Upon antigen sensitization *in vitro*, these CAR T cells released pro-inflammatory cytokines and completely eliminated PSMA-positive tumor cells at a low effector-to-target ratio. Moreover, as determined histologically and by *in vivo* imaging, the generated CAR T cells were able to eradicate solid PSMA-positive tumors in a mouse xenotransplantation model in combination with chemotherapy. In conclusion, the preclinical evaluation of our novel PSMA targeting CARs demonstrates that the derived CAR T cells combine excellent specificity with high antigen-specific cytolytic activity *in vitro* and *in vivo*, hence supporting the translation of these CAR T cells to a clinical setting.

OR002

Allele-specific editing of STAT3 mutations with CRISPR/Cas in primary cells of hyper-IgE syndrome patients

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Autosomal dominant hyper-IgE syndrome (AD-HIES) due to mutations in the STAT3 locus is characterized by high serum IgE, recurring staphylococcal skin abscesses, and recurrent pneumonia. While allogeneic hematopoietic stem cell transplantation is generally not recommended due to potential severe side effects, adoptive transfer of autologous T cells in which the mutated STAT3 allele is inactivated or corrected may represent a promising alternative. We have designed allele-specific CRISPR/Cas nucleases to target the DNA sequence corresponding to some of the most common STAT3 mutations (H58Y, C328_P330dup, V463del and V637M). After identification of the best performing CRISPR/Cas candidates in an episomal reporter assay, the selected designer nucleases were tested on genomic level in a human reporter cell line harboring integrated copies of the mutated STAT3 locus. Allele-specific nucleases disrupted 30 % – 70 % of the mutant sequences without altering the endogenous wild-type STAT3 gene. When applied to patient-derived peripheral blood mononuclear cells (PBMCs), the mutation-specific CRISPR/Cas nucleases where able to selectively disrupt up to 70 % of the mutated STAT3 alleles, as assessed by next generation sequencing. A thorough analysis of STAT3 target gene expression by ddPCR suggests that monoallelic STAT3 expression is not sufficient to restore functionality. These results are supported by STAT3 knockout experiments in healthy donor PBMCs, implying that inactivation of the dominant-negative allele in AD-HIES cells might result in haploinsufficiency. Ongoing experiments aiming at allele-specific correction of the underlying mutations will substantiate our hypothesis and help us to establish a novel treatment option for HIES patients.

OR003

Effective targeting of ROR1$^+$ solid tumours with next-generation Chimeric Antigen Receptor therapy

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Cancer immunotherapy with Chimeric Antigen Receptor (CAR)-T cells shows remarkable efficacy in haematological malignancies but limited success in solid tumours, where the hostile tumour microenvironment actively hampers the immune response. This is in part mediated through the PD-1/PD-L1 axis and in line with other studies, we hypothesise that PD-1 blockade mediates improvement of CAR-T cell therapy in solid tumours. Receptor tyrosine kinase-like orphan receptor-1 (ROR1) is overexpressed in several haematological and solid malignancies with limited expression on normal tissues, making it an attractive target for immunotherapy. Focusing on a monotherapy approach, we developed a next-generation CAR-T system where engineered cells target ROR1 and, upon activation, secrete anti-PD-1 single chain variable fragments (scFvs). These scFvs bind to surface PD-1, protecting activated T cells from PD-L1-mediated inhibition in the confines of the tumour microenvironment. In *in vitro* assays we measured significant and targeted cytotoxicity at various E:T ratios (1:1-1:20) against the ROR1$^+$ leukaemia cell line Kasumi2 and the breast cancer cell line MDA-MB-231. CAR-T cells secreting scFvs showed superior pro-inflammatory cytokine production and killing compared to

CAR-T cells alone: 5%-70% versus 9%-90% live Kasumi2 left and 3%-25% versus 5%-45% live MDA-MB-231 left, respectively. Further studies are ongoing to demonstrate superior efficacy and safety of this therapy *in vivo*. Overall, with our ROR1 targeting CAR-T cells with inducible aPD-1 scFvs we present a promising therapeutic approach to more effectively target ROR1+ solid malignancies, where there remains a high unmet therapeutic need.

OR004

In vivo antitumor activity of a hCD44v6-specific chimeric antigen receptor in syngeneic models of solid tumors

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CD44v6, the adhesive receptor CD44 variant isoform 6, is widely expressed on various hematological and solid cancers. T cells targeted to the human CD44v6 (hCD44v6) by means of a specific chimeric antigen receptor (CAR) containing as spacer the extracellular domain of the low-affinity nerve-growth-factor receptor (LNGFR) and a CD28 signaling domain, has been shown to mediate a potent antitumor effects against primary human acute myeloid leukemia (AML) and multiple myeloma (MM) cells in immunocompromised mice. However, human xenograft tumor models only partially reproduce the human setting because they lack an intact immune system. Therefore, in this study, several murine tumor types of different genetic background were modified to express the hCD44v6, in order to assess and characterize the antitumor activity of syngeneic hCD44v6 CAR T cells. hCD44v6-specific CAR T cells were endowed with a robust cellular cytotoxicity when co-cultured with hCD44v6+ murine tumor cells. *In vivo*, hCD44v6-specific CAR T cells greatly enhanced the survival of mice bearing RMA/hCD44v6 lymphoma or B16F10/hCD44v6 melanoma in primary (subcutaneous tumor) or established pulmonary metastases. In conclusion, hCD44v6-specific CAR T cell therapy has the potential to treat both hematologic and primary or metastatic solid tumors expressing hCD44v6, mobilizing the adaptive and innate immunity against tumor cells. These results provide a strong rationale for undertaking future studies to assess hCD44v6-targeted T cells in patients with primary and metastatic solid cancers.

OR005

AVXS-101 phase 1 gene replacement therapy clinical trial in spinal muscular atrophy type 1 (SMA1): 24-month event-free survival and achievement of developmental milestones

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Children with SMA1 are unable to sit unassisted, almost none achieve a Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) score ≥40 by 6 months, and 92% die or require permanent ventilatory support by 20 months. In the onasemnogene abeparvovec (AVXS-101) phase 1 trial (NCT02122952), SMA1 patients received a one-time intravenous dose of AVXS-101 gene replacement therapy at low (cohort 1, n = 3) or proposed therapeutic dose (cohort 2, n = 12). The primary objective was safety; secondary objectives included survival (avoidance of death/permanent ventilatory support) and ability to sit unassisted. CHOP-INTEND scores and other motor milestones were recorded. At 24 months follow-up, all patients were alive and without need for permanent ventilatory support. Cohort 2 patients showed improved motor function: 11/12 had CHOP-INTEND scores ≥40 points; 11/12 sat unassisted for ≥5s, 10 for ≥10s, and 9 for ≥30s; 11/12 achieved head control, and 9 could roll over. Two patients crawled, pulled to a stand, stood, and walked independently. In the long-term follow-up study, 2 additional patients sat unassisted for ≥30s, and 2 stood with support; 3/4 received no therapy besides AVXS-101. No patients received concomitant nusinersen during the 24-month study period. Asymptomatic transient rise in serum aminotransferase levels occurred in 4 patients and were attenuated by prednisolone. In contrast with natural history, AVXS-101 improved survival of both cohorts, and motor function of cohort 2; 11/12 patients achieved CHOP-INTEND scores and motor milestones rarely or never seen in this population. No waning of effect or regression in motor function was reported.

OR006

Gene supplementation therapy for CNGA3-linked achromatospia

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Achromatopsia type 2 (ACHM2) is a genetically and clinically well-defined inherited retinal disorder caused by mutations in the CNGA3 gene. ACHM2 patients suffer from day blindness, poor visual acuity, photophobia, nystagmus, and lack the ability to discriminate colours. We developed AAV8.CNGA3, a recombinant adeno-associated virus (AAV) vector for gene supplementation therapy of ACHM2. The vector expresses human CNGA3 under control of a short human ARR3 promoter and was packaged with AAV8 capsid. A first-in-man dose escalation clinical trial with nine ACHM2 patients (NCT02610582) was conducted focusing on safety and efficacy of a single subretinal injection of AAV8.CNGA3. Three patients were treated per dose group (0.1-1.0 × 10e11 total vector genomes). Safety as primary endpoint was assessed by clinical examination of ocular inflammation. The primary endpoint was met with an excellent safety profile and no serious adverse event. Ocular adverse events were either unrelated or associated with the surgical procedure, but not the study drug. Analysis of vital signs, blood chemistry, immunopathology and shedding supported the

excellent safety profile. Secondary outcomes were change in visual function from baseline in terms of spatial and temporal resolution, chromatic-, luminance-, and contrast sensitivity over a period of 12 months post treatment. Analysis of secondary endpoints supports the notion that the treatment improved clinical features in nine out of nine patients. In conclusion, the first clinical gene therapy trial for achromatopsia in man was well tolerated, safe and provides evidence that cone photoreceptors in ACHM2 can be reactivated by gene supplementation.

OR007

Generation of novel immune-evading AAVs through identification and mutation of immunogenic epitopes in the variable capsid regions of adeno-associated virus 9

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AAVs are small, non-pathogenic viruses of the Parvoviridae family, predominantly isolated from human and primate tissues. Due to their unique properties, such as tropism for specific tissues, low toxicity and low immunogenicity, AAV vectors have been evaluated extensively in animal models and in humans for treatment of a variety of diseases such as hemophilia, ocular and cardiovascular diseases. Despite their efficiency and widespread use in human gene therapy, clinical application is limited by the presence of pre-existing antibodies against the capsid, mainly due to its human origin. In fact, about 50% of patients who could be enrolled in clinical trials have to be excluded due to the presence of antibodies against AAVs. Consequently, there is an urgent need for new experimental strategies permitting the generation of immune-evading AAVs. Towards this goal, we first sought to identify immunogenic epitopes on the capsid surface of AAV serotype 9. Surface-exposed variable regions III to IX, which are known to be immunogenic, were modified by introducing site-specific point mutations. The resulting AAV variants were then evaluated for packaging efficiency and transduction on different cell types. Subsequently, they were screened against eight individual or pooled human sera as well as IVIG, using a cell-based neutralizing antibody assay. This allowed us to identify several immunogenic hotspots including novel candidates, and to generate a combinatorial variant whose properties were evaluated. More generally, this work provides a versatile platform for similar targeted approaches to generate immune-evading vectors derived from alternative AAV serotypes or other viruses.

OR008

The impact of vector integration on chromatin architecture

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In this work, we investigate the impact of Lentiviral Vectors (LV) carrying enhancers and chromatin insulators (CI), features able to interact long-range with the host genome, and whose effects on chromatin architecture are so far unexplored. We devised a LV-specific Circular Chromosome Conformation Capture (LV4C) method, able to retrieve the LV integration site (IS) together with the corresponding host-genomic long-range interacting site. We applied LV4C on K562 cell clones marked by multiple (30-60) LV IS harboring a strong enhancer and/or an *in vivo* characterized CI bound by the CCCTC-binding-factor (CTCF). In an *in vivo* genotoxicity study, we show this CI to improve the mouse survival by reducing vector-mediated oncogene activation. By LV4C, both LVs display long-range interactions with gene-dense regions, spanning 100-500Kb from the IS, where 50% of IS have multiple interaction-targets (2-5). Interestingly, while the LV without CI interacts with genomic loci upstream and downstream the IS, inducing gene deregulation at such sites, the CI in the LV fully redirects long-range chromatin interactions towards genomic loci upstream the LV IS that are enriched for CTCF-motives in convergent orientation to the ones within the LV ($p < 0.01$). At these interaction-sites, genes upstream the IS are not deregulated, while for genes downstream the IS, where no chromatin-loops are formed, we observe vector-mediated aberrant transcription of host genes. Summarizing, we show the mechanism underlying insulators' function in LVs, promoting their use for gene therapy applications. Furthermore, our approach can serve to study any regulatory element, carried by LVs, on the host chromatin conformation.

OR009

Cross-packaging control in multiplexed AAV libraries

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The generation of pooled libraries of AAV variants and subsequent parallel screening has emerged as a potent method for identifying capsids with novel and diverse phenotypes for gene therapy applications. One important confounder of this process is the potential of generating protein capsids that package a mismatched genome, thereby giving an erroneous readout when screened. This process of cross-packaging is a known consequence of pooled library production, and while several conceptual solutions have been proposed to mitigate this concern, few studies have quantitatively assessed cross-packaging in a library setting in order to minimize its prevalence in pooled vector production. To better understand the relationship between transfection conditions and cross-packaging, we developed qPCR-based assays to measure rates of cross-packaging in simplified library production contexts. We then use these assays to establish optimal transfection conditions which minimize cross-packaging while preserving vector yield. Finally, we demonstrate that more complex libraries produced with these optimized conditions are minimally cross-packaged through a functional *in vivo* assay. Interestingly, while optimal transfection conditions introduce potentially thousands of library plasmids to each cell, rates of cross-packaging are surprisingly low, possibly suggesting a preference of capsids for packaging of their own genomes. Together, these experiments demonstrate a quantitative

measure of incorrectly packaged virions in a simple mixture and indicate optimal conditions for pooled production of high quality AAV vector libraries.

OR010

Development of a next generation synthetic promoter for liver directed gene therapy

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Delivering a successful gene therapy solution is not only dependent on the tropism and biochemical characteristics of the vector, but also the strength and specificity of the promoter driving the gene of interest (GOI). Until recently, promoters selected to drive the GOI were either constitutive or originating from rational design optimization of identified organ specific promoters. Here we report on a collaboration between uniQure and Synpromics to generate and validate a selection of next generation synthetic promoters. Target specifications for the liver specific promoter was to be no larger than 250bp and with a 5-fold increase in strength relative to the in-house reference promoter. Two libraries and several data driven rational designs were analyzed *in vitro* for strength and specificity. Subsequently, selected promoters underwent iterative rational design optimizations and were further validated through transfection and transduction of immortal and primary liver cell lines. Concomitantly, an *in vivo* comparison study was performed with C57BL/6JRj mice injected with AAV harbouring a reporter gene driven by the selected promoters at $5 \times$ E12 gc/kg. From the 15 validated promoter designs all performed better that the reference in expressing the reporter gene *in vitro* and *in vivo*. The lead candidate was able to drive expression of the reporter up to 40-fold over the reference promoter with less than 5% off-target activity when compared to CMV. This is the first report on the successful development of a synthetic promoter for the use in the gene therapy landscape adding valuable instruments to the toolbox.

OR011

Developing stem cell-based gene therapy for RAG1 deficient-SCID

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Recombinase-activating gene (RAG) deficient SCID patients lack B and T lymphocytes due to the inability to rearrange immunoglobulin and T-cell receptor genes. The two RAG genes are acting as a required dimer to initiate gene recombination. Gene therapy is a valid treatment alternative for RAG-SCID patients, who lack a suitable bone marrow donor, but developing such therapy for RAG-SCID has proven challenging. We tested clinically relevant lentiviral SIN vectors with different internal promoters (UCOE, PGK, MND, and UCOE-MND) driving codon optimized versions of the RAG1 gene to ensure optimal expression. We used Rag1-/- mice as a clinically relevant model for RAG1-SCID to assess the efficacy of the various vectors at low vector copy number. In parallel, the conditioning regimen in these mice was optimized using busulfan instead of commonly used total body irradiation. We observed that B- and T-cell reconstitution directly correlated with RAG1 expression. Mice receiving low RAG1 expression showed poor immune reconstitution; however high RAG1 expression resulted in a lymphocyte reconstitution comparable to mice receiving wild type stem cells. Efficacy and safety of our clinical, MND-driven, RAG1 lentivirus batch was assessed in Rag1-/- mice model showing that phenotypic and functional restoration of RAG1-deficiency can be achieved. Additionally, RAG1-SCID patient CD34+ cells transduced with our clinical MND-RAG1 vector and transplanted into NSG mice led to fully restored human B and T cell development. Together with favourable safety data (IVIM, murine pathology), these results substantiate a clinical trial for RAG1 SCID which is currently in preparation.

OR012

Gene therapy trial in non-conditioned Fanconi anemia patients

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Fanconi anemia (FA) is a bone marrow failure disorder characterized by DNA repair defects, congenital abnormalities and cancer predisposition. Based on previous results showing the efficiency of lentiviral vectors to correct FA patients' hematopoietic stem cells (HSCs) (Rio, Navarro et al. Blood 2017) a gene therapy trial was initiated in 2016 for FA-A patients. Six patients have been treated to-date with fresh or cryopreserved CD34+ cells transduced with the PGK-FANCA.Wpre* lentiviral vector. In the four patients with at least 12 months of follow up 0.6-1.4 million CD34+ cells/kg, transduced at efficacies of 0.17 to 0.53 copies/cell, were infused. The analysis of the provirus in BM and PB cells evidenced the engraftment and a marked *in vivo* expansion of gene-corrected cells in treated patients (2.8 to 44% corrected cells were determined in PB at 12 to 24 months post-infusion). Insertion site analysis in PB cells showed no

evidence of insertion-site mediated clonal expansion, and revealed an oligoclonal pattern of reconstitution and engraftment of multipotent corrected HSCs. Functional studies showed 4.3-70.2% of BM colony forming cells resistant to mitomycin C and a significant correction of diepoxybutane-induced chromosomal fragility in PB T cells. Additionally, evidence of stabilization of the BMF was observed in these patients. Our studies demonstrate for the first time that lentiviral-mediated gene therapy results in progressive engraftment and phenotypic correction of HSCs in non-conditioned FA patients.

OR013

Evolutionarily recent transposable elements and their controllers regulate human early embryonic transcriptional networks

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Sequences derived from transposable elements (TEs) prominently contribute to the human genome and are increasingly recognized as essential evolutionary forces. Here, we describe how recent TE invaders and their trans-acting controllers govern the earliest steps of human embryogenesis. The same sets of young retrotransposons from the SVA, HERVK and HERVH subgroups are accessible and transiently expressed during early genome activation and strongly acetylated in naïve human embryonic stem cells (hESC). Activation of these TE loci is under the opposing controls of KLFs/OCT4 pioneer factors and largely primate-restricted KRAB zinc finger protein repressors, the expression of which coincides with that of their targets. TEs expressed in naïve hESC mostly act as enhancers and, for some, as promoters or splicing modifiers subsequently affecting human-specific gene expression network.

OR014

NextGenAAV: a mix of organic chemistry and vectorology

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AAV vectors are becoming therapeutic products. However, clinical trials using AAV have shown critical limitations: (i) high doses are required to achieve therapeutic effect; (ii) broad biodistribution to non-target tissues; (iii) loss of efficacy in the presence of pre-existing neutralizing antibodies. In an attempt to overcome these barriers, we have used organic chemistry to improve the ''therapeutic index'' of rAAVs. For this purpose, N-acetylgalactosamine and mannose ligands (GalNAc, Man), with an isothiocyanate (-NCS) coupling function, were synthesized. GalNAc recognizes the asialoglycoprotein receptor present in hepatocytes, whereas mannose receptors are present in the retina. NCS-ligands were grafted on AAV2 by reacting with amino groups at the surface of the capsid. Analytical tools were used to show, unambiguously, a covalent coupling of the ligands to the surface of the capsid by formation of a thiourea function and not an adsorption. Notably, we also demonstrated the possibility to modulate the number of ligands grafted to the AAV particles according to conditions used in the chemical reaction (i.e. number of ligands Equivalents (Eq)/ AAV particles). Indeed, Dot and Western blot analysis showed higher amounts of ligands covalently coupled on the surface of the AAV2 capsid by using increasing amounts of ligands (3e5 vs 3e6 Eq). Importantly, GalNAc-AAV2 were more efficient in transducing mice primary hepatocytes in culture than wild-type AAV2. Similarly, Mannose-AAV2 showed an improved transduction efficiency in the retina compared to non-modified AAV2 upon subretinal injection in rats. Hence, our data open novel possibilities for AAV-mediated targeted gene transfer.

OR015

Evolution of recombinant adeno-associated viral vectors with favorable retinal penetration properties

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Most retinal degenerative diseases, including retinitis pigmentosa and age-related macular degeneration, are characterized by the progressive loss of photoreceptors. Various AAV-based gene therapies to prevent or slow down retinal degeneration are targeted at the photoreceptors. Typically, AAVs are injected subretinally, thus restricting therapeutic intervention to the injection site. Further, subretinal injections have resulted in a decrease in retinal thickness and visual acuity. A safer method that targets cells along the entire width of the retina is intravitreal injection. We developed AAVs with the ability to penetrate through the retina when applied intravitreally. We applied an *in vivo* directed evolution strategy using a novel peptide display design that enabled us to iteratively enrich for AAV2 variants with the desired properties. After four selection rounds we determined and ranked the receptor-binding motifs of the converged library by next-generation sequencing. Fifteen capsids containing the highest rank motifs were subsequently analysed for their transduction fingerprints following intravitreal injection into wildtype C57/BL6 and blind rd1 retinitis pigmentosa mouse eyes. For evaluation, a transgene encoding the green fluorescent protein mCitrine under the ubiquitous CMV and EF1a promoters was packaged into the novel capsids. The *in vivo* expression screen resulted in three synthetic AAV capsids with much improved transretinal penetration abilities compared to wildtype AAV2, mediating panretinal mCitrine expression. Our novel synthetic AAV2 capsids may have clinical relevance for the implementation of photoreceptor-targeted gene therapies, but also optogenetic approaches targeting dormant photoreceptors or inner retinal cells.

OR016

Correction of autosomal recessive disorders via CRISPR-associated base editors in adult animals

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The CRISPR/Cas genome-editing tool holds great promise for treatment of genetic disorders. Clinical translation, however, is hampered by the low frequency of precise repair of Cas9-induced DNA double-stranded breaks (DSB) via homology-directed repair (HDR). We have a strong interest in developing CRISPR approaches with enhanced precision for *ex vivo* and *in vivo* gene editing therapies. I will first present a novel CRISPR system, in which we co-localized the DNA repair template to the Cas9 nuclease via covalent linkage. Using this system we increased HDR rates up to 24-fold in cell lines and dividing primary cells, supporting potential application in *ex vivo* gene therapies. I will then provide insights into our efforts of developing *in vivo* gene editing approaches. Precise gene editing *in vivo* in adult organisms is challenging, since most adult tissues primarily consist of non-dividing cells in which HDR is inactive. In a recent study we circumvented this problem by applying CRISPR-associated base editors. These systems enable direct conversion of C•G to T•A base pairs and vice versa independent of DSB formation and HDR. We targeted a mouse model for the metabolic liver disease Phenylketonuria (Pahenu2) by systemic AAV-mediated delivery of Pahenu2-targeting base editors. This resulted in gene correction rates that fully restored physiological blood phenylalanine levels. We observed mRNA correction rates up to 63%, restoration of the phenylalanine hydroxylase enzyme activity, and a reversion of the light fur phenotype. Our findings suggest the feasibility of using CRISPR-associated base editors to repair genetic diseases *in vivo* in adult patients.

OR017

CRISPR/Cas9-mediated downregulation of PMP22 ameliorates Charcot-Marie-Tooth disease 1A in mice

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Charcot-Marie-Tooth 1A (CMT1A) is the most common inherited neuropathy without a known therapy, which is caused by duplication of the gene encoding the peripheral myelin protein of 22 kDa (PMP22). Overexpression of PMP22 is thought to cause demyelination and subsequently axonal degeneration in the peripheral nervous system (PNS). Here, we investigated whether downregulation of PMP22 by targeting gene regulatory region of PMP22 using CRISPR/Cas9 could provide a therapeutic strategy for treating CMT1A. In this study, we utilize human PMP22 overexpressing transgenic mouse model of CMT1A to test the efficacy of CRISPR/Cas9-mediated suppression of PMP22 expression. For this, CRISPR/Cas9 was designed to target the gene regulatory region of human PMP22 to normalize overexpressed PMP22 level. Using primary human Schwann cells, we screened CRISPR-associated guide RNA (gRNA) and a lead gRNA was then tested for their ability to reduce PMP22 transcripts in mouse model of CMT1A. Efficient editing of gene regulatory region of PMP22 was achieved via single intraneural injection of CRISPR/Cas9 in ribonucleoprotein complexes, which downregulated PMP22 transcripts in sciatic nerves. Treatment led to preservation of both myelin and axons which resulted in improvement in motor nerve conduction velocity and compound motor action potentials. PMP22 downregulating CRISPR/Cas9 showed no aberrant cleavage of off-target sites predicted by unbiased genome-wide identification. These results demonstrate that our approach utilizing CRISPR/Cas9 to target gene regulatory region of PMP22 efficiently and specifically downregulates PMP22 expression to therapeutic level, providing a compelling therapeutic approach for CMT1A.

OR018

Supra-therapeutic levels of transgene expression achieved *in vivo* by CRISPR/Cas9 mediated targeted gene insertion

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CRISPR/Cas9 mediated genome editing holds tremendous promise for the treatment and potential cure of human diseases by addressing their underlying genetic cause. Many genome editing studies to date have focused on knocking out genes by the error-prone repair mechanisms of non-homologous end joining (NHEJ) that predominate in most cell types. We present data showing that the combination of lipid-nanoparticle (LNP) and AAV delivery of CRISPR/Cas9 components and donor template DNA can achieve robust levels of targeted gene insertion in terminally differentiated primary hepatocytes as well as mouse liver *in vivo*. Targeted insertion in hepatocytes included engineering the donor template, identifying an ideal target site, and defining the delivery timing of LNP and AAV. This strategy has resulted in supra-therapeutic levels of gene expression for multiple transgenes. The gene insertion efficiencies we report are at least an order of magnitude higher than previously reported in the literature. This set of technologies and refinements enables the development of therapies for a wide range of genetic diseases that require stable gene insertion and expression.

OR019

Successful *in vivo* editing of patient-derived primary human hepatocytes

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Powered by promising clinical results, recombinant adeno-associated viral (rAAV) vectors have become the vectors of choice in gene therapy applications targeting the liver. The

recent development of novel synthetic capsids has markedly improved the efficiency with which human hepatocytes can be transduced, bringing an increasing number of liver disease phenotypes within reach by conventional gene addition and enhancing the prospects for more challenging strategies such as gene repair. Precise gene repair involves the correction of the genetic defect directly at the mutant locus, allowing retention of physiological expression under endogenous control elements. To this end, we sought to correct a disease-causing mutation in the ornithine transcarbamylase (*OTC*) gene in patient-derived primary human hepatocytes using AAV-CRISPR/Cas9-mediated genome editing. Using the FRG mouse model (*Fah-/-Rag2-/-IL2rg-/-*) xenografted with human hepatocytes, we delivered rAAV-based editing reagents designed to cut the target locus and introduce a precise single nucleotide change by homology-directed repair. Following delivery of the editing reagents packaged into the highly human hepatotropic AAV capsid, NP59, we detected efficient cutting at the target locus, with up to 70% of *OTC* alleles modified. Importantly, the desired precise single nucleotide change was present in 11.4% to 16.6% of alleles with resultant restoration of OTC enzymatic activity. This is the first demonstration of successful correction of a disease-causing mutation in patient-derived primary human hepatocytes *in vivo*. Furthermore, we have developed a platform suitable for evaluation of human-specific editing reagents that has the potential to be adapted to validate gene editing vectors before clinical use.

OR020

Towards clinical application of a lentiviral gene therapy protocol for p47phox deficient chronic granulomatous disease

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Chronic Granulomatous Disease (CGD) is an inherited primary immunodeficiency disorder with an incidence of ~1:200,000 live births. This disease is caused by mutations in any of the genes encoding subunits of the NADPH oxidase, the enzyme complex involved in pathogen killing. Mutations in the p47phox subunit of the NADPH oxidase cause the most common form of autosomal recessive CGD called p47phox deficient CGD, a disorder amenable to haematopoietic stem cell (HSC) gene therapy. To this aim, we have developed a self-inactivating lentiviral vector containing a codon-optimized p47phox transgene under the transcriptional control of a myeloid promoter (pCCLChim-p47). Our lentiviral gene therapy protocol was able to restore the NADPH oxidase activity in CD34-derived granulocytes from p47phox deficient CGD patients. When tested in an animal model of p47phox deficient CGD, the pCCLChim-p47 vector induced high and stable levels of p47phox expression in granulocytes from blood of gene therapy-treated mice and rescued the ability of p47phox deficient CGD mice to fight Salmonella Tiphymurium, a frequent cause of death for septicaemia among CGD patients. Presence of functional neutrophils in secondary transplanted p47phox deficient CGD mice (at 6 months post-transplantation) also suggests that the protocol is able to correct hematopoietic stem cells and that the vector is not subjected to epigenetic inactivation. Overall our experiments pave the way to a clinical application of the pCCLChimp47 vector. Biodistribution and genotoxicity studies are underway.

OR021

Toward gene editing in Rett syndrome

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Mutations in MECP2 and FOXG1 genes respectively cause the classic form and the congenital variant of Rett syndrome, one of the most common genetic causes of intellectual disability in girls. Both genes are transcriptional regulators and both under- and over-expression cause disease in humans. Therefore, a classical gene replacement approach could not be appropriate for such disorders. We present here a successful application of CRISPR/Cas9 gene editing technology in patient-specific human cellular models. We have engineered a two-plasmid system to correct FOXG1 (c.688C>T-p.Arg230Cys and c.765G>A-p.Trp255*) and MECP2 (c.473C>T-p.Thr158Met and c.916C>T-p.Arg306Cys) mutations. Mutation-specific sgRNAs and donor DNAs have been selected and cloned together with an mCherry/GFP reporter system. Cas9 flanked by sgRNA recognition sequences for auto-cleaving has been cloned in a second plasmid. Cell types relevant to the project (fibroblasts, induced Pluripotent Stem Cells (iPSCs), iPSC-derived neurons) have been infected with AAVs to select the most appropriate serotype. AAV9 infection in terminally differentiated iPSC-derived neurons demonstrated that these cells can be efficiently co-infected. mCherry+/GFP+ cells isolated by Fluorescent Activated Cell Sorting and analyzed by Next Generation Sequencing have shown a change from 50% to 70-80 % of normal alleles. Our studies will open a real opportunity toward the application of gene therapy for the treatment of Rett syndrome and related disorders and experiments in the mouse model are ongoing.

OR022

In utero liver-directed lentiviral gene therapy cures a pig model of hereditary tyrosinemia type 1

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In utero gene therapy is an advanced approach that offers significant advantages over postnatal therapy. In liver disease, it can be curative while also addressing disease before any clinical consequences occur. We show here for the first time that *in utero* gene therapy can cure a large animal model of liver disease, in this case hereditary tyrosinemia type I (HT1). We performed

ultrasound-guided intrahepatic injections of a lentiviral vector carrying the human fumarylacetoacetate hydrolase (Fah) gene under the alpha-1 antitrypsin promoter in 16 Fah-/- pig fetuses at E70 ± 5. Doses ranged from 2.4×10E8 to 1.6×10E9 transducing units/fetus. Sows were maintained on the protective drug, 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), throughout gestation, and piglets were maintained on NTBC until weaning. Sixteen treated and seventeen control Fah-/- piglets were born from four pregnancies. Six treated piglets were euthanized for early data collection, and five died from complications due to surrogate sows. Positive FAH immunohistochemistry was seen at birth. Remaining treated piglets and control littermates are being followed long-term, and some treated piglets have already demonstrated NTBC-independent growth. Liver biopsies at 50 days of life show expansion of FAH+ hepatocytes with no evidence of fibrosis, cirrhosis, or HCC, and plasma tyrosine levels have begun to normalize. Lentiviral integration was seen in only one of 4 sows and in the liver as well as several other tissues in treated piglets with a benign integration profile. This report is the first ever to use *in utero* gene therapy to cure a large animal model of a human liver disease.

OR023

Brain targeted stem cell gene therapy corrects mucopolysaccharidosis type II via multiple mechanisms

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Mucopolysaccharidosis type II (MPS II) is an X-linked lysosomal storage disorder characterized by mutations in the iduronate-2-sulphatase (IDS) gene, leading to cellular accumulation of heparan and dermatan sulphate, with severe neurodegeneration, skeletal disease, and cardiorespiratory disease. Most patients manifest with cognitive symptoms which are non-responsive to enzyme replacement therapy or standard haematopoietic stem cell transplantation. We report a novel lentiviral-mediated haematopoietic stem cell gene therapy approach to target the brain using lentiviral IDS fused to ApoEII (IDS.ApoEII) compared to a lentivirus expressing normal IDS or a normal bone marrow transplant. Transduced haematopoietic stem cells (HSCs) were transplanted into 6-8 week-old MPS II mice after full myeloablative conditioning using busulfan. IDS and IDS.ApoEII treated mice showed increases in IDS enzyme levels of 124-fold and 152-fold over wild-type by 8 months post-transplant. We achieved vector copy numbers in HSCs of 3.1 and 3.8 in IDS and IDS.ApoEII treated mice. We show complete correction of skeletal abnormalities and motor function in MPSII mice by all treatments at 8 months of age. In contrast, only IDS.ApoEII mediated complete normalization of brain pathology and behaviour, providing significantly enhanced correction compared to IDS. A normal bone marrow transplant achieved no brain correction. Whilst corrected macrophages traffic to the brain, secreting IDS/IDS.ApoEII enzyme for cross-correction, IDS.ApoEII was more active in plasma, and was taken up and transcytosed across brain endothelia significantly better than IDS via both heparan sulfate/ApoE-dependent receptors and mannose-6-phosphate receptors. Brain targeted hematopoietic stem cell gene therapy provides a promising therapy for MPS II patients.

OR024

Comprehensive preclinical studies for the gene therapy of patients with leukocyte adhesion deficiency type I (LAD-I)

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Leukocyte Adhesion Deficiency Type I (LAD-I) is a severe primary immunodeficiency caused by mutations in the ITGB2 gene that encodes for the common subunit of β2 integrins (CD18), leading to defects in the ability of phagocytic cells to extravasate to infection sites. To restore the functional ability of leukocytes from LAD-I patients, we developed a lentiviral vector (LV) that has recently obtained Orphan Drug designation (EU/3/16/1753 and DRU-2016-5430). In this LV, the expression of hCD18 is driven by a chimeric promoter with a higher activity in mature myeloid cells. Comprehensive safety- and efficacy-focused preclinical LV-mediated gene therapy studies have been conducted in LAD-I mouse models harboring hypomorphic and knock-out mutations in the ITGB2 gene. Our studies showed stable engraftment of gene corrected LAD-I mouse hematopoietic stem cells (HSCs) in LAD-I recipients and demonstrated the phenotypic correction of peripheral blood neutrophils. Safety studies demonstrated the absence of hematotoxic and genotoxic effects in treated animals and showed the restriction of the therapeutic provirus to hematopoietic tissues. Further studies have been conducted with GMP-produced LVs in human CD34+ cells aimed at optimizing the transduction of these cells. The use of specific transduction enhancers showed the efficacy of these molecules to increase the transduction of the HSCs. Moreover, transplantation experiments in NSG mice provided evidence of sustained engraftment of hematopoietic repopulating cells harboring 1-4 copies of the therapeutic gene. Taken together, these results demonstrate the efficacy and safety of a preclinical gene therapy approach enabling clinical investigation of this gene therapy in LAD-I patients.

OR025

Haematopoietic stem cell targeted neonatal gene therapy by a clinically applicable lentiviral vector corrects osteopetrosis in oc/oc mice

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Infantile malignant osteopetrosis (IMO) is an autosomal recessive disorder characterized by nonfunctional osteoclasts. About 50% of the patients have mutations in the TCIRG1 gene, encoding for a subunit of the osteoclast proton pump. Gene therapy could be an alternative treatment to allogeneic stem cell transplantation. The oc/oc mouse is a model of IMO which has a 1500 bp deletion in the tcirg1 gene, severe osteopetrosis and a lifespan of only 3-4 weeks. Here we show that the osteopetrotic phenotype in oc/oc mice can be reversed by HSC targeted gene therapy with a clinically applicable lentiviral vector expressing a

non-mutated form of human tcirg1 under the mammalian promoter elongation factor 1α short (LV-EFS/TCIRG1). oc/oc c-kit+ fetal liver cells transduced with LV-EFS/TCIRG1 were transplanted into sublethally irradiated oc/oc mice by i.v. temporal vein injection one day after birth, 9/12 survived longterm (4-6 months) and displayed tooth eruption. Splenocytes from the transplanted mice were harvested 19-25 weeks after transplantation and differentiated to osteoclasts on bone slices. Vector corrected osteoclasts showed TCIRG1 expression by WB; their average Ca2+ and CTX-I release was increased relative to that from oc/oc derived osteoclasts, but this increase was not statistically significant. Histopathology of the bones of surviving animals showed varying degrees of rescued phenotype. The average vector copy number per cell in the bone marrow was 1.8 ± 0.5. In conclusion, 75% of mice exhibited long-term survival, tooth eruption and reversal of osteopetrotic phenotype and this is a significant step towards the development of gene therapy for osteopetrosis.

OR026

DPPA2 and DPPA4 regulate expression of Dux in mouse embryonic stem cells

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In metazoans, the fertilized egg contains RNAs and proteins produced during oogenesis but is initially transcriptionally silent. These reservoirs are sufficient to sustain the first embryonic cell divisions until degradation of the maternal products and production of a wave of embryonic transcripts, known as zygotic genome activation (ZGA). Our recent work identified the DUX family of transcription factors as master regulators of ZGA in human, mouse, and likely all placental mammals. In contrast to the previously identified ZGA-triggering factors, expression of murine and human DUX is activated upon fertilization of the oocyte. The maternally inherited transcriptional activators responsible for embryonic transcription of Dux remain completely unknown. Mouse embryonic stem cells (mESCs) represent the best available *in vitro* model to study preimplantation embryo development, owing to their innate ability to cycle through a 2-cell (2C)-like state, where Dux and ZGA genes are transiently expressed. We identified DPPA2 and DPPA4 as activators of Dux transcription. Notably, depletion of DPPA2 or DPPA4 from mESCs prevents formation of 2C-like cells by inhibiting Dux transcription. In contrast, ectopic DPPA2 or DPPA4 expression in mESCs has no effect on the establishment of a 2C-like state, suggesting that these proteins are necessary but not sufficient for Dux expression. Dppa2 and Dppa4 are expressed to different extents in oocytes and pre-implantation embryos. Interestingly, two independent studies found that embryonic development is impaired in embryos lacking DPPA2 or DPPA4. Further experiments are necessary to determine if DPPA2 and DPPA4 are necessary for transcription of Dux in zygotes.

OR027

T cell immunity towards CRISPR-associated nucleases

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CRISPR/Cas9 gene editing is a promising technology for the treatment of inherited diseases. Previous gene therapy trials suggest that pre-existing T cell immunity towards the delivery vector leads to decreased efficacy (e.g. gene therapy with AdV or AAV). We investigated the prevalence of T cell responses towards different CRISPR-associated nucleases (Cas) (Streptococcus pyogenes SpCas9, Acidominococcus Sp. AsCpf1) in the peripheral blood of healthy human donors and detected a ubiquitous effector/memory T cell response with multi-functional cytokine profile and cytolytic activity towards Cas9-transduced target cells. Cas-specific effector T cells could potentially migrate to CRISPR/Cas9-edited tissues and inflict damage to the targeted organ. In depth analysis of the activated T cells revealed a high frequency of Cas-reactive regulatory T cells. Cas-reactive regulatory T cells were able mitigate Cas-specific effector T cell proliferation and function *in vitro*. In conclusion, immunosuppressive treatment should be considered in first clinical trials using the current CRISPR/Cas9 technology *in vivo*. Our results emphasize that immune monitoring before, during and after application of CRISPR/Cas9 must be performed to identify high risk patients (potentially with low Treg/Teff ratio) and stratify immunosuppressive treatment accordingly. Further studies should investigate how pre-existing immune responses to Cas can be adequately prevented or delayed. We propose that adoptive transfer of Cas-specific regulatory T cells may circumvent the need for global immunosuppression in patients with pronounced pre-existing T cell immune reactivity to Cas.

OR028

Alphaviral trans-replicating RNA is a low dose vaccine vector

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Trans-replicating RNA (TR) is a split-vector system engineered from alphaviral self-replicating RNA (srRNA) comprising one RNA encoding the alphaviral replicase, and another encoding the transgene only. In the past TR helped to elucidate alphaviral replication. In those studies, TR was well expressed, but although the parental srRNA is promoted as vaccine vector, to our knowledge TR has not yet been evaluated for this purpose. Hence, we addressed this knowledge gap and found that TR is a promising vaccine vector. Transgene expression started within 2h after transfer and rapidly increased, eventually reaching expression levels of srRNA *in vitro*. To benchmark immune response induced by TR we compared a total dose of 20 μg TR - only 50 ng encoding Influenza virus hemagglutinin (HA), 20 μg encoding replicase - to 20 μg mRNA or 1.25 μg srRNA encoding entirely for HA. All these vaccine RNAs elicited comparable humoral immune responses and protected mice from influenza virus infection. Thus, with respect to the HA-coding RNA dose, TR was the most effective, being approximately 25x more potent

than srRNA and 400x more potent than mRNA, confirming that seasonal batch sizes of a TR vaccine could be reduced accordingly. Therefore, we believe that TR is the optimal format for a vaccine that requires seasonal adjustments within short production times like needed against influenza.

OR029

Modeling functional and dysfunctional brain circuits with human iPSC-derived neurons in microfluidic chambers

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We have recently generated iPSC-derived neurons from Parkinson's disease (PD) patients with mutations in the OPA1 gene, encoding for a crucial mitochondrial protein. OPA1 mutant neurons showed altered mitochondrial dynamics, oxidative stress and limited survival. However, conventional *in vitro* culturing of neurons failed to reconstitute the physiological connectivity between neurons and hampers manipulation of single neuronal compartments. We have fabricated microfluidic devices for the patterned and compartmentalized growth of human iPSC-derived neurons. We employed these devices to establish an *in vitro* model of the nigral-striatal connection, which selectively degenerates in PD. In fact, the demise of nigral dopaminergic neurons occurring in PD might be the final result of the dysfunctions of the dopaminergic terminals contacting the striatal medium spiny (MS) neurons. Thus, establishing an *in vitro* model of the dopaminergic-striatal connection will provide a superior system for addressing mechanisms of neurodegeneration. To establish this model, we optimized differentiation of PD and control iPSCs into both MS and midbrain DA neurons. In this system, DA neurons extended their axons within the microchannel to meet in the central chamber with the SM neuronal dendrites and generate synaptic contacts. Thus, we were able for the first time to generate the nigral-striatal circuit in a compartmentalized and accessible *in vitro* system. We have developed similar cultures starting from OPA1 mutant iPSC-derived neurons for generating a PD nigral-striatal neuronal circuit. Establishing *in vitro* brain circuits using patient specific human iPSC-derived neurons will provide an invaluable model to decipher the pathological mechanisms leading to neurodegenerative diseases.

OR030

Gene therapy for AADC deficiency results in de novo dopamine production and supports durable improvement in major motor milestones

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Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare, genetic disorder of neurotransmitter synthesis. Children with severe AADC deficiency fail to achieve motor milestones. AGIL-AADC is a recombinant, AAV2 vector containing the human cDNA encoding the AADC enzyme. We have treated 25 patients using a single administration of AGIL-AADC delivered bilaterally to the putamen by stereotactic infusions during a single, operative session in single-arm, open label clinical studies in our hospital. Patients received a total dose of either 1.8×1011 vg total of AGIL-AADC (n = 21) or 2.4x1011 vg total of AGIL-AADC (n = 4). Of the 25 children, 3 are now more than seven-year, 4 are more than six-year, and 9 are more than two-year post-gene therapy. Clinical results of the first 18 patients were compared to natural history cohort. At baseline (21 months to 8.5 years old), no child had developed full head control, sitting unassisted or standing capability, consistent with the published natural history cohort of severe AADC patients. After gene therapy, all patients exhibited increase scores in motor scales and gained new motor function. Objective evidence for de novo dopamine production was obtained from cerebrospinal fluid (CSF) homovanillic acid (HVA) levels and 6-[1^8F]-fluoro-L-DOPA (FDOPA) positron emission tomography (PET). CSF HVA levels increased significantly one-year after gene therapy. FDOPA uptake in bilateral putamens also increased one-year after gene therapy, and was stable over five-years after gene therapy. These findings indicate that gene therapy with AGIL-AADC is a potential therapeutic for patients with AADC deficiency to achieve and maintain motor milestones.

OR031

Shielding lentiviral vectors from phagocytosis increases hepatocyte gene transfer in non-human primates

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Lentiviral vectors (LV) are attractive vehicles for liver-directed gene therapy by virtue of their ability to stably integrate in the genome of target cells and the absence of pre-existing anti-vector immunity in most humans. Over the past years, we have developed LV that achieve stable transgene expression in the liver, induce transgene-specific immune tolerance and establish correction of hemophilia in mouse and dog models upon systemic administration. We have recently treated 3 dogs as puppies by peripheral vein administration of escalating doses of LV expressing a hyper-functional canine clotting factor IX (FIX) and achieved stable reconstitution of FIX activity up to 10% of normal at plateau. We modified the vector envelope, by changing the protein composition of the producer cell plasma membrane and obtained novel LV that are more resistant to phagocytosis and innate immune sensing, because of increased surface content of CD47, a natural phagocytosis inhibitor. We have administered these CD47hi-LV to non-human primates (NHP, M. nemestrina). Administration was well tolerated, without significant elevation of serum aminotransferases or increase in body temperature and only caused a transient self-limiting leukopenia. Human-specific FIX activity reached up to 300% of normal and was nearly 3-fold higher in the CD47hi-LV compared to LV-treated animals. Overall, our studies show a favorable efficacy and safety profile of these LV in NHP and position them to address some of the outstanding challenges in liver-directed gene therapy for hemophilia and conceivably other diseases.

Poster Presentations

P001

Development of an AAV-specific plasmapheresis device for the selective removal of anti-capsid antibodies

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Adeno-associated viral (AAV) vectors have shown great potential in the treatment of monogenic diseases and, for some applications, marketing authorization was obtained. Despite these achievements, a major limitation in implementing AAV gene therapies is represented by anti-AAV antibodies, which frequently prevent seropositive patients from being included in gene therapy trials. Plasmapheresis has been proposed as a solution to this problem. Though this technology is efficient, plasmapheresis is not specific, and nonspecific removal of immunoglobulins can lead to hypogammaglobulinemia and consequently to an increased risk of contracting infections. Thus, novel technologies are required to enhance the safety and efficacy of plasmapheresis. In this study, we investigated the possibility to specifically remove anti-AAV antibodies using immuno-affinity chromatography with AAV particles grafted to a chromatographic matrix. This separation method was optimized with respect to ligand (AAV) density per column, column matrix, and flow rate: for instance, at a high ligand density (6×1013 vg/5ml column), 98% and 93% of anti-AAV8 antibodies could be removed from a preparation of human IgG (IVIg, 72mg/ml) and from anti-AAV positive human plasma, respectively. Importantly, only AAV antibodies were depleted whereas total IgG concentration was not affected. Furthermore, we observed that, probably due to their higher structural flexibility, empty AAV particles retain a more efficient anti-AAV capture potential compared to full AAV particles. Preliminary testing of the technology in a mouse model of liver gene transfer confirmed the feasibility of the approach and supports the continuation of the work towards large animal models and, in the future, to humans.

P002

Engineering of 5′ UTR to control the expression and incorporation level of VP1 during rAAV vector production using a baculovirus system

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Recombinant Adeno Associated Virus (rAAV) is a growing vector of choice for gene therapy, and clinical trials are ongoing for multiple diseases. The lack of efficient and cost-effective methods of production was a bottleneck, until a baculovirus /Sf9 cells expression system was developed for AAV. The baculovirus/S9 system is fully scalable, enabling the production of AAV from 25ml flask up to 10,000L with very good reproducibility and at high quality. Nevertheless, published reports have described reduced level of VP1 incorporation into the AAV capsids for several tested serotypes when produced in baculovirus/Sf9 cells. This seems to result in lower cellular transduction when compared with vectors produced in HEK293 cells. To overcome this problem and have a more stable and homogeneous AAV vector production with baculovirus/Sf9 cells, we switched the original ACG stating codon to a regular ATG and modulated the translation of VP1 by engineering the 5′UTR and Kozak sequences. We use these novel engineered designs to modulate the expression of VP1 for several of vectors of interest like AAV2, AAV9, Caltech AAV capsids, and AAVRh10. Compared to the original sequence using non-canonical starting codon, this sequence is more universal and allows a straightforward tuning of VP1 expression to reach levels comparable and even higher than vectors produced with HEK293 cells.

P003

Interaction of implanted zinc-containing cryogel with tissue environment

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Zinc compounds have multiple regulatory effects on mammalian cells and proven ability to promote regeneration of damaged tissues. The combination of zinc compounds with (bio)polymers is a promising approach to develop bioactive and tissue engineered materials. In our study, zinc ions were stably introduced into composite gelatin cryogels to produce biomaterials with improved physicochemical and cell-modulating properties. Resorption and biointegration of the biomaterials with pre-optimized characteristics were studied using rat models of skin incision and skin flap. The cryogel membranes of appropriate size were implanted beneath skin followed by wound suturing. The implanted biomaterials and surrounded tissues were collected during one-week period and analyzed after histological and immunofluorescent staining. We found that monocytes/macrophages and subsequently fibroblasts effectively infiltrated into metal-free cryogel resulting in gradual resorption and substitution of the biomaterial. The introduction of metal component into cryogels did not provide local adverse effects but modulated behavior of leukocytes, promoted fibroblast migration and formation of collagen matrix in the area of implanted biomaterial. Our results show that zinc ions within developed cryogels display sufficient availability and activity *in vivo*. This component effectively modulates the ability of biomaterials to interact with living cells and tissues. Potential applications of zinc-containing cryogels in wound healing and tissue engineering applications are also considered. This work was performed according to Russian Government Program of Competitive Growth of Kazan Federal University.

P004

Industrialization and large scale manufacturing of AAV vectors for gene therapy using baculovirus/Sf9 system

ABSTRACT WITHDRAWN

P005

Cell manufacturing facility at Lausanne university hospital: large experience of autologous skin cells for burns patients

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Regenerative medicine is a challenging task for clinical application into public institution since the manufacturing of ATMP requires specific conditions for such production according to GMP rules. Due to its large experience into production of autologous skin cells, the Lausanne University Hospital, implemented a Cell Production Center (CPC) that is now accredited by Swissmedic (authorization n°507482). The main challenge was to transfer the activity of the skin cells production done in a classical room for cell cultures since 1985 to a GMP facility that put in place the concept of bioconfinement with hermetic modules and automated management for check-list (SOP) and batch of production. We described here, the workflow put in place from the demand by plastic surgeons to the release of final product with the in-process control required.

P006

Development of nanosized hydroxyapatite-containing cryogels as a matrix for osteogenic cells

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Development of effective osteoinductive materials is an important problem in tissue engineering and regenerative medicine. The use of collagens and hydroxyapatite (HAP) as natural bone-forming components is preferable due to safety and bioactivity reasons, however, they should be well-organized at micro- and nanolevels to increase regenerative potential of the biomaterials. Liquid-phase method for HAP preparation was optimized to produce well-defined nanosized HAP particles. The HAP samples were characterized by physicochemical techniques; according to SEM, the nanoparticles were less than 50 nm in diameter, had variable shapes and Ca/P ratios. The procedure for introduction of the HAP nanoparticles into chemically linked gelatin cryogels was developed. The composite HAP/gelatin cryogels exhibited increased mechanical strength along with decreased porosity and hydration properties, which partially inhibited migration of human skin fibroblasts compared with the cryogels without HAP. The conditions for growth and differentiation of chondrogenic/osteogenic teratocarcinoma cells (ATDC-5 line) on culture plates were studied. The differentiation was induced in the medium containing transferrin, pyruvate, selenite and ascorbate at optimal concentrations with human insulin supplement (10 μg/ml) and accompanied by alteration of cell morphology and activity. Histological staining, ICC for type X collagen and determination of alkaline phosphatase activity were performed to reveal differentiation of ATDC-5 cells. The cells effectively migrated into composite cryogels and colonized the matrix. The effect of structure of the composite HAP/gelatin cryogels on proliferation and differentiation of ATDC-5 cells was further assessed using LSCM and other techniques. This work supported by Program of Competitive Growth of Kazan Federal University.

P007

HEK-derived AAV purification: comparison of small scale laboratory production towards industrial format using monoliths

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Purification of recombinant adeno associated virus (rAAV) is designed to remove the large amount of host-cell and product related impurities which are produced alongside the target particle. Different downstream processing options are available for this purpose, such as gradient centrifugation, TFF and chromatography. Although effective at laboratory scale, methods such as Caesium chloride gradient purification lack scalability. Consequently, they cannot be used for purification of large batches and cannot keep up with the increasing demand for gene therapy vectors. The aim of the study was to develop a robust, fast and efficient rAAV virus purification platform, which can be used for several AAV serotypes with various inserts. The example presented introduces a chromatography based approach for purification of AAV9. Lysed harvest and culture supernatant of rAAV9 were first captured and concentrated on CIMmultus™ OH column, followed by an intermediate purification step on CIMmultus™ SO3 column and finally polished on CIMmultus™ QA column. The process presented avoids the use of ultrafiltration and affinity purification steps. The method is fully scalable and suitable for a cGMP environment. The purity of this industrial scale monolith purification product was compared to a laboratory scale purification using a ''conventional'' method.

P008

3D-derivation of uncommitted human muscle stem cells from iPSCs

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One of the most fundamental problems associated with stem cell therapy of skeletal muscle is the limited availably of cells that can robustly engraft into the stem cell compartment. It has extensively been attempted to isolate adult muscle stem cells (MuSCs) and expand them in culture to obtain sufficient cell numbers for such treatments. The challenge associated with this approach is that, once isolated from their niche and maintained in culture, MuSCs

become terminally committed to myogenic differentiation and show a dramatically reduced engraftment potential. However, the recent discovery of induced pluripotent stem cells (iPSCs) has opened new avenues for the in-vitro derivation of cell types that are more suitable for transplantation. Here, we report a highly efficient approach for the scalable derivation of uncommitted MuSCs from human iPSCs in a biologically faithful 3D environment. We employed human iPSCs and a spectrum of immortalized cell lines to generate 3D aggregation conditions promoting mesoderm formation and subsequent specification to the myogenic lineage without the parallel upregulation of myogenic commitment markers. Taken together, our work reveals a seven-day derivation protocol for the generation uncommitted MuSCs from human iPSCs that can easily be scaled up to the bioreactor level.

P009

rAAV large scale manufacturing using BEVS technology

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Recombinant Adeno Associated Virus is becoming a vector of choice for a variety of human gene therapy applications. Therefore, there is an increasing requirement for generation of high quantity of potent rAAV vectors based on various serotypes. Baculoviruses and insect cells has been shown to be amenable of generation of massive quantity of a high quality rAAV. In order to exploit a full potential of the Baculovirus Expression Vector System to support the large scale rAAV manufacturing, uniQure engaged into development of a single use stirred tank bioreactors (STR) process for rAAV manufacturing. We will review our comparative efforts to demonstrate the similarity between the small-scale (50L) wave and STR processes. Furthermore, the visibility of scaling uniQure's proprietary platform to large scale manufacturing (500L) will be discussed.

P010

Effects of curcumin-loaded halloysite on C.elegans nematodes in host-microbiome interactions

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Curcumin is a natural compound with anticancer, antioxidant and antimicrobial properties. However, pharmacological applications of curcumin are limited by its low water solubility and bioavailability. Thus, a carefully designed nanovehicles are required to significantly facilitate the delivery of curcumin and broaden the range of its perspective therapeutic applications. To encapsulate curcumin we used natural clay halloysite nanotubes (HNTs). HNTs lumens were loaded with curcumin using a vacuum process and functionalized with enzyme-responsive dextrin stoppers. The efficiency of curcumin loading into halloysite was estimated by thermal gravimetric analysis. The morphology of nanocontainers was studied using electron microscopy. The *in vivo* antibacterial effect of curcumin-loaded and dextrin-coated halloysite nanotubes (curcumin@HNTsD) against Serratia marcescens ATCC 9986 investigated using Caenorhabditis elegans nematodes. The loading of 10 mg curcumin in 100 mg HNTs formed a stable and strongly yellow-colored homogeny dispersion. The drug loading efficiency was $9{,}93 \pm 0{,}68\%$ by mass. Typical tubular shape of the modified nanotubes of the nanoclay preserved and lumen filled with curcumin. Results showed a significant reduce in intestinal colonization of S. marcescens in the presence of nanocontainers when compared to control. In the presence of curcumin@HNTsD during 72 hours S. marcescens failed to produce prodigiosin in the intestine of C. elegans. Furthermore, curcumin@HNTsD twofold increased the lifespan of nematodes infected by S. marcescens. We believe that curcumin-loaded clay nanocontainers could be potentially developed into an effective antimicrobial drug. The work was performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and RFBR 17-04-02182 grant.

P011

Molecular evaluation of neuroprotective properties of WJ-MSC in different microenvironmental conditions

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Mesenchymal stem cells (MSC) applied in regenerative medicine are exposed to the recipient strong immune response, which significantly hinder their survival and tissue regeneration after transplantation. New studies focused on the enhanced neuroprotective and anti-inflammatory role of 3D cultured cells are proposed. The aim of *in vitro* studies was to test the effect of different microenvironmental conditions (oxygen concentration and 3D hydrogel scaffolds) on WJ-MSCs proliferation, viability and gene expression profile. The *ex vivo* studies in organotypic hippocampal slice culture (OHC) with experimental model of oxygen glucose deprivation aimed to mimic an ischemic injury of neural tissue. MSC-induced neuroprotection was evaluated in OHC co-cultured indirectly with WJ-MSCs either in 2D or on the 3D hydrogel scaffold. To assess cell death in CA1 and CA2/3 hippocampal regions, the fluorescent marker propidium iodide was used. The secretion profile of WJ-MSC in 2D vs 3D was characterized with qRT-PCR. WJ-MSCs in hydrogel scaffolds show high survivability, and are able to migrate beyond the 3D structures. The increased expression of e.g. BDNF, VEGF-A compared with 2D cell cultures was also observed. *Ex vivo* studies indicated strong neuroprotective effect of WJ-MSCs on injured hippocampal slices. WJ-MSCs seeded on the scaffolds as compared to 2D culture, revealed increased expression of several neurotrophins, decreased expression of pro-inflammatory cytokines (IL-1β) and elevated expression of anti-inflammatory TGF-β. The results have indicated that optimizing and standardizing culture conditions is necessary for generation of therapeutically competent cell population. Furthermore, the analyzed scaffold models are promising for future use in MSC-based therapy.

P012

The mechanism of the nanoparticles penetration into human cells

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Cell culture is one of the experimental models of genetic and cellular engineering. Also, cell culture is an ideal object in studies of the toxicity of various substances, including nanoparticles. At this time, the mechanism of the penetration and exposure of nanoparticles into cells is not clear enough. We used the cell line A549 in our work. The cell line was cultured in the presence of nanoparticles of halloysite, kaolin and graphene oxide. Then the cells were fixed and dried. AFM images were made using Dimension Icon microscopes in Peak Force Tapping mode. The result data was processed using NanoscopeAnalysis1.7. In our work we analyzed the surface of cells with different nanoparticles using atomic force microscopy. High-resolution AFM images demonstrate typical morphological characteristics. Scanning the surface gives a more accurate three-dimensional image. In Peak Force Tapping mode, we can not only demonstrate the surface structure, but also obtain data on the mechanical properties of the surface (elasticity, stiffness, adhesion). Stiffness and adhesion indicate that the adhesion cells have not changed, but they have become tougher. It can be assumed that the particles penetrated the cell, thereby changing the rigidity of the cell itself. A partial absorption of nanoparticles by the cell was detected. By mechanical characteristics, it can be seen that the cell absorbs nanoparticles, most likely, by endocytosis. This data is also confirmed by electron microscopy. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and RFBR 18-44-160001grant.

P013

Identification of nano particle mixtures in human cell culture using dark-field and hyperspectral imaging

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We report the use of dark-field microscopy and hyperspectral analysis for the fast and effective detection of combinations of nanoparticles in human cell culture. Unlike the conventional approaches, dark-field and hyperspectral imaging allows for the direct observation and mapping of nanoparticles distribution in cells under ambient conditions. We used human lung carcinoma cells incubated with several combinations of nanoparticles, namely gold, titanium oxide, zinc oxide nanoparticles and carbon nanotubes.To visualize the nanoparticles inside the cells, we first fixated cells on coverslips and stained the nuclei of the NP-incubated cells with DAPI. After mounting the samples, we used CytoViva hyperspectral microscopy based on enhanced dark field condenser attached to Olympus BX51 upright microscope equipped with a fluorite 100×objective, to obtain dark-field and hyperspectral images of the nanoparticles directly samples. Hyperspectral analysis of the data and mapping of nanoparticles was performed in the cells. We obtained dark-field images of human cell culture. Hyperspectral images of cells were obtained using the ENVI software. The subsequent analysis of the three-dimensional array of hyperspectral data and the mapping of nanomaterials allows investigating the distribution and quasi-quantitative uptake distribution of nanoparticles in cell cultures. We were able to distinguish the different types of nanoparticles based on hyperspectral libraries collected using pure nanoparticles. We demonstrate the detection of various nanoparticles in human cell cultures based on dark-field imaging and corresponding hyperspectral mapping. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and funded by Russian presidential grant MD-6655.2018.4.

P014

Characterization of microelement-doped hydrogels as a bioactive cellular matrix

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Modification of tissue-engineered materials with organic and inorganic components is an important approach to improving their physicochemical and regenerative properties. Microelements have versatile regulatory effects *in vitro* and *in vivo* and great potential for promoting tissue regeneration in acute and chronic lesions. In our study, bioactive transition metals (Zn, Co, Mn, Cu etc.) were combined with model hydrogels of different structure prepared by chemical cross-linking of gelatin. Cryopolymerization technique was used to produce cryogels with large interconnected pores. The conditions for controllable introduction of metal compounds into the hydrogels were studied. The distribution and stability of the metal component in biomaterials were determined by ICP-MS and micro-X-ray fluorescence analysis. The structure of biomaterials was characterized by rotational rheometry, SEM and LSCM. The results showed that the metals were stably and uniformly incorporated into the hydrogels, forming additional bonds in the hydrogel network, and therefore modulating pore size, swelling and physicochemical properties of the biomaterials. The effect of metal component on migration and proliferation of human skin fibroblasts within the modified cryogels was studied using MTS assay and LSCM. The concentration range of metals in the matrix, which did not provide cytotoxic effect on cultured cells, was estimated and compared with the effect of compounds supplemented in culture medium. Our results demonstrate that transition metal-doped hydrogels may serve as cell-compatible and modulating matrices and are also of interest in wound healing applications. This work was performed according to Russian Government Program of Competitive Growth of Kazan Federal University.

P015

Silver-infused halloysite nanotubes as an antibacterial nanocomposite in cell therapy

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Microorganisms can acquire resistance to specific antibacterial substances and form bacterial films on various surfaces. Therefore, the development of new nanocomposite materials with sustained release of antibacterial agents has become crucially important in modern medicine and cell therapy. The use of silver nitrate can contribute to solving this problem because it has a wide spectrum of antibacterial activity and low toxicity for mammalian cells. Different concentrations of silver nitrate and halloysite were investigated. The loading of silver nitrate into

halloysite nanotubes was made with vacuum infiltration. The antibacterial activity was tested on the ATCC 9986 strain of S. marcescens (0.4 OD at 595 nm) using a nutrient agar medium. The plates were incubated at 28°C for 24 h. We found that all the investigated concentrations of silver nitrate and halloysite nanotubes have antibacterial activity, but the best effect was obtained using a concentration of 100 mg: 50 mg (silver nitrate: halloysite). This investigation showed that silver-doped halloysite nanotubes exhibit antibacterial effect against S. marcescens and they will be used in further studies to develop an antimicrobial coating for devices applied in cell culturing and manipulation. The work was performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and 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).

P016

in vitro optimization of a cryogel based tumour model

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Development of three-dimensional models of tumor tissues is of particular interest for studying different aspects of cancer progression and therapy. Effective formation of tumor-like structures *in vitro* requires optimization of matrix structure as well as conditions for cell seeding and culturing. We aimed to develop a prototype of tumor model by combining gelatin based macroporous cryogels and different cancer cells. The cells were seeded within the cryogel membranes by three different ways: migration from cell monolayer, top seeding and syringe injection. Using the MTS assay cell quantity in the cryogels was determined, and the effect of diffusion retardation was estimated. Cell proliferation, morphology and microenvironment in whole and cryosectioned matrices were analyzed. The results showed that, in comparison with the migration method, top seeding provided increased number of the cells, which were predominantly located at the periphery of matrix. Direct injection of cell suspension inside the cryogel resulted in much higher cell density; furthermore, the cells were detected both along the borders and in the bulk of cryogel. The number of injected cells and time of culturing were optimized to ensure linear proliferation rate in the matrix. The cells grown in the cryogels were characterized by increased cytotoxic concentrations of doxorubicin, and hence, decreased drug sensitivity attributed to the supporting effect of 3D matrix. Our results will be used to further develop specific cryogel based tumor models for *in vitro* and *in vivo* studies. This work was performed according to Russian Government Program of Competitive Growth of Kazan Federal University.

P017

Effect of titanium nickelide reticular membranes on mesenchymal stem cells *in vitro*

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One of the problems in dentistry is the study of the interaction of membranes from artificial materials with mesenchymal stem cells for osteoregeneration. Adipose derived mesenchymal stem cells (MSC) were isolated from the visceral fat tissue of a dog using enzyme treatment. The fat tissue was obtained during the planned sterilization surgery under sterile conditions in a veterinary operating room. All experimental procedures were performed with the written informed consent of the dog's owner. In order to study the influence of titanium nickelide membranes on MSC, cells were seeded on top of membranes and cultured for 96 h. Control cells were cultured in standard tissue culture dishes without membranes. During the whole period of culturing, membranes floated freely in the culture medium without settling to the bottom. The dog's cells underwent adhesion on the culture dishes and proliferated actively in both control and experimental groups. When examined under the microscope, adhesive cells were seen at the porous surface of a braided filament of the nickelide reticular membrane. The proliferation index in the control ($n=3$) and experimental ($n=3$) groups was 1.8 ± 0.1 and 1.7 ± 0.1, respectively. Therefore, the titanium nickelide reticular membrane had no cytotoxic effect on the dog's MSC in the case of *in vitro* culturing, which makes it possible to create combined tissue-engineered membrane constructs for better osteoregeneration.

P018

Magnetic nanomaterials for 3D spheroids formation

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Background Development of 3D cultures of mammalian cells is actual for cell therapy because of their possible application in experimental probing of anti-cancer drugs (Theodoraki et al., 2015; Friedrich et al., 2009), nanomaterials toxicity screening as well as understanding the 3D development of mammalian organs (Achili et al., 2012; Leong et al., 2014). The impact of magnetic nanoparticles (MNPs) stabilized by a positively charged polyelectrolyte poly(allylamine)hydrochloride (PAH) on spheroid formation from cancer and primary cell lines was investigated. Material and methods Magnetic nanoparticles were synthesized using aqueous solutions of FeCl2/FeCl3 as reported previously (Dzamukova et al., 2015). The MNPs were stabilized with cationic polyelectrolyte PAH (poly(allylamine hydrochloride) Mw 70 kDa)). The hanging drop method was realized for spheroid formation (Kelm et al., 2003). Results Absence of adverse effects of nanoparticles on the formation of 3D cell structures was shown. Using magnetic nanoparticles (MNPs) allows controlling spheroid movement, which can be used in cell biology, tissue engineering and nanomedicine. Development of 3D cultures is very important especially that of mixed spheroids because they retain many essential properties of natural tissues and organs. Nanoparticles are now widely applied as drug delivery systems and in tissue engineering. Conclusions Non-hindrance of synthesized MNPs with the formation of spheroids from both tumor and primary cells make these nanomaterials highly promising candidates for applications in tissue engineering. Acknowledgments: This work is supported by Russian Government Program of Competitive Growth of Kazan Federal University and funded by Russian presidential grant MK- 4498.2018.4 and RFBR 18-34-00306 grant

P019

CARAAVs - a novel class of CAR-antagonists in cancer immunotherapy

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Adoptive immunotherapy using chimeric antigen receptors (CARs) has come of age recently, culminating in FDA-approval of two CAR T cell products. Despite impressive results, clinical management of severe side effects like cytokine release syndrome (CRS) still remains challenging. Current intervention approaches that not only reduce symptoms but focus on the effector cells are either not effecting all CAR T cells or induce their apoptosis. Interestingly, the occurrence of a cytokine storm is positively correlated with treatment outcome, indicating the need of a drug that interferes with the CAR while keeping the T cell viable. We successfully developed capsid-engineered AAV2 particles displaying the binding epitope of a CAR (CARAAVs) that promise to fulfil these criteria. CAR-AAVs specifically bind to the scFv of the cognate CAR in ELISA and cellular binding assays independent AAV2's attachment receptor HSPG. Strikingly, already 4 hours after co-incubation, surface CAR expression is significantly reduced in T cell lines and primary T cells without activation of T cells. This inhibitory effect is solely dictated by the inserted epitope irrespective of the AAV serotype and is completely reversible. Moreover, we could define a therapeutic window of 10 hours in which CARAAVs inhibit CAR T cell-mediated killing of breast cancer cells. Thus, we consider CARAAVs as potent CAR antagonists that preserve CAR T cell viability. Owing to their specific, fast and reversible mode of action, we propose that CARAAVs could be clinically tested as prospective regulators of CRS or emergency medication to prevent cross-reactivity of high-risk CAR T cells.

P023

dmPGE2 and poloxamer-F108 enhance transduction of human hematopoietic stem and progenitor cells with a β-globin lentiviral vector

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Lentiviral vector (LV)-based hematopoietic stem and progenitor cell (HSPC) gene therapy is becoming a promising option for curing hemoglobinopathies. However, the large size and complexity of the human β- globin gene leads to LV products with low titers and stifled transduction efficiency. Thus, LV products often fail to achieve a clinically beneficial vector copy number (VCN) in patient HSPC. Here we investigated the potential of two compounds (10 μM dmPGE2 and 1 mg/mL poloxamer-F108) to enhance transduction efficiency of a GMP-grade preparation of Lenti/G-betaAS3-FB. Transduction efficiency with enhancers or vehicle control was evaluated in three G-CSF mobilized peripheral blood CD34+ cell lots exhibiting poor LV transduction (*in vitro* VCN <0.3 at 2E7 TU/mL, MOI = 20). Strikingly, the addition of dmPGE2/poloxamer-F108 increased the *in vitro* VCN of bulk myeloid cultures $\sim$10-fold (VCN without enhancers = 0.2 ± 0.02 [mean $\pm$ SD], VCN with enhancers = 1.94 ± 0.07) while using a 10-fold lower LV dose of 2E6 TU/mL (MOI = 2). This was accompanied by an increased percentage of LV+ colony forming units (CFUs) from $18 \pm 8\%$ to $76 \pm 8\%$. Significantly increased transduction efficiency was also maintained in the bone marrow of 12 week NSG xenografts. Importantly, the addition of dmPGE2/poloxamer-F108 did not skew hematopoietic differentiation (in CFUs and NSG xenografts) nor result in any detectable toxicity as determined by cell counts, percentage of apoptotic cells, CFU clonogenic potential, and NSG engraftment levels. These data suggest that inclusion of dmPGE2/poloxamer-F108 during LV transduction can increase gene transfer and therapeutic efficacy in gene therapy for hemoglobinopathies.

P024

Reversal of the thalassemic phenotype in mice post *in vivo* transduction of mobilized hematopoietic stem cells (HSCs) with an integrating hybrid adenovirus vector system

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To overcome the cost and complexity of current thalassemia *ex vivo* gene therapy protocols, we developed a minimally invasive and readily translatable approach for *in vivo* HSC gene delivery. It involves HSC mobilization with G-CSF/AMD3100 and intravenous injections of a hybrid 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 γ-globin and mgmtP140K genes. We tested our approach in a mouse model of β-thalassemia intermedia (Hbbth-3/hCD46++mice). At week 8 post transduction, hCD46+/+/Hbbth-3mice expressed HbF in $31.2 \pm 2.7\%$ of circulating erythrocytes. Due to a significant drop in HbF expression by week 16 ($11.9 \pm 3.0\%$), a 4-dose O6BG/BCNU treatment was applied recovering the HbF expression in circulating erythrocytes ($76.0 \pm 5.7\%$). With an average vector copy number of 1.4/cell, the human γ-globin to mouse α-globin expression was $\sim$10% by HPLC and the human γ-globin to mouse β-globin mRNA ratio $\sim$10%, by qRT-PCR. Hematological parameters at week 29 post *in vivo* transduction were significantly improved over baseline or were indistinguishable from normal values, suggesting near to complete phenotypic correction. Treated mice showed significant reduction of spleen size, extramedullary erythropoiesis and parenchymal hemosiderosis. At week 16 after secondary transplantation, more than 90% of donor-derived erythrocytes (CD46+) were γ-globin-positive. Safety was demonstrated by the absence of alterations in hematopoiesis, the normal colony-forming potential of bone marrow cells and the random integration pattern of our vector system. Overall, we present a simplified platform for gene therapy of thalassemia, which can serve as a cost-efficient and ''portable'' approach.

P025

Oncogene-induced senescence in haematopoietic progenitors features myeloid-restricted hematopoiesis and histiocytosis

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Hematopoietic stem cell gene therapy has brought unprecedented clinical benefits; however, oncogenic activation remains a concerning side effect. Activation of the MAPK pathway by insertional mutagenesis is a major culprit of tumorigenesis, particularly when vectors carried strong enhancers/promoters. Oncogene-induced senescence (OIS) responds to MAPK pathway activation and results in proliferation arrest and activation of a pro-inflammatory program. OIS establishment is beneficial and leads to benign cancer lesions; however, cytokines may exacerbate many diseases contributing to chronic inflammation. The effects of OIS on hematopoiesis have been poorly studied, but MAPK mutations are associated to histiocytosis, a potentially lethal disease characterized by multi-organ accumulation of pro-inflammatory dendritic cells with elusive pathogenesis. To investigate the role of MAPK activation in the hematopoietic system, we ectopically expressed in human hematopoietic progenitors the oncogene BRAF-V600E, well-established inducer of OIS and most frequent mutation found in patients, and transplanted them into immunocompromised mice. Mice showed severely hampered hematopoiesis, which gave rise almost exclusively to monocytes and dendritic cells, and quickly developed an aggressive histiocytosis that recapitulated all the main features of OIS. Senescence features were not restricted to BRAF-V600E-expressing cells, because cytokines (and in particularly TNFa) could transfer them to non-mutated bystander cells. Overall, OIS in hematopoietic progenitors acts as an anticancer mechanism but, contrary to what has been observed in other tissues, its chronic inflammatory response and the continuous availability of cells from peripheral blood can allow lesion growth and can lead to a life-threatening disease.

P026

Surgery and bleed management in patients receiving AMT-060 in a Phase I/II trial: evaluation of the safety of exogenous FIX treatment after gene transfer

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Gene transfer for haemophilia offers the potential to convert the disease phenotype from severe to mild with a single treatment. It is unknown whether additional factor replacement, in the context of surgery or traumatic bleeds, interacts with the effects of gene therapy. Patients enrolled in CT-AMT-060-01 (NCT02396342) received a single intravenous infusion of AMT-060, an investigational adeno-associated virus serotype 5 vector containing a codon-optimized wildtype human FIX gene and liver-specific promoter, of either 5e12 gc/kg (n=5) or 2e13 gc/kg (n=5). Patients who successfully discontinued pre-trial prophylaxis and who had post-treatment episodes of cumulative factor use >15000 IU were selected for examination of adverse events and clinical outcomes (patient-reported bleeds, endogenous FIX activity, FIX consumption). Five patients met criteria for inclusion in the analysis. Three patients had 1 episode >15000 IU each: 2 surgeries (IUs FIX used/over days/days post gene-transfer: 32,500/7/512 and 83,000/21/108) and 1 bleed (19,000/3/256). One had 4 episodes due to bleeds (16,000/3/25, 44,000/6/52, 20,000/3/274, and 16,000/4/345); and 1 had 3 episodes (27,000/6/181 for a bleed; 62,000/46/225 and 87,000/76/382 for bleed followed by short-term prophylaxis). No adverse events, FIX inhibitors or thrombotic events were associated with the FIX replacement. Mean(SD) endogenous FIX activity ranged from 5.1(0.8) to 12.0(1.3) in these patients, corresponded to a 1:1 antigen:activity ratio in the 3 CRM- patients, and remained stable after replacement for these episodes. These cases suggest FIX replacement in the peri-operative setting or for acute bleed management following AMT-060 was safe and did not impact transgene-driven FIX expression.

P027

Preclinical studies towards the gene therapy of Diamond-Blackfan anemia

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Diamond Blackfan anemia (DBA) is an inherited bone marrow failure syndrome (iBMF), associated with congenital anomalies and cancer predisposition. Allogenic hematopoietic stem cell transplantation (HSCT) currently represents the unique definitive curative treatment of DBA. As an alternative, gene therapy (GT) could constitute an innovative therapeutic strategy for DBA patients. Nevertheless, many open questions still arise in the development of DBA GT, including potential limitations in the availability of hematopoietic stem cells and progenitor cells (HSPC) and in their repopulating properties after genetic correction. To answer to these key points in the GT of DBA, we have first characterized the content and functionality of HSPCs from bone marrow samples of DBA patients. In contrast to the very low number of CD34+ and colony forming cells observed in the BM from Fanconi anemia patients, we have observed significantly higher numbers of these progenitor cells in BM samples from DBA patients. Additionally, we observed hematopoietic engraftment in NSG immunodeficient mice transplanted with BM cells from DBA patients. Aiming at correcting the phenotype of DBA HSCs, we have constructed two therapeutic lentiviral vectors (LV) carrying a codon-optimized version of the *RPS19*cDNA driven by the PGK or the EF1alpha promoter. Studies carried out in DBA-like cells (K562

transduced with anti-RPS19 shRNA-LVs) and in CD34+ cells from DBA patients showed that transduction of either of the therapeutic LVs, suppressed the pre-rRNA processing phenotype associated with reduced RPS19 in DBA-like cells, and restored the expression of RPS19 in hematopoietic cells from DBA patients. These studies support that gene therapy may constitute suitable approach for the treatment of DBA patients.

P028

Impairment in the hematopoietic supportive capacity of bone marrow stroma in beta-thalassemia patients is associated with niche iron overload and oxidative stress

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The human bone marrow (BM) niche contains a population of mesenchymal stromal cells (MSCs) that provide physical support and specific molecular signals to sustain hematopoietic stem cell (HSC) homeostasis. Beta-thalassemia (BT) is an hereditary blood disorder characterized by reduced or absent synthesis of hemoglobin beta-chains amenable to allogeneic HSC transplantation and HSC-gene therapy. Iron overload is a common complication in BT patients caused by chronic blood transfusion affecting several organs. However, data on the BM stromal compartment of BT patients are scarce. Here, we reported high levels of total iron and ferritin in BT BM niche, indicative of increased iron accumulation. We isolated BT-MSCs showing a reduced clonogenic capacity, longer population doubling time and altered differentiation capacity compared to healthy donor (HD) controls. *in vitro* experiments confirmed that these alterations correlated with a pauperization of the most primitive MSC pool caused by an iron-dependent increased production of ROS and impaired anti-oxidant response in BT-MSCs. Similarly, we found a reduced frequency of primitive MSCs in patient's BM samples. Finally, we discovered an altered expression of BM niche associated genes associated with a reduced hematopoietic supportive capacity of BT-MSCs *in vitro* and *in vivo*. The results of this work suggest an impairment in the MSCs of BT-BM niche possibly associated with prolonged iron exposure and underline the importance of iron level for normal MSC function. Whether the BT stromal niche impairment influences HSC engraftment and support after allogeneic and autologous transplantation procedures is being further investigated to improve treatment outcome.

P029

BaEV pseudotyped LVs confer FVIII gene transfer in HSCs, allowing secretion of functional factor FVIII from a B-cell specific promoter *in vivo* in NSG FVIII $^{-/-}$ mice

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Previously, we demonstrated that our baboon envelope pseudotyped lentiviral vectors (BaEV-LVs) are potent tools for high transduction of human hematopoietic stem (hHSC) and B cells. Here, we assessed BaEV-LVs for a possible treatment of hemophilia A (HA). We designed an LV containing human FVIII and a GFP reporter under the control of a B-cell specific promoter, FEEK (BaEV-Feek-FVIII-GFP). This vector was produced at high titer (1E8 IU/ml) and allowed efficient expression and secretion of active FVIII in the Raji B cell line and the plasma B cell line, U266. In addition, we confirmed high levels transduction and secretion of FVIII with BaEV-FEEK-FVIII-GFP in primary human plasma cells. We then tested the vector *in vivo* by transplantion of umbilical hHSC transduced with BaEV-FEEK-FVIII-GFP LV into immunodeficient hemophilic mice (NSG-HA, n = 8) after sublethal conditioning with busulfan. Transplanted mice showed an average of 37% of human engraftment in bone marrow (BM) while the chimerism peaked at 23% at week 12 in peripheral blood (PB). Most of the human cells were $CD19^{+}$ B cells and aPTT assay confirmed up to 5 % hFVIII activity in the NSG-HA and partial bleeding phenotype correction. In contrast, FVIII activity close to zero was observed in NSG-HA mice injected with either untransduced or BaEV-FEEK-GFP transduced hHSC used as control. Concluding, BaEV-FEEK-FVIII LVs permit partial correction of the bleeding phenotype in a preclinical model for FVIII deficiency through FVIII secretion from hB cells

P030

The NPY-Y1 receptor for neuropeptide Y is highly expressed on hematopoietic stem cells from bone marrow and cord blood

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Neuropeptide Y (NPY) is a neurotransmitter, secreted by sympathic nerves of the central and peripheral nervous system. However, NPY also affects hematopoietic stem cells (HSCs) by activating different G-protein couples receptors, present on bone marrow niche cells, including macrophages, osteoblasts and endothelial cells. Absence of NPY or its receptors results in a decrease in the numbers of HSCs, but does not affect HSC differentiation or maturation. Here, we studied the direct effects of NPY on HSC proliferation and expansion. Healthy donor bone marrow cells and cord blood cells were assessed for the expression of NPY receptor Y1 (NPY-Y1). The effect of different concentrations of NPY (0.01 nM to 10 nM) on HSC proliferation was tested using WST-1 cell proliferation agent (Roche) and Propidium Iodide (Sigma) to assess cell cycle activity. In both bone marrow and cord blood cells, NPY-Y1 receptor was highly expressed on CD34+ cells, but also on mature hematopoietic cells, including CD19+ B-cells, CD3+ T-cells and CD16+ Natural Killer cells. Concentrations of 0.3 nM NPY resulted in the highest proliferative activity of HSCs after 4 days of serum-free culture in StemMACS HSC expansion medium supplemented with TPO, SCF and Flt3-ligand based on WST and cell cycle measurements. High expression of the NPY-Y1 receptor on HSCs and mature hematopoietic cells, as well as a proliferative effect in response to NPY treatment in the absence of other niche cells indicates a direct regulatory effect of NPY on HSCs.

P031

Preclinical biosafety studies of lentiviral vector-mediated gene therapy in erythrocyte pyruvate kinase deficiency

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Red blood cell pyruvate kinase (RPK) deficiency (PKD) is an autosomal recessive disease caused by mutations in the PKLR gene that produce chronic non-spherocytic hemolytic anemia (CNSHA). Currently there is no specific curative therapy, although allogeneic bone marrow transplantation, has been reported to reverse the disorder in a small number of patients with severe disease. Supportive treatments, including regular blood transfusions, iron chelation and splenectomy, provide palliation for severe PKD patients. Previously, we demonstrated that the transplantation of lentivirally-transduced hematopoietic stem cells provides a definitive correction of the disorder in PKD mice (Garcia-Gomez, Calabria et al. 2016). In order to support the clinical application of Hematopoietic Stem Cell (HSC) gene therapy for PKD, we conducted preclinical biosafety and biodistribution studies to assess the safety and tolerability of lentiviral vector-based PKD gene therapy. Lineage negative bone marrow cells from male and female P3D2F1 mice (B6.SJL-Ptprca Pepcb/BoyJ × DBA/2j) were transduced with 4x107 hPGK-coRPK-Wpre* lentiviral particles (LV) and transplanted into lethally irradiated B6D2F1 (C57Bl/6j-Ptprcb × DBA/2j) congenic recipients of both sexes. We did not detect any toxicity associated with the HSC gene therapy protocol. Additionally, mice transplanted with transduced cells showed efficient hematopoietic reconstitution and a high vector copy number (range X to Y copies/cells) in different hematopoietic organs. Only residual copies were identified in non-hematopoietic organs. Additionally, no viral particles were detected in the serum of these animals. These data confirm the safety of our preclinical lentiviral vector-based PKD gene therapy prior to clinical testing.

P032

Kinetics and quality assessment of mobilized stem cell product upon G-CSF and Plerixafor administration in patients with hematological disorders for gene therapy approaches

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Mobilized Hematopoietic Stem/Progenitor Cells (mHSPC) are becoming the major source for HSPC-gene therapy (GT) due to superior accessibility and higher number of collected cells compared to bone marrow (BM). Current mobilization protocols are based on administration of G-CSF (G) and the CXCR4 antagonist Plerixafor (P), often combined in poor mobilizers or in GT trials. To dissect the mobilization kinetics in patients with hematopoietic disorders we are now deep-phenotyping and quantifying HSPC subsets and mature lineages in peripheral blood (PB) before, after G and G+P administration in 5 WAS, 4 ADA-SCID and 2 CGD patients. As reference we collected data from 8 adult healthy donors and 2 pediatric subjects with non-immune disease. Our analyses showed similar kinetic of mobilization in all groups with enrichment of both primitive and myeloid progenitors upon G, followed by an increase of lymphoid progenitor contribution after P. Interestingly, the G+P mHSPC resembled their BM counterpart before mobilization. We calculated Mobilization Index (MI) for each HSPC subpopulation based on the absolute count of each subset in PB before and after mobilization with respect to BM before mobilization. We estimated that multi-potent progenitors were the subset with the highest ratio of mobilized cells over BM counts whereas hematopoietic stem cells displayed an increase of their MI after P. Finally, we are correlating mHSPC content and phenotype after mobilization with the hematopoietic reconstitution in patients receiving GT. These information will be fundamental to assess the impact of the mHSPC quality using different mobilization strategies on the outcome of GT.

P033

Efficient and cost effective transduction of hematopoietic stem cells with lentiviral vectors for the treatment of leukocyte adhesion deficiency type I

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The efficacy of hematopoietic gene therapy (HGT) relies on the stable engraftment of gene-corrected hematopoietic cells in patients. Although transduction at high multiplicities of infection (MOIs) or with multiple infection cycles has been employed to increase the transduction of hematopoietic stem cells (HSCs), prolonged transduction periods have been shown to impair the subsequent engraftment of *ex vivo* manipulated HSCs. Additionally, the very high cost of therapeutic vectors limits the application of high MOI-based strategies for clinical HGT. As an alternative approach, here we have investigated the efficacy of different transduction enhancers (TEs) to genetically correct mouse and human HSCs using low MOIs of lentiviral vectors (LVs) developed for the treatment of Leukocyte Adhesion Deficiency Type I (LAD-I), with a defective or null expression of CD18. The combined use of two different TEs increased more than 2-fold the stable expression of CD18 in recipient LAD-I mice. Similar results were obtained when optimized combinations of TEs were used in human $CD34^+$ cells. As observed in mouse HSCs, TEs increased 2-3 folds the transduction of $hCD34^+$ cells and preserved the repopulating ability of these cells when transplanted into

immunodeficient mice. On average, transduction of hCD34$^+$ cells with GMP-produced LVs used at MOIs of 10-20 IU/cell resulted in 1-3 copies of the therapeutic gene per genome of engrafted cells. Our results indicate that the use of optimized combinations of TEs will constitute practical and cost-effective methods for transduction of human HSCs in HGT protocols, including imminent gene therapy investigations in LAD-I patients.

P034

Efficient *ex vivo* gene transfer of γ-globin in human thalassemic CD34$^+$ cells using an integrating hybrid adenoviral vector system

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Ex vivo approaches for gene therapy of hemoglobinopathies traditionally rely on the use of self-inactivating lentiviral vectors (SIN-LVs). We here propose an alternative platform to effectively address some of the current challenges of LV gene therapy, including suboptimal titers due, in part, to limited transgene accommodating capacity and the semi-random integration of SIN-LVs showing preference for active genes. A CD46-targeting, high-capacity adenovirus HDAd5/35++ vector system, capable of achieving random integration of a full-length human γ-globin gene by an enhanced Sleeping Beauty transposase (SB100X), was tested in human CD34$^+$ cells from thalassemia-major patients (n=2), previously enrolled in mobilization trials. After overnight transduction (MOI 500), 1×10^6 CD34$^+$ cells were transplanted into partially myeloablated NSG mice. Multilineage reconstitution was achieved in all mice, suggesting that transduction with the HDAd5/35++ vector system does not negatively affect the engraftment potential of CD34+ cells. Human CD45+ cells isolated from the chimeric transduced NSG bone marrow (BM) and seeded in methylcellulose and in erythroid differentiation culture (EC) expressed higher levels of HbF over the untransduced BM samples (BFU-E %: 66±9.09% vs 11±0.65%, EC: 81.05% vs 6.69%). Importantly, myeloid colonies (CFU-GM) derived from either the untransduced or the transduced samples were expressing HbF at similar, low levels, supporting the erythroid-specific expression of our vector system. We here show that the HDAd535++ vectors targeted HSCs with high efficiency and may serve as an alternative vector system for gene therapy of hemoglobinopathies over the traditionally used SIN-LVs.

P035

Proof of concept for autologous cell/gene therapy of hemophilia B using patient's specific iPSC-derived hepatocytes after genetic correction with CRISPR/Cas9 technology

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Hemophilia B (HB) is a genetic disorder due to an impaired activity of circulating clotting factor IX (FIX), synthesized by hepatocytes. Current treatment based on regular intravenous injections of FIX is very restrictive, costly and only palliative. Gene therapy trials show promising results but their long-term efficacy is still unknown. It is thus important to explore other therapeutic strategies. To demonstrate the feasibility of a gene/cell therapy approach using patient's iPSCs, we reprogrammed skin fibroblasts from a severe hemophilia B patient (FIX activity <1%). Using the CRISPR/Cas9 technology, we then targeted the genomic insertion of a cassette including the hepatic specific apolipoprotein AII (APOAII) promoter driving the expression of a F9 mini-gene. One third of the amplified clones showed accurate monoallelic integration at the targeted AAVS1 safe harbor locus. Non-corrected and corrected iPSCs were differentiated into hepatocytes (HB-Heps and Corr-HB-Heps, respectively) to study the expression of FIX. Due to the promoter used, the FIX mRNA expressed by the therapeutic cassette is detected earlier than the endogenous one during the differentiation. Both HB-Heps and Corr-HB-Heps produced FIX detectable both by immunostaining and western blot. FIX activity is currently studied and preliminary results confirm clotting activity in corrected cells. In parallel, the therapeutic efficacy of the correction is assessed *in vivo* by transplantation of Corr-HB-Heps into the liver of newborn HB mice. Immunohistochemistry analyses show a good engraftment of the cells and activity measurements on mouse plasmas are currently performed to confirm the therapeutic efficacy of our approach.

P036

Systematic comparison of culture media and transduction enhancers for optimised CD34$^+$ cell-based retroviral gene therapy protocols

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Ex-vivo retrovirus-based cell modification has demonstrated considerable success in treating monogenetic haematological diseases. However, current gene therapy protocols may profit from further optimisation of CD34$^+$ HSPCs culture and infection to improve cell quality, increase efficiency and reduce cost, and to allow the application of difficult-to-transduce cells or vectors. To identify ideal culture conditions, three GMP and one animal-cell-free media were compared in terms of HSPC expansion, stemness and CFU profiles. Among them, SCGM media led to the highest HSPC percentage and promoted the strongest expansion in absolute CD34$^+$CD38$^-$CD90$^+$ HSPC counts, while CFU counts were comparable across the tested media. For optimised HSPC infection, a panel of 9 previously identified transduction enhancers (TE) were side-by-side compared employing EGFP-reporter vectors. Out of the tested compounds, 6 positively affected lentiviral and 5 increased alpharetroviral transduction rates. Systematic combination of up to three of the most promising compounds even further increased gene transfer

rates, reflected by an up 2.5-fold increase of MFI in $EGFP^+$ live cells, 1.7-fold increase in the percentage of $EGFP^+$ $CD34^+CD38^-CD90^+$ HSPC and >10-fold increase in VCN. The increase of the VCN was also true when selected TE combinations were tested with a clinical grade vector, yielding a >2.2-fold increase in VCN. Finally, selected TEs were included in the GMP manufacturing process of a HSPC ATIMP for X1-SCID. The inclusion of these TEs led to a 6.4-fold increase in VCN.

P037

Safety and dose escalation of BAX 888 (SHP654), an AAV8 vector expressing B-domain deleted factor VIII in patients with severe haemophilia A: design of a global, open-label, multicentre Phase 1/2 study

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BAX 888 (SHP654) is an investigational coagulation factor VIII (FVIII) gene therapy for the treatment of haemophilia A (HA), a rare congenital disease characterized by reduced levels of FVIII. The primary objective of this multicentre Phase 1/2 first-in-human study (NCT03370172) is to evaluate the safety of BAX 888 (SHP654) in patients with severe HA (FVIII activity <1%). Up to 10 adult male patients with severe HA using either prophylaxis or on-demand factor replacement therapy will receive BAX 888 (SHP654) as a single peripheral IV infusion. Patients with inhibitors to FVIII, haemostatic defects other than severe HA, chronic hepatic dysfunction or severe renal impairment will not be eligible. Initially, 2 patients will receive BAX 888 (SHP654) at a dose of 2.0×10^12 cp/kg (cohort 1). Based on safety and FVIII expression data (centrally determined with a one-stage clotting assay at weeks 4 and 14), cohort 1 can be expanded to 5 patients and/or dose escalation will be performed and patients in cohort 2 will receive BAX 888 (SHP654) at a higher dose (6.0×10^12 cp/kg). The primary outcome measure will be the incidence of BAX 888 (SHP654)-related AEs. Secondary outcome measures will include additional safety and efficacy assessments, including plasma FVIII activity levels and annual bleed rate. The study will collect data for 3 years (with long-term data from an additional 2-year extension), and will be conducted in accordance with all applicable regulatory requirements. Study and writing assistance funded by Shire. All authors are employees of, and may own stock in, Shire.

P038

The prevalence of pre-existing humoral immunity to AAV in adults with severe haemophilia: interim results from an ongoing global epidemiology study

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AAV2 and AAV8 are effective vectors for transgene delivery in clinical studies. Capsid-specific antibodies, however, can bind to AAV vectors and prevent transgene expression. Patients with pre-existing anti-AAV antibodies are excluded from receiving this therapy. The geographic prevalence of immunity to wild-type AAV is therefore important to identify eligible populations. Here we present interim data from an ongoing prospective epidemiology study in males aged 18–75 with severe haemophilia A or B. AAV8 is currently used in Shire's BAX 888 study (NCT03370172) as a liver-targeted capsid. Peripheral blood samples were collected for markers of immunity towards AAV. Titres of anti-AAV antibodies were measured by cell-based transduction inhibition assay (neutralizing antibodies; NAbs) and ELISA (binding antibodies; BAbs). Antibody titres with cut-offs of ≥1:5 (NAbs) and ≥1:80 (BAbs) defined seropositivity. Anti-AAV titres were compared across three geographies. Forty-one patients (mean age 33.8 ± 11.07 years) were enrolled across France, Spain, and the USA; data were available for 40 (NAb) and 31 (BAb) patients. While 3/14 (21.4%) US patients were positive for AAV8 NAbs, 14/23 (60.9%) patients in France and 3/3 patients in Spain were positive. Prevalence of NAbs to AAV2 was higher (25/40 [62.5%]) with a similar geographic distribution. AAV8 BAbs were present in 12/31 (38.7%) and AAV2 BAbs in 11/31 (35.5%) patients. This interim analysis demonstrates geographic differences in AAV seropositivity that should be recognized when identifying patients for gene therapy studies. Study and writing assistance funded by Shire. All authors were employees of, and may have owned stock in, Shire at study initiation.

P039

Developing SIN lentiviral vectors to correct RAG2 deficiency

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Recombinase-activating gene 2 (RAG2) deficient SCID patients lack B and T lymphocytes due to the inability to rearrange immunoglobulin and T-cell receptor (TCR) genes. RAG2, together with RAG1, are required as a dimer to initiate lymphoid-specific V(D)J recombination. Curative treatment option for this form of SCID is limited and confined to allogenic hematopoietic stem cell transplantation; however gene therapy (GT) might be a valid alternative, especially for patients lacking a suitable bone marrow donor. We focused on clinically relevant lentivirus SIN vectors containing different internal promoters (EFS, PGK, MND, UCOE), driving codon optimized version of the RAG2 gene to ensure optimal expression at low vector copy numbers (VCN). Lineage-depleted bone marrow cells were transduced with the novel lentiviral SIN vectors and transplanted into Rag2-/- mice, which were used as a preclinical model to assess efficacy by immunophenotyping, antibody production and immune spectratyping analysis. We observed that lentivirus-mediated GT allowed immunologic reconstitution which directly correlate with RAG2 expression levels. Indeed, mice having low RAG2 expression showed poor immune reconstitution; however higher RAG2 expression resulted in a lymphocyte reconstitution comparable to mice receiving wild type cells. Interestingly, we observed that a high RAG2 expression in the BM could be detrimental for B-cell development, highlighting the importance of RAG2 expression using a suitable promoter. Functional restoration of Rag2-deficiency was obtained restoring immunoglobulin levels and TCR rearrangements comparable to control mice. We conclude that functional restoration of RAG2-deficiency at low VCN can be achieved with clinically acceptable vectors utilizing the MND or PGK promoter.

P040

The *ex vivo* transduction of human hematopoietic stem cells induces the expression of NKG2D ligands

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The *ex vivo* transduction of hematopoietic stem cells (HSCs) with gamma-retroviral or lentiviral vectors (LVs) requires the activation of these cells with hematopoietic growth factors (HGFs) and their interaction with the therapeutic viral vectors. NKG2D ligands (NKG2D-Ls) are induced in several processes of cellular stress and may result in the activation of cytotoxic cells, such as NK or CD8 T cells. Due to the proliferative stress induced by HGFs, and given that viral vectors may trigger an additional stress to transduced cells, we investigated whether ligands for the NKG2D receptor were activated during the *ex vivo* transduction of human $CD34^+$ cells. Our studies showed that the 24-72h *ex vivo* stimulation with HGFs of either cord blood or mobilized peripheral blood $hCD34^+$ cells progressively induces the expression of NKG2D-Ls, particularly ULBP3, ULBP4 and MICA, in these cells. Significantly, this observation was more evident when LVs were present during the *ex vivo* incubation of $hCD34^+$ cells. We hypothesize that the infusion of *ex vivo* transduced cells expressing NKG2D-Ls might induce the secretion of inflammatory cytokines (TNFα and INFγ) or trigger a cytotoxic response against gene-corrected HSCs.

P041

Addressing the impact of vector genotoxicity on the dynamics of hematopoietic reconstitution by integration site analyses

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In hematopoietic stem cells (HSCs) gene therapy (GT) applications, patients are re-infused with millions of transduced cells, many of which harbor vector insertions leading to the deregulation of host genes including oncogenes and tumor suppressors. Despite the potential risk posed by these cells, leukemia occurs rarely and the behavior of these cells after transplantation is unknown. To study the fate of cells harboring genotoxic insertions in-vivo, we performed clonal tracking studies in wild type (WT) mice transplanted with WT or tumor prone Cdkn2a-/- HSCs marked with a genotoxic Lentiviral Vector (LV) with active LTRs or an LV with SIN-LTRs. Mice receiving Cdkn2a-/- HSCs transduced with genotoxic-LV ($N=24$) developed tumors earlier than mock- or SIN-LV controls ($N=25$ and 19 respectively, $p<0.0001$). No tumors developed in mice transplanted with WT HSCs transduced with any vector ($N=19$ and 23). The hematopoietic reconstitution of transplanted mice was monitored by FACS analysis of peripheral blood (PB) monocytes, B and T cells and at the molecular level by integration site analysis (>100.000 IS). Mice transplanted with WT cells transduced with the genotoxic-LV showed a marked increase in myeloid cells at 350 days post-transplant with a strong reduction in clonality. This phenotype was significantly less pronounced when SIN-LV was used ($p<0.0001$), or not observed in mice transplanted with Cdkn2a-/- HSCs. Our results suggest that genotoxic insertions in WT HSCs impact on the hematopoietic reconstitution of the transplanted mice even in absence of tumor formation and induce non-cell autonomous mechanisms leading to premature HSC ageing.

P042

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

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Sickle cell disease (SCD) is caused by a mutation in the β-globin gene leading to polymerization of the sickle haemoglobin (HbS) and deformation of red blood cells. Autologous transplantation of haematopoietic stem cells genetically modified using lentiviral vectors (LV) to express an anti-sickling β-globin leads to some clinical benefit in SCD patients but requires high levels of transgene expression to counteract HbS polymerization. Here, we aimed at developing a potent therapeutic approach for SCD by combining a LV-based gene addition strategy with CRISPR/Cas9-mediated knock-down of the sickle β-globin or reactivation of the natural anti-sickling foetal γ-globin. We transduced an adult erythroid progenitor cell line with LV carrying an anti-sickling β-globin transgene and a gRNA targeting the endogenous β-globin gene, or a region within the γ-globin promoters involved in foetal haemoglobin (HbF) silencing, or the erythroid specific enhancer of the HbF repressor BCL11A. Cas9 delivery in transduced cells resulted in high genome editing efficiency leading to either a robust knock-down of endogenous β-globin or a strong γ-globin reactivation in mature erythroblasts, thus increasing the production of tetramers containing anti-sickling chains. Neither counter-selection of genome edited cells nor impairment in the differentiation were observed during erythroid maturation. Importantly, knock-down of the endogenous β-globin gene did not result in generation of α-globin precipitates typical of β-globin deficiencies. These results pave the way for future pre-clinical studies to demonstrate the efficiency, efficacy and safety of our novel therapeutic approaches for SCD.

P043

The engraftment of lentivirally transduced $hCD34^+$ cells in non-conditioned NSG mice is not altered due to a mobilization regimen with G-CSF/AMD3100

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We are currently conducting a gene therapy clinical trial in patients with Fanconi anemia (FA). Because this trial considers the infusion of gene corrected $CD34^+$ cells 24 hours after finalizing HSC mobilization with G-CSF and plerixafor (AMD3100), we reasoned that this mobilization regimen might modify the natural engraftment of HSCs in non-conditioned patients. To address this question, NSG mice were first treated with HSC mobilizing drugs according to the regimen used in the clinical trial. Twenty four hours afterwards, aliquots of 400,000 cord blood $CD34^+$ cells (either non-transduced or transduced with the FA therapeutic vector) were transplanted in two groups of non-conditioned NSG mice: untreated mice (control group) and mice that had been treated with the mobilizing drugs (treated group). Non-significant differences ($p > 0.05$) of human hematopoietic engraftment were observed between the control and treated groups of NSG mice at 3 months post-infusion (mean values of $hCD45^+$ cells were respectively 53.8 to 77.7% in BM of mice transplanted with fresh $CD34^+$ cells, and 63.1 to 65.1% in BM of mice transplanted with transduced cells). Additionally, when mice were transplanted with transduced $CD34^+$ cells, similar vector copy numbers per cell were determined in the control and the treated groups (mean values: 0.56 to 0.48 copies/cell in BM; differences were not significant at $p > 0.05$). These studies strongly suggest that the infusion of fresh or transduced $hCD34^+$ cells 24 hours after finalizing a mobilization regimen with G-CSF and AM3100 does not modify the engraftment ability of transplanted HSCs.

P044

Flow-cytometry platform for intracellular detection of FVIII in blood cells: a new tool to assess gene therapy efficiency for haemophilia-A

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Myeloid-derived Kupffer cells and platelets naturally produce FVIII, making hematopoietic stem cells an attractive target for Hemophilia-A (HA) gene therapy. Detection of intracellular FVIII in cells by flow-cytometry is unreliable. Here, we optimized such assay, using extensive blocking steps, and successfully report the detection of different FVIII domains in various cell lines (Hela, HECV, U937) and in PBMCs (%±SEM = $2.73\% \pm 0.65$, $n = 3$). We applied our method to U937 cells transduced with a PGK-FVIII lentiviral vector at MOIs of 5 and 30 and compared gene expression and protein detection. Q-PCR showed that higher MOI resulted in higher amount of mRNA. However, this increase was not mirrored by FVIII protein detection using flow-cytometry (%±SEM in untransduced cells = $11.36\% \pm 7.1$; MOI-5 = $18.9\% \pm 8.2$; MOI-30 = $12.6\% \pm 4.3$, $n = 3$, $p = 0.0046$). We speculated that the higher MOI transduction could be inefficient for the cells, due to a factor limiting proper folding processing. To test this hypothesis, we interfered with the Unfolded Protein Response (UPR), by blocking the proteasomal degradation with Lactacystin. Interestingly, a low MOI was enough to overload the folding pathway (collective FVIII MOI-5 = 30.65%; MOI-30 = 28.65%), suggesting that even a slight overexpression of FVIII triggers the UPR and consequent cell toxicity in ''non-professional FVIII-producers''. We showed that a flow-cytometry platform for FVIII protein detection can be reliable and sheds light on the functional status of cells after gene transfer, serving as a read-out method for optimizing gene therapy protocols for HA.

P045

Lyophilisation cycle development of AAV gene therapy product

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Biopharmaceuticals show varying levels of stability in aqueous solutions. Lyophilisation can provide the possibility of long-term storage/shipment of a drug product at $+5 \pm 3°C$. The gene therapy with AAV subtype 8 containing Factor IX (FIX) (BAX335) was formulated in a new proprietary buffer and lyophilised. A stability study was established with the lyophilised material to determine its stability profile at the accelerated temperature $+5 \pm 3°C$ for up to 10 months. The stability of the samples were tested using the following analytical methods: pH, appearance, AAV ELISA, FIX-qPCR (vector genome), SEC (aggregates), WAX (%full AAV), *in vivo* and *in vitro* biopotency and residual moisture, to determine the stability profile of the product over the course of the study. A second stability study was performed at the 6-month testing time point, which investigated 2 different lyocycles developed specifically for AAV subtypes. No changes in the viral drug product or activity were observed in the feasibility and stability studies performed, which demonstrates the optimal composition of this formulation for AAV.

P046

Gene therapy of breast cancer related anemia by delivering of erythropoietin in mice model

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Cancer-related anemia (CRA) is a frequent primary consequence of tumor burden which occurs in more than 40% of cancer patients at the moment of diagnosis and can be reached to 90% in patients who go under aggressive chemo radio therapy. Because of the negative effect of anemia on mental and physical functions that markedly lead to decrease the quality of life in cancer patients and poor prognosis, developing effective therapeutics for treating CRA is in great demand. To develop a potential gene therapy for this condition, we used a lentivirus to deliver the EPO gene into mice. The recombinant 4t1 cell line expressing thymidine kinase gene was used to induce cancer in mice and after confirming anemia, we administered ganciclovir intravenously to clear cancer cells in one group of animal models and other groups didn't receive the drug. Recombinant lentivirus expressing Epo gene were injected into the leg muscle of cancer-

related anemia mouse model. Collecting blood samples from treating mice and analyzing them chemically show increasing serum level of Hb after injection of rLenti-EPO compared with controls. We also observed that serum level of EPO was increased 24 hours after injections and remain stable in the sample blood of treated mice in compared with controls until the maximum level of serum EPO was reached after two weeks. Our study provides a proof of principle for the development of clinical gene therapy to cure CRA.

P047

Biological activity of additional AAV subpopulation in AAV gene therapy product

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The Factor IX (FIX) gene therapy (BAX335) was produced in small-scale for hemophilia-B patients in clinical phase I/II. In the course of the internal BAX335 process development, the small-scale clinical product was compared to BAX335 developed using a large-scale process. During this comparability study, an additional AAV-subpopulation was identified by analytical ultracentrifugation and found only in the BAX335 produced with the small-scale process. The BAX335 was produced in the HEK293 cell line and was based on triple transient transfection. The small-scale production process employed downstream purification with iodixanol gradient in a benchtop ultracentrifuge. Whereas the large-scale production process employed a proprietary large-scale ultracentrifugation step. The AAV-subpopulation detected in the iodixanol process was separated from the main full AAV fraction and further enriched in order to obtain isolated AAV-subpopulation fraction for analytical characterization (by qPCR, analytical ultracentrifugation, agarose-gel-electrophoresis, *in vitro* potency [FIX], *in vivo* potency in FIX ko mice, and SDS-PAGE). The analytical ultracentrifugation of BAX335 from the small-scale process detected the following fractions: full AAV capsids at 80S (sedimentation coefficient), AAV subpopulation at 70S, and empty AAV capsids detected at 50S. The biological activity was evaluated by measuring the *in vitro* and *in vivo* potency and was significantly lower in the AAV-subpopulation compared to the full AAV capsids. This AAV-subpopulation with poor activity and can be removed during the downstream process. In contrast to the small-scale iodixanol ultracentrifuge method, this large-scale ultracentrifugation process was shown to be capable of removing the AAV-subpopulations that did not possess the desired biological activity.

P048

The risk allele A of rs200395694 associated with SLE in Swedish patients affects on MEF2D gene regulation and alternative splicing

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Systemic lupus erythematosus (SLE) is a complex autoimmune disorder with hormonal, genetic, and environmental factors involved in disease aetiology. Since GWA studies lack the capability of identifying low frequency variants, we used next generation sequencing to search for novel rare variants which might contribute to disease heritability. We identified a novel rare variant rs200395694 located in the MEF2D gene encoding for the myocyte-specific enhancer factor 2D transcription factor as being associated with SLE in Swedish patients (504 SLE patients and 839 healthy controls, $p = 0.013$). The regulatory potential of rs200395694 was investigated by EMSA and luciferase reporter assay and indicated allelic differences both in protein-DNA binding and reporter transcription. The MEF2D gene splicing was studied by qRT-PCR using RNA purified from PBMC and different cell lines. Two major α1 и α2 isoforms were detected in PBMC, THP-1, K562 and Daudi, but only the α1 transcript was detected in Jurkat T cell line, HeLa and HEK293. Next, we used minigenes with different genotypes in order to assess the effect of the SNP on splicing. There was no allelic difference for the α1 isoform in neither of the cell lines analysed, while the α2 isoform was significantly suppressed by the rare A allele. In summary, we identified the association of the rare regulatory variant rs200395694 with SLE in Swedish patients. The risk allele exerts an effect on gene regulation and also inhibits the splicing of the alternative MEF2D transcript. Abramov SN was supported by the program of competitive growth of Kazan Federal University.

P049

Exploring the HIV-1 integration sites with different methods

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Combined Antiretroviral therapy (cART) is successful in the suppression of HIV replication but cannot target and eradicate the latent proviral reservoir. Integration is a non-random process and proviruses in specific genes are supposed to be linked to clonal expansion and persistence of infected cells in patients under long-term antiretroviral treatment. A careful monitoring of integration sites in HIV-1 patients will provide knowledge on clonal expansion of infected T cell and will uncover molecular mechanism maintaining HIV latency. A standardized and validated assay to detect HIV integration sites is still missing. High-resolution viral IS analysis by conventional LAM-PCR has been performed in CD4+ cell populations obtained from two subjects who under cART. To overcome the bias introduced by restriction enzyme digestion in LAM-PCR, we have also performed

sheared extension primer tag selection ligation mediated (S-EPTS/LM) PCR and a non-PCR biased direct sequencing approach upon capture of vector sequences (target enrichment sequencing; TES). To establish the baseline for evaluating the IS retrieval assays, we have also performed whole genome sequencing (WGS) on the same samples Preliminary data analyzed using adapted Gene-IS pipeline demonstrated that HIV integration site characteristics and genes were compared to previously report patient data sets. All samples showed a polyclonal, not-clustered integration profile. No substantial hotspots of HIV IS were identified. Notably, a moderate increase of clustering near cancer associated genes was observed along with the treatment period increasing. A detailed validation and comparison between non-PCR based assays and WGS is still on going.

P050

The role of autophagy expression in T-lymphocytes of patients with severe asthma

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The leading role in the formation of an allergic reaction belongs to T-lymphocytes. Prolongation of allergic inflammation in bronchial asthma is associated with increased survival of T-cells and their loss of apoptosis ability. It was proposed that resistance to apoptosis in T-lymphocytes in asthmatic patients could be due to increased autophagy rate in these cells. It has been shown that autophagy is involved in the pathogenesis of asthma. However, the role of it in severe atopic asthma (SAA) is not well understood. To further explore this, we investigated T-lymphocytes autophagy in SAA patients and healthy controls by utilizing transmission electron microscopy (TEM) and immunoblotting analyses. We found an increased number of autophagic T-lymphocytes in the patients with SAA versus healthy controls. The activation of autophagy in T-lymphocytes of patients with asthma was confirmed by the results of fluorescence microscopy and flow cytometry. They showed the LC3-II protein expression (an autophagy marker) and the presence of lysosomes in T-cells of patients with SAA. The results of immunoblotting were consider with electron and fluorescence microscopy data. These data suggest that autophagy may play an important role in the pathogenesis of SAA, facilitate T-lymphocytes activation and survival, and ultimately increase the level of airway inflammation in patients with this disease. The reported study was funded by RFBR according to the research project No 18-34-00739. The part of this study was supported by the Program of Competitive Growth of Kazan Federal University.

P051

Effect of dexamethasone on autophagy induction in T-lymphocytes of patients with mild bronchial asthma

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The concept of the role of programmed cell death (PCD) in the pathogenesis of bronchial asthma still remains incompletely understood. According to the data of different groups the apoptosis in T-cells from asthmatic patients is inhibited. Thus, the aim of this study was to analyze of autophagy in T-cells from asthmatic patients with mild form and healthy donors. The results showed that stress conditions (depletion of nutrients in the culture medium) induce autophagy in T-cells of asthmatic patients, but not in control group. In the same time the LC3 protein is represented by form I only. In contrast, in T-cells from healthy donors the most of cells has a morphology corresponding to apoptotic changes and autophagosomes were not identified. Autophagic marker protein is present in total LC3-I form. Corticosteroids promote the apoptotic death of unstimulated and stimulated T-lymphocytes. Therefore the second aim was to analyze the effect of dexamethasone on T-cells autophagy in asthmatic individuals. Culturing of T-cells with Dex led to stimulation of the apoptotic process. The activation of autophagy was not detected. Dex treatment also showed that the glucocorticoid is not responsible for LC3-II protein formation in the group with mild asthma. So we can conclude that stress conditions induce autophagy in T-cells of asthmatic patients. But the Dex treating is not responsible for autophagy induction. The reported study was funded by RFBR according to the research project No 18-34-00739. The part of this study was supported by the Program of Competitive Growth of Kazan Federal University.

P052

Towards the gene therapy of the bone marrow failure in patients with dyskeratosis congenita

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Mutations in the DKC1 gene cause X-linked dyskeratosis congenita (X-DC), a low prevalent disease classified as a bone marrow failure (BMF) syndrome. DKC1 encodes for the dyskerin nucleolar protein, which is constitutively expressed in human cells and essential for telomerase function and rRNA pseudouridylation. To date, hematopoietic stem and progenitor cell (HSPC) transplantation constitutes the only curative treatment for the BMF of these patients. Nevertheless, the reduced number of compatible donors, complications derived from conditioning regimes and graft versus host disease postulate gene therapy as a promising alternative for the treatment of DC patients. We have previously described a dyskerin derived peptide, called Genetic Suppressor Element 4 (GSE4), which efficiently correct different phenotypic features of DC fibroblasts. Since the insertion of the whole DKC1 cDNA did not correct the phenotype of DC cells, we envisaged a lentiviral-mediated gene therapy approach aiming at stably expressing GSE4 HSPCs from X-DC for preventing and/or treating the BMF that takes place in most of these patients. To achieve this goal, we transduced X-DC like $CD34^{+}$ cells and X-DC patients' hematopoietic primary cells with lentiviral vectors carrying the GSE4 cDNA. Remarkably, the lentiviral-mediated expression of GSE4 rescued the expression of telomerase related genes, DKC1, TERC and

TERT. Moreover, a reduced p21 expression was conferred by this LV, consistent with a lower rate of senescence and DNA damage in transduced cells. Our results suggested that the lentiviral delivery of GSE4 may constitute an efficient gene therapy approach to correct the BMF characteristic of X-DC patients.

P053

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

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Most Hematopoietic Stem/Progenitor Cells (HSPC) reside in the bone marrow (BM) after birth. Few circulating HSPC (cHSPC) are found in peripheral blood (PB) of un-mobilized individuals and their amount is emerging as marker of altered BM function. However, cHSPC role both in physiological and stressed conditions, such as transplantation, is largely unknown. Combining a multi-parametric phenotyping with *in vitro* functional assays, we are dissecting cHSPC subset composition and differentiation potential during hematopoietic physiological maturation in healthy donors (HD) of different ages and during hematopoietic reconstitution in patients treated with HSPC gene therapy (GT). Preliminary data on 7 newborns, 21 pediatric (0-18 years), 9 adult (20-60 years) and 12 aged (>75 years) subjects unveiled consistent changes in cHSPC subset absolute numbers with progressive loss of clonogenic potential during ageing. Analyzing cHSPC in 6 Wiskott-Aldrich syndrome (WAS) and 6 Metachromatic Leukodistrophy (MLD) patients before treatment and at different time points after GT we observed an enrichment of lymphoid and myeloid cHSPC progenitors during initial hematopoietic reconstitution followed by progressive stabilization of cHSPC count and composition. Interestingly, WAS patients displayed a higher content of cHSPC compared to age-matched HD before GT which normalized after treatment, suggesting restoration of functional BM niche. We are currently collecting integration sites from both BM and PB HSPC at different time points after GT to analyze their hierarchical relationship and to track their differentiation potential at single clonal level. These studies will be essential to unveil the contribution of cHSPC to hematopoietic reconstitution and maintenance after GT.

P054

The potential use of nanobodies delivered via AAV vectors in the treatment of haemophilia

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Several gene therapy approaches for haemophilia have emerged in recent years. However, some challenges remain regarding the potential vector and/or transgene immunogenicity, especially for haemophilia A (HA) patients who often develop inhibitory antibodies. We propose an innovative AAV-based gene therapy approach using single domain antibody fragments (nanobodies) to target the anti-thrombin (AT) anticoagulant and re-balance the hemostasis. Several anti-AT nanobody sequences were isolated following llama immunization, and subsequently engineered to produce multi-valent variants. *in vitro*, the bi-valent KB-AT-02/03 and the tri-valent KB-AT-113 nanobodies were able to correct thrombin generation in haemophilic plasma, to the same extent as factor VIII. In a tail vein transection model, HA mice administered with the purified nanobodies showed a strongly reduced blood loss (<300ul on average) compared to controls (>750μl on average). We subsequently injected a liver-specific AAV8 vector expressing KB AT-02/03 in HA mice, where we observed a decreased AT-activity in plasma over time (up to 0.7% at 8 weeks post-injection). In a different model, AAV8 vectors expressing KB AT-02/03 or KB-AT-113 were administered to haemophilia B mice. In a tail clip assay, we observed a significant reduction in blood loss in animals stably expressing KB-AT-02/03 and 113 (<300ul and <600ul, respectively) compared to the vehicle-treated group (>800μl on average). No anti-nanobody immune-response was observed. We demonstrated that AT-nanobodies showed pro-coagulant activity, both *in vitro* and *in vivo*. Importantly, this gene therapy approach provides the potential of a long-term therapeutic solution in haemophilia patients with or without inhibitory antibodies to the therapeutic clotting factor.

P060

Organotypic multicellular spheres (OMS) as a 3D model system to study oncolytic adenovirus responses in glioblastoma tumours

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Glioblastomas are the most common and deadliest type of primary brain cancer. Despite surgery, radiotherapy and chemotherapy, glioblastoma patients witness a median survival of only 15 months. Oncolytic viruses (OVs), such as the adenovirus-based Ad5.d24.RGD virus, have great promise as novel treatment regimen. Although clinical trials for glioblastoma have shown safety, responses are observed in only a subset of patients. The reasons for the differences in responses are unclear. Lack of immunocompetent animal models for adenovirus hampers studying differences in response between patients. Here we show that we have established Organotypic Multicellular Spheres (OMS), 3D organoids derived from the tumour tissue of GBM patients, as a model system for studying OV efficacy and specificity. The success rate of OMS establishment was increased by using defined serum-free medium. OMS could be established from resected tumor pieces as well as from cavitron ultrasonic surgical aspirator (CUSA)-derived glioblastoma

tissue. Immunohistochemistry on the OMS demonstrated the prolonged (up to 4 weeks) presence of tumour cells and several other cell types, including endothelial cells and macrophages. Fluorescent confocal microscopy and immunohistochemistry demonstrated infectivity of the Ad5.d24.RGD.GFP virus in the OMS, albeit with lower efficacy as compared to the infection of traditional glioblastoma sphere model, which consists of tumour cells only. The OMS model can serve as a valuable model to better understand the heterogeneity in response to OVs and can be used to shed light on factors in the tumor-microenvironment that can hinder the oncolytic activity.

P061

The antitumoral activity of TLR7 ligands is corrupted by the microenvironment of pancreatic tumors

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Toll like receptors (TLRs) are key players in the innate immune system, and recent studies have suggested that TLRs may impact pancreatic adenocarcinoma (PDAC), a disease with no cure. We aim to better understand the mechanism of TLRs in PDAC biology, to open door for clinical applications. We used novel TLR7 ligands, that form positively charged liposomes for non-viral transfection. *in vitro*, TLR7 ligands strongly inhibit the proliferation and induce cell death by apoptosis of murine and human PDAC cells but fail to induce IFN response, nor to sensitize tumor cells to chemotherapy following therapeutic genes delivery. *In vivo*, TLR7 ligands significantly delayed the growth of very aggressive experimental murine tumors in immunodeficient mice but surprisingly promoted tumor growth in syngenic models. Transcriptomic signatures advocate for the presence of tumor-promoting macrophages following treatment with TLR7 in immune competent mice. Thus, we demonstrate for the first time that TLR ligands have great potential to inhibit PDAC cell proliferation and growth *in vivo*. However, TLR7 ligands may also recruit tumor-promoting macrophages that drive pancreatic fibrogenesis and tumorigenesis. Thus, TLR7 agonists-based therapies must be carefully considered for PDAC management and should be further combined with agents that antagonize the accumulation of activated macrophages at these lesions.

P062

Evaluation of proapoptotic effects of MSC expressing endostatin and TRAIL on SVEC and 4T1 cell lines and mouse model

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Breast cancer is the second of the most common cancer. Targeted killing can be achieved by induction of apoptosis and inhibition of angiogenesis in the cancer cells Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) can selectively kill tumor cells and, in combination with other agents, for example, Endostatin that is the most potent inhibitor of angiogenesis could enhance the efficiency of cancer therapy. Also, it has been determined that MSCs is a good vehicle for targeting tumor cells because of their tropism to tumor lesions. In this study, the anti-angiogenesis and proapoptotic effect of recombinant MCSs expressing Endostatin, TRAIL and Endostatin/TRAIL *in vitro* and *in vivo* on tumor cells were assayed. Gene construct contains Endostatin and TRAIL was ordered to synthesize to Genecust company. HEK293T was transfected by helper plasmids and three transfer plasmid, containing Endostatin, TRAIL and Endo/TRAIL, and produced three recombinant lentiviruses. MSCs were transduced by the three lentiviruses, and their potential for the production of genes and inducing of cell death on SVEC and 4T1 was evaluated *in vitro* and *in vivo*. Western blot analysis on rMSC culture medium revealed a 30kDa and 22kDa protein bands for TRAIL and Endostatin respectively. The results of flow cytometry showed that recombinant MSCs significantly enhanced cell death in 4T1 cell line but not in SVEC cell line. Also, the result of mouse model revealed that expression of TRAIL and Endostatin simultaneously could decrease in tumor volume, but the expression of these genes separately did not show the same effect.

P063

Vaccinia virus shuffling: deVV5, a novel chimeric poxvirus with improved oncolytic potency

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Oncolytic virus (OV) therapy has emerged as a promising approach for cancer treatment with the potential to be less toxic and more efficient than classic cancer therapies. Various types of OVs in clinical development, including Vaccinia virus (VACV)-derived OVs, have shown good safety profiles, but limited therapeutic efficacy as monotherapy in some cancer models. Many different methods have been employed to improve the oncolytic potency of OVs. We used a directed evolution process, pooling different strains of VACV, including Copenhagen, Western Reserve and Wyeth strains and the attenuated modified vaccinia virus Ankara (MVA), to generate a new recombinant poxvirus with increased oncolytic properties. Through selective pressure, a chimeric VACV, deVV5, with increased cancer cell killing capacity and tumor selectivity *in vitro* was derived. The chimeric viral genome contains sequences of all parental strains. To further improve the tumor selectivity and anti-tumor activity of deVV5, we generated a thymidine kinase (TK)-deleted chimeric virus armed with the suicide gene FCU1. This TK-deleted virus, deVV5-fcu1 replicated efficiently in human tumor cells, and was notably attenuated in normal primary cells. These studies demonstrate the potential of directed evolution as an efficient way to generate recombinant poxviruses with increased oncolytic potency, and with high therapeutic index to improve cancer therapy.

P064

Adenovirus-mediated transfer of shRNA against Elovl6 reduces the progression of hepatocellular carcinoma

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The elongation of long-chain fatty acids family member 6 (Elovl6), a key enzyme in lipogenesis, catalyzes the elongation of saturated and monounsaturated fatty acids. Insulin resistance is associated with upregulation of Elovl6, which has been linked to obesity-related malignancies, including hepatocellular carcinoma (HCC). However, the role of Elovl6 in HCC progression remains unclear. In this study, we analyzed the expression of Elovl6 in 61 clinical HCC specimens. Patients with Elovl6 high-expressing tumors were associated with shorter disease-free survival and overall survival compared with those with Elovl6 low-expressing tumors. Adenovirus-mediated knockdown of Elovl6 reduced cell proliferation and Akt phosphorylation, leading to cell cycle arrest, as well as increased lipid accumulation in HCC cells. Intratumoral treatment with 108 PFU of adenoviral vectors encoding shRNA against Elovl6 (Ad. shElovl6) at days 10 and 16 significantly suppressed tumor growth and prolonged the survival of BALB/c mice bearing syngeneic HCC, as compared to treatment with the control vectors. Taken together, our results indicate that Elovl6 enhances oncogenic activity of HCC and is associated with poor prognosis in patients with HCC. Thus, targeting Elovl6 may be a novel therapeutic strategy for HCC.

P065

Laminin receptor dependent gold nanoparticles for mitochondrial targeted delivery in cancer

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Nanocarriers with their array of tunable physicochemical properties have continually shown potential for efficient gene and drug delivery. The exciting potential of the mitochondria as an invariable target present in all tumours has made it a focus of cancer research in recent times. We studied the efficient delivery of Betulinic acid (BA) to the mitochondria of cancer cells by the biocompatible Epigallocatechin gallate reduced gold nanoparticles (Eg-AuNPs). Pertinent to our objective is the high affinity of EGCg for the 67 kDa laminin receptor over expressed in certain tumours. Synthesised AuNPs were either coated with Poly(Ethylene Glycol) (PEG) (p-Eg-AuNP) or Poly(L-Lysine)-graft-Poly(Ethylene Glycol] copolymers (PLL-g-PEG) (Pp-Eg-AuNP) for biocompatibility, then with triphenylphosphonium cation (TPP+), for mitochondrial targeting. Following characterization with TEM, NTA zetasiser, FTIR and UV spectroscopy, we determined the cytotoxic and mitochondrial targeting properties of drug-NP conjugates in 67LR negative and positive cancer cell lines, Hep G2 and Caco-2 respectively. Furthermore, a mechanistic study that included cell cycle analysis, apoptosis, and caspase activation assays were conducted to ascertain the possible mechanism of action. Enhanced cellular uptake, with significant localization to the mitochondria were recorded for targeted nanoparticles compared to the non-targeted NPs, with uptake levels markedly higher in the 67LR positive cell line. Consistent with these results were the significant apoptotic effects of targeted T-Pp-BA-Eg-AuNP and T-p-BA-Eg-AuNP compared to the untargeted constructs. These findings emphasize the innate targeting property of Eg-AuNP, their capacity to modulate drug pharmacodynamics and efficiently deliver therapeutics to cancer mitochondria.

P066

Development of a chimeric form of IFNa for "on demand" *in vivo* cancer gene therapy

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The immunosuppressive tumor microenvironment is the major hurdle for cancer therapy. Our lab previously developed a strategy for targeted gene-based delivery of interferon alpha (IFNa) to tumors through Tie2 expressing monocytes/macrophages able to reprogram the tumor microenvironment. Whereas a sustained output could ensure long-term protection from tumor recurrence, it may raise concerns for long-term effects, especially in case of cancer eradication. To overcome this issue, we are developing inducible strategies to control the timing and amount of IFNa secreted in the tumor microenvironment. By fusing a destabilizing domain (DD) to a protein of interest (POI) the former can confer its instability to the latter. This destabilization can be rescued in a reversible and dose dependent manner with the addition of a small molecule specifically binding to the DD. To apply this technology to our strategy we have designed and *in vitro* tested different fusion proteins of IFNa (DD-IFNa) with or without the addition of flexible or cleavable linkers and selected them for their capacity to be stabilized in presence of their specific ligand *in vitro*. Through this approach, we have identified effective fusion proteins with low basal activity and high fold induction upon ligand treatment. These novel regulated forms of IFNa are functional and their specific activities are comparable to the wild type. Based on promising preliminary results *in vivo*, we are now testing the safety and efficacy of our new platforms in inducing anti-tumor responses in melanoma, colon and glioma models of cancer.

P067

TG6002: a novel oncolytic and vectorised gene-prodrug therapy approach to target and treat cancer

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TG6002 is a recombinant vaccinia virus Copenhagen strain deleted in two genes: thymidine kinase (TK; J2R) and a subunit of Ribonucleotide Reductase (RR; I4L). TG6002 has demonstrated strong tumour selectivity and retained full capacity to replicate and lyse human cancer cell lines. The double deletion leads to an improved safety profile, as a result of very high attenuation in healthy tissues. TG6002 is also armed with the suicide gene FCU1. FCU1 gene encodes a bifunctional chimeric protein that efficiently catalyses the direct conversion of the nontoxic 5-fluorocytosine (5-FC) into the toxic metabolites 5-fluorouracil (5-FU) and 5-fluorouridine monophosphate (5-FUMP). The expression of the FCU1 gene by the virus allowed to obtain a targeted chemotherapy within the tumour, with a higher level of efficiency than traditional treatment with 5-FU. TG6002 has been evaluated in numerous sub-cutaneous and orthotopic therapeutic human tumour models in nude mice, where it demonstrated potent and significant tumour regression, which is improved by combination with 5-FC administration. Following the encouraging preclinical results, a dose-escalation Phase 1 trial of intravenous TG6002 delivery in combination with 5-FC was initiated in patients with recurrent glioblastoma. Moreover, the ideal features of the VVTK-I4L- backbone characterised during the preclinical evaluation of TG6002, allowed the creation of InvirIO, a platform using this backbone for the delivery of immunological playloads aimed at modulating the tumour microenvironment.

P068

HSV-tk mediated suicide gene therapy leads to an immunogenic cell death of glioblastoma cells and a T-cell mediated immune response

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Suicide gene therapies for glioblastoma (GBM) have shown promising results in early phase clinical studies. A part of the therapeutic response is likely mediated by an immunological bystander effect. We investigated if HSV-tk induces an immunogenic cell death in 3 different human GBM cell lines and 1 mouse glioma cell line. After treatment with Ganciclovir tumor cells upregulated HMGB1 and Calrecticulin, which are important markers indicating an immunogenic cell death. In addition, we investigated the immune response induced by HSV-tk mediated killing *in vivo* in a syngeneic mouse glioma model. We found infiltration of higher numbers of CD4+ and CD8+ T cells compared to controls. This was in particular observed with CD4+ T cells as verified also by FACS analysis. In addition, there was a higher number of Granzyme B+ cells in treated tumors compared to controls. In conclusion, HSV-tk mediated suicide gene therapy leads to an immunogenic cell death and a T cell mediated immune response in GBM, however arginase+ suppressor cells may interfere with this anti-tumor immune response. Therapeutic targeting of this cell population in the future might enhance the effect of suicide gene therapy.

P069

Role of components of microRNA machinery in carcinogenesis: targeting DGCR8 impairs pancreatic tumors growth

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MicroRNAs are key players in cancer, including pancreatic cancer (PDAC), a disease with no cure. We have previously demonstrated that microRNA expression is early altered during carcinogenesis, that oncomiRs such as miR-21 are promising therapeutic targets, and that circulating microRNA may help manage patients with PDAC. However, the role of proteins involved in microRNA biogenesis is still largely unknown in cancer, including PDAC. In this study, we generated DICER, DROSHA and DGCR8-deficient PDAC cells lines using stable shRNA expression. We found that cell proliferation and migration was not altered following DICER or DROSHA targeting; on the other hand, depleting DGCR8 from cells significantly inhibited cancer cell proliferation, tumorsphere formation and migration, *in vitro*. FACS analysis revealed that DGCR8-deficient cells were blocked in G1 phase of cell cycle following p27 KIP1 induction and Rb phosphorylation. *In vivo*, DGCR8-null PDAC cell lines generated microtumors in the pancreas, as compared to control cell lines, and failed to metastasize to the liver. DGCR8-null PDAC cell lines derived tumors were positive for p27 KIP1, with low proliferative index (KI67 staining), as compared to control tumors. We performed transcriptomic studies and found that miR-24-3p levels were significantly inhibited in DGCR8-null PDAC cell lines. Restoring miR-24 levels using mimics rescued cell proliferation, tumorsphere growth, cell migration, cell cycle arrest and inhibited p27 KIP1 expression in DGCR8-null PDAC cell lines. Taken together, this study demonstrates that DGCR8 has a critical role in PDAC oncogenesis and may help identify new targets, such as miR-24-3p, for the therapy of cancer.

P070

H-1 parvovirus inhibits both primary tumor and metastatic growth of human pancreatic tumours

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Pancreatic cancer (PDAC) is a disease with no cure that ranks second worldwide in cancer-related death. Among candidate oncolytic viruses, H-1 parvovirus (H-1PV) shown promise and is safe in humans. However, H-1PV therapeutic potential hasn't been fully explored in human PDAC preclinical models. In this study, we show that H-1PV inhibits cell proliferation using real-time monitoring and induces cell death of PDAC cell lines and primary cultures. H-1PV replicates efficiently in PDAC cells but failed to form plaques, strongly suggesting minimal propagation. Mice with exponentially growing tumors received intracardiac injection of H-1PV, to achieve maximal delivery of the virus to the pancreas. H-1PV injections are well tolerated, strongly inhibits the growth of primary tumors and the size and number of hepatic metastasis as monitored by ultrasonography and diminishes the numbers of circulating tumoral cells (CTC). Mice receiving H-1PV survived longer as compared to mock treated mice. Collectively, our results confirm that H-1PV replicates and

kills PDAC cells but fails to propagate in cultures. Our next objective is to generate tumor-adapted, H-1PV variants using serial passaging for optimal spread *in vitro* and *in vivo*. We demonstrate for the first time that H-1PV systemic administration inhibits both human preclinical primary pancreatic tumors and metastases, but may also target CTCs, a unique feature in the field of virotherapy. The role of the immune system in H-1PV therapeutic activity is currently under investigation. Thus, our study reveals novel antitumoral properties of H-1PV and may stem for future, rationally-designed, clinical trials in patients with PDAC.

P071

Development of RNA drug to induce antitumour immunity and cancer selective apoptosis

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Inactivated Sendai virus (hemmaglutinating virus of Japan) envelope (HVJ-E) stimulates anticancer immunity and cancer cell-selective apoptosis through the recognition of viral RNA genome fragments by RIG-I (retinoic acid-inducible gene-I). 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 for further investigation because the viral fractions containing more DI particle which showed stronger anti-cancer effects than other strains. DI particles of Sendai virus Cantell strain include incomplete RNA genome (approximately 550 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 RIG-I/MAVS signal-related proapoptotic proteins expressed in cancer cells such as PC3, human prostate cancer cells. Furthermore, DI particle-derived RNA was synthesized by *in vitro* transcription (IVT-B2). IVT-B2 RNA with double-strand stem region and single strand loop in secondary structure showed stronger anti-cancer effect both *in vitro* and *in vivo* than IVT-HN RNA without stem region (negative control). The 5′-triphosphate of IVT-B2 was also crucial for the expression of proapoptotic genes and IFN-b. Additionally, tumor growth was significantly suppressed by electroporation of IVT-B2 RNA to human prostate cancer xenograft mice. Intra-tumor transfection of IVT-B2 RNA showed intra-tumoral apoptosis and potential to recruit immune cells to tumor microenvironments with the activation of NK cells. IVT-B2 RNA will be a novel nucleic acid medicine for cancer treatment.

P072

Synergistic antitumour effects of temozolomide in combination with suicide gene expressing mesenchymal stem cells in orthotopic xenograft glioma models

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Glioblastoma multiforme (GBM), the most common and aggressive primary brain tumor, has a poor prognosis even with combined surgery, radiotherapy and chemotherapies. Previously, we demonstrated that mesenchymal stem cells (MSCs) have a high tropism for brain tumors, and engineered MSCs to express a bacterial CD gene (MSC/CD) that successfully suppressed tumor growth in a rat glioma model. In this study, the efficacy of MSC/CD in a multi-modal combination regimen with temozolomide (TMZ) was evaluated. Combined treatment of TMZ and MSC/CD with 5-FC synergistically inhibited the proliferation and induced the G2/M arrest of GBM cells. In an orthotopic xenograft glioma model, TMZ treatment alone induced moderate improvement in tumor growth inhibition; however, this effect was more intense with MSC/CD transplantation followed by the sequential treatment with 5-FC and TMZ. Consistently, combination therapy significantly prolonged survival of orthotopic glioma-bearing mice compared to either treatment alone. These results illustrate the potential of combining *ex vivo* gene therapy and chemotherapy for synergistic effects that more effectively treat glioblastoma.

P073

Extracellular vesicles enhance the targeted delivery of immunogenic oncolytic adenovirus in immunocompetent mice

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Oncolytic viruses (OVs) are engineered to preferentially infect, replicate in and kill cancer cells instead of normal cells. Even though promising results have been observed in preclinical studies, the efficacy in clinical trials has remained limited. Intratumoral therapy with OVs achieves tumor lysis, immunogenic cell death and production of type I IFN, creating a microenvironment favorable for the activation of anti-tumor immune responses. However, many solid tumors cannot be treated using this approach, thus new strategies for the tumor targeted delivery of OVs are in high demand. Extracellular vesicles (EVs), are naturally occurring cargo delivery agents with the potential to be used as drug delivery vehicles. We previously demonstrated that human lung cancer cell-derived EVs could be used for systemic delivery of OVs by efficiently reducing tumor growth in nude mice. Herein, we evaluated the effects of EV-Virus formulations on the immune system; to this aim, we used a mouse lung cancer cell line (LL-2) to produce the EV-Virus formulations and to generate a tumor model in immunocompetent syngeneic mice (C57Bl/6). Our results show that EV-Virus formulations induced tumor associated inflammation *in vivo* suggesting a specific tropism of EVs towards the tumor. Fluorescent labeled EV-DID-Virus showed a specific signal for the tumor and merging of the fluorescent and bioluminescent signals indicates the homing of EV-DID-Virus within the tumor and a peritumoral immune-response associated with the targeted delivery of the Virus. Our findings strongly support the systemic administration of OVs encapsulated into EVs as a strategy aimed at treating lung cancer.

P074

WNT signaling pathway regulates Bmp4 expression in mesenchymal stromal cells from acute myeloid leukemia patients

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Acute Myeloid Leukemia (AML) is a clonal myeloid disease characterized by hematopoietic insufficiency. A molecular signature from Mesenchymal stromal cells from AML patients (hMSC-AML) has been proposed by our group, and its gene expression could be related to the leukemic transformation process. We highlight BMP4, which has its expression decreased in hMSC-AML and this expression could be regulated in silico by WNT signaling pathway. In this context, the aim of this work was to verify if WNT signaling is capable of regulating the BMP4 gene in hMSCs. To evaluate the expression profile of the WNT signaling we performed PCR Array assay with hMSC-AML compared to hMSCs from healthy donors (hMSC-HD), both isolated from bone marrow. We found 26 differentially expressed genes, and these genes are mostly related to the WNT canonical pathway. The results were confirmed by real-time PCR (RT-qPCR) in a larger number of samples. WNT canonical pathway mediates regulation through the formation of the β-catenin/TCF-LEF complex. No differences in expression and localization of β-CATENIN protein by immunofluorescence were found. However, Tcf7 and Lef1 transcription factors' mRNA and LEF1 protein were found down-regulated in hMSC-AML. After in silico analysis of BMP4 promoter region, we found six consensus binding sites for TCF/LEF, and through chromatin immunoprecipitation assay followed by RT-qPCR, we observed less binding of LEF1 in two TCF/LEF consensus binding sites in hMSC-AML in comparison to hMSC-HD. Altogether, we suggest that the WNT canonical pathway is potentially capable of acting in the regulation of the BMP4 gene in hMSC-AML.

P075

Human mesenchymal stem cells as cellular vehicles to deliver retroviral replicating vectors for cancer gene therapy

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Retroviral replicating vectors (RRV) have shown promising clinical results for gene therapy of cancer and are currently being evaluated in an international Phase III trial for recurrent high-grade glioma. RRV have been shown to achieve tumor-selective replication, efficient tumor transduction and therapeutic benefit in a wide variety of cancer models. However, retrovirus-based vectors are produced at only low titers *in vitro* and are easily inactivated by complement in the blood. For efficient delivery of RRV to tumors, we evaluated mesenchymal stem cell (MSC)-based RRV producer cells as 'RRV carrier vehicles' which preferentially accumulate and engraft at tumor sites. Human MSCs derived from adipose tissue, bone marrow and umbilical cord were infected efficiently with RRVs but produced virus progenies less efficiently than tumor cells. We then assessed tumor cell tropism of the MSCs, using a Transwell plate migration assay with mesothelioma cells as the targets. All three types of the MSCs showed significant transmigration toward the mesothelioma cells. Furthermore, when co-cultured, RRVs were found to be transmitted efficiently from MSCs to mesothelioma cells, thereby achieving high levels of tumor cell transduction. These data indicate the potential utility of the MSC-mediated delivery of RRVs for cancer gene therapy.

P076

Gene replacement based on RNA reprogramming as an effective approach for personalized cancer theranostics

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Group I intron-based trans-splicing ribozyme enables to sense and reprogram target RNA into therapeutic transgene. Previously, we proposed hTERT-targeting trans-splicing ribozyme downstream 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 of the ribozyme through microRNA regulation. 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.

P077

Breast cancer stem cells (BCSCs) associate with aggressive tumors and cause field cancerization in breast cancer

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Functional similarity between normal stem cells and cancer stem cells prompted us to evaluate the role of cancer stem cells in imparting aggressive behavior to the tumors and also contribute to field cancerization in human breast cancer. The study included primary tumor and adjacent normal specimens from 37 chemotherapy naïve female breast carcinoma patients with low and high grade breast carcinoma. BCSCs were identified and sorted using cell surface markers $CD44^{+}$ & $CD24^{-/low}$ by flow cytometry. Sorted BCSCs were characterized by mammosphere formation, side population assay and staining for AlDH1A1. Higher percentage of BCSCs was observed in histopathologically defined aggressive tumors (Grade II & III) as compared to Grade I tumors. Number of BCSCs didn't correlate well with metastatic lymph node, tumor size, patient age, presence or absence of lymphovascular emboli & proliferative index. Higher frequency in Triple Negative and HER 2^{+} cases. High ($p < 0.05$) IHC score for AlDH1A1 in higher grade tumors (score 2-3})

than grade I tumors (≤ 1). The frequency of BCSCs showed an increasing trend in normal adjacent tissue also with histopathological grade of tumor. No such cells were however found in normal breast tissues obtained from reduction mammoplasties. H&E stained serial sections were used to validate the flow cytometry results. Our findings reveals that higher number of BCSCs in the tumor and adjacent normal tissue associate positively with the aggressive behavior of tumor. BCSCs's presence in normal adjacent tissue substantiates epithelial component's role in field cancerization. Has important clinical implications.

P078

The search for the primary tumor in patients with metastases of malignant tumors without identified primary tumor

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The problem of finding the primary tumor in patients with metastases of malignant tumors without identified primary tumor is important and relevant today. Therefore, the aim of our work is the creation of mathematical models with high probability assume localization of the primary tumor. Retrospective analysis of case histories and ambulatory cards of 581 patients with metastases of malignant tumors without identified primary tumor was made on the base of Tatarstan Cancer Center from 1996 to 2008. Statistical results were evaluated in the program Excel and Statistika-6.0. In 69 patients in the observation process were revealed a primary lesion in the lung. For this example, the patients proposed a mathematical model identification of the primary tumor. Were selected factorial signs: gender, age, morphology of the metastasis, localization of metastases the prevalence of metastatic lesions. The only effective basis identified the primary tumor in the lung. Data were summarized in contingency tables, which allowed to establish the relationship between two or more characteristics. The primary focus in the lung with high probability we can assume in men, mostly aged under 50 years, presence of metastasis, neuroendocrine tumor, squamous cell carcinoma and suspected bronchioloalveolar cancer in the lymph nodes of the mediastinum and neck, metastases to the lungs and the pleura. Using this mathematical model appears able to predict the localization of the primary tumor. Work supported by Program of Competitive Growth of KFU.

P079

Topical administration of rAAV/carboxymethylocellulose formulations – *in vitro* and *in vivo* tests

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Skin cancer is already a considerable problem of modern medicine and public health. Moreover, the treatment of many skin diseases is based solely on relief of symptoms because its genetic or autoimmune etiology. Topical application is one of the most convenient methods of applying preparations. The aim of the study was to evaluate the possibility of introducing rAAV into skin cells after a local application of gene CMC formulation. Gene preparations were applied on the depilated mouse skin. The study also took into account the influence of the sorption promoter on the efficiency of vector administration. After 14 days, the animals were sacrificed and skin exposed for preparations was removed. After DNA isolation, grade of penetration of vectors into skin was evaluated by qPCR method. In addition, analysis of the release of rAAV from the gel base and its stability was also performed *in vitro*. It was demonstrated that stability of CMC formulations is independent on their concentration. The 2% gel was selected for *in vivo* tests due to its optimal physicochemical properties for topical administration. Functional gene formulations were obtained, as a result of the research (app. 60 copies of rAAV were detected in 50ng DNA). It has been shown that used absorption promoter has no effect on the efficiency of transduction. Introduction of viral vectors to pharmaceutical vehicle to obtain a topical formulation is possible. The received results encourage further research in the field of local treatment of genetic disorders. Work supported by a grant Strategmed 1/233264/4/WCBR/2014

P080

Transposable element-driven transcripts as new cancer biomarkers

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Cancer management is in need of biomarkers to trace the origin of tumors, predict their sensitivity to chemotherapy, monitor therapeutic responses, detect relapses as early as possible and identify new therapeutic targets. Transposable elements (TEs) have been related to oncogenic processes, and, together with their KZFP controllers, could regulate transcriptional networks important for tumor cell identity and differentiation, as observed in human embryonic stem cells. Here, we studied the role of TEs in cancer, taking colorectal cancer (CRC) as a paradigm. First we discovered several CRC-restricted transcripts resulting from splicing of TE-initiated RNAs into protein-coding sequences. The most prevalent of these CRC-specific transcripts, which is never present in normal colon, appears in more than 60% of the patients and its expression is tightly correlated with the transition from adenoma to carcinoma, and linked to a lower survival time and to a decrease in relapse-free survival. This TE-initiated transcript encodes for a stem cell-like gene highly similar to OCT4. The increased colonogenicity capacity of CRC cell lines overexpressing this stem cell gene and the correlation of its expression with the CRC stemness signature suggests that our candidate displays features of stem cell marker in CRC. Our results point to the interest of TE-based CRC biomarkers, some of which could be involved in disease progression.

P081

Generation of genetically engineered canine mesenchymal stem cells co-expressing immunomodulating cytokines and tumour suppressors

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The similar pathophysiology of canine and human cancer makes dogs a promising model to investigate the effectiveness of antitumor therapy. Mesenchymal stem cells (MSCs) are non-hematopoietic progenitor cells. They have low immunogenicity and exhibits a homing behavior toward tumor sites. Thus MSCs can

be used for targeted delivery of anti-cancer agents. Cytokines are molecular messengers that allow cells of the immune system to interact with each other. Numerous cell cultures and animal tumor models studies have shown that cytokines have broad anti-tumor activity. Recombinant plasmid encoding canine tumor necrosis factor ligand superfamily member 10 (cTRAIL), canine interferon beta-17 (cIFNB1) and canine phosphatase and tensin homolog deleted on chromosome 10 (cPTEN) was used to generate recombinant lentivirus, which was produced by co-transfection of the HEK293FT packing cell line. Canine mesenchymal stem cells were isolated from adipose tissue. Cells were largely positive for mesenchymal stem cell surface markers and negative for hematopoietic stem cell surface markers. The multipotency was confirmed via differentiation into chondrocytes, osteoblasts and adipocytes. MSCs were transduced with described recombinant lentiviral vector to concurrently express cTRAIL, cIFNB1 and cPTEN. Resulting cell line was selected with blasticidin S (5 μg/ml) for 10 days. The genes expression was confirmed by quantitative PCR. The functionality and antitumor activity of the cell culture will be further investigated in various transformed cell cultures *in vitro*.

P082

Quantification and functional evaluation of CD40L production from an adenovirus vector ONCOS-401

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Adaptive immunity involves activation of T cells via antigen presentation by antigen presenting cells (APCs) along with the action of co-stimulatory molecules and pattern recognition receptors. Cluster of differentiation 40 (CD40) is one such costimulatory molecule that is expressed on APCs that binds to CD40 ligand (CD40L) on T helper cells and activates a signaling cascade, subsequently resulting in a wide range of immune and inflammatory responses. Considering its important role in regulation of immune response, CD40/40L has been used for developing antitumor vaccines. In this study, we developed methods for evaluating and quantifying the activity of CD40L expressed from an adenovirus vector ONCOS-401. Our results show that the ONCOS-401 vector produces functional CD40L, which can bind and activate a NF-kB-dependent signaling cascade, leading to secreted embryonic alkaline phosphatase reporter production in HEK293-BLUE cells. In this study, we demonstrated that the HEK293-BLUE assay can be used for determination of the biological function of CD40L expressed from a recombinant virus vector. Both HEK293-BLUE and ELISA could be used for accurate quantification of secreted CD40L levels although the mechanisms of action of the assays are different.

P083

Self-organization and cell proliferation of adipose derived stem cells, HeLa and mononuclear cells after co-culture

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Developing of *in vitro* tumor models is important for preclinical drug screening and study of intercellular interactions in tumor microenvironment. In our study we analyzed self-organization and proliferation activity of HeLa, adipose-derived mesenchymal stem cells (ADSCs) and mononuclear cells (MCs) after co-culture (Hela+MCs, HeLa+ADSCs, HeLa+ADSCs+MCs). Cells were labeled by Vybrant DiD, DiO and DiI membrane fluorescent dyes and mixed at 1:1:1 ratio. Fluorescent microscopy revealed high interaction between different cell types, which led to self-organization and formation of cell aggregates. After 96 hours of co-culture, cells were dissociated, sorted by FACS into individual populations, seeded on 96-well plate and analyzed after 24 hours incubation using MTS Cell Proliferation Assay (Promega). After co-culture (with Hela, MCs, Hela+MCs) separated ADSCs had 2.3, 1.6 and 2.2-fold higher proliferation rate, respectively, compared to native ADSCs. HeLa cell proliferation was reduced after co-culture with ADSCs or MCs, but not with ADSCs+MCs. MCs proliferation was reduced in all co-culture experiments. Thus, co-culture with HeLa and/or MCs increases proliferation activity of ADSCs possibly due to the presence of secreted factors and metabolites in culture medium. MCs proliferation decrease in co-cultures can be explained by cytokine/chemokine secretion by MSCs/HeLa, which mediates the inhibition of MCs proliferation. HeLa proliferation decrease after co-cultivation with ADSCs or MCs can be explained by the antitumor effect of stromal/stem cells previously described in the literature.

P084

Mitofusin-2 expression is implicated in cervical cancer pathogenesis

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Crucial roles for mitofusin-2 (MFN2) in cancer progression have recently been identified, yet it remains unclear whether this protein functions as a tumor suppressor or oncogene in this process. Moreover, its influence in cervical cancer remains to be studied. In the present work, we therefore aimed to investigate the effect of MFN2 expression on the pathogenesis of this disease. MFN2 expression in 7 healthy cervical, 64 cervical intraepithelial neoplasia (CIN), and 120 cervical squamous cell carcinoma (SCC) tissue samples was tested by immunohistochemistry. Levels of this protein exhibited a tendency to gradually increase from healthy cervical tissue to CIN to SCC. Moreover, its expression was significantly associated with poor prognostic indicators, such as higher T stage ($P = 0.008$) and lymph node metastasis ($P < 0.001$). The influence exerted by MFN2 on the biological behavior of the cells was also investigated using MFN2-knockdown cervical cancer cell lines *in vitro*. The proliferative, migratory, and invasive abilities of these MFN2-knockdown cells were significantly lower ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively) than those of scrambled control-treated cells. Thus, we concluded that MFN2 may be involved in cervical cancer pathogenesis as an oncogene and might serve as a biomarker of cervical SCC.

P085

Non-small lung cancer cells exhibit signs of EMT in response to cisplatin treatment and after development of cisplatin resistance

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Non-small lung cancer (NSCLC) is the leading cause of cancer death worldwide. Effectiveness of NSCLC chemotherapy, including cisplatin, is often limited by acquired drug resistance. Numerous studies demonstrated the link between NSCLC cisplatin sensitivity and epithelial-mesenchymal transition (EMT). Here, we used a lung adenocarcinoma cell line (A549) to directly assess if cisplatin treatment and acquired cisplatin resistance induces EMT. Experiments were performed in ''physiologic'' (10% FBS medium – common for the cell line) and ''metabolic stress'' (1% FBS medium to induce starvation). In ''stress'' conditions, presence of 8uM cisplatin in medium for 24 hours resulted in downregulation of E-cadherin (epithelial marker) and upregulation of vimentin (mesenchymal marker) in NSCLC cells, as well as increased cell migration in wound healing assay. None of these were observed when experiment was performed in ''physiologic'' conditions. In parallel, A549 cells were incubated in increasing concentrations of cisplatin for 5 months, resulting in generating A549R cells with 4.5 folds increase in cisplatin IC50 (18.5uM versus 4uM for parental cells, analyzed for 72 hours). The A549R cells exhibited downregulation of E-cadherin and upregulation of vimentin proteins in both ''physiologic'' and ''stress'' conditions. In summary, our data is another demonstration of that EMT may play role both in cisplatin treatment response and development of cisplatin resistance of NSCLC cells. The reported study was funded by RFBR according to the research project No 18-015-00495. The part of this study was supported by the Program of Competitive Growth of Kazan Federal University.

P086

HuR knockdown decreases membrane expression of DR5 and reduces apoptosis levels

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TRAIL (TNF-Related Apoptosis Inducing Ligand) is a potent apoptotic ligand which induces apoptosis in susceptible cells via the DR4 and DR5 death receptors. Although the detailed pathways of TRAIL-induced signaling have been elucidated, regulatory mechanisms acting on its receptors are less clearly understood. Furthermore, unlike that of DR4, the means of transfer of DR5 to the cell membrane have not been clearly defined. Failure of DR5 translocation to the cell membrane, and its heading towards nucleus instead, is thought to constitute a significant resistance mechanism against TRAIL-induced apoptosis. We hypothesized that the 3′UTR region along with the HuR protein that binds to it, and Vps39, which functions in the endosomal trafficking, may play a role in this setting. To test our hypothesis, we suppressed the expressions of HuR and Vps39 via siRNAs in LNCaP and HeLa cells; isolated cytoplasmic, nuclear, and membrane fractions; and investigated the effects of HuR and Vps39 knockdown on DR5 expression in different subcellular fractions using Western Blot and ELISA techniques. TRAIL-induced apoptosis and necroptosis levels were determined via ELISA. HuR knockdown decreased DR5 surface expression levels in both cell lines. Reduced apoptosis levels were also evident in HeLa cells. Nuclear DR5 expression was found to be altered in both cell lines following Vps39 suppression. Understanding the mechanisms behind reduced DR5 expression on cell surface along with its proliferative function in nucleus will bring out new targets for gene therapy approaches that aim to utilize TRAIL as an inducer of selective apoptosis in cancer cells.

P087

Possibilities of application of photodynamic therapy for the treatment of squamous cell carcinoma of the oesophagus and bronchus

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Limitations of photodynamic therapy in providing high-tech medical care and the relatively expensive photosensitizer in most cases constitute an obstacle to the full implementation of the method in the clinical practice. Thus, the aim of our report is to describe the clinical case of effective use of the method of photodynamic therapy as a method of choice in a patiesnt with synchronous locally advanced cancer. Patient V., 68 years old, was observed in the «Tatarstan Cancer Center» (Kazan, Russia) from December 2011 with a diagnosis of cancer of the larynx T3N1M0 after successful complex treatment. In March 2017 patient was diagnosed squamous cell carcinoma of the esophagus and right upper lobe bronchus. The patient had metastases to the retroperitoneal lymph nodes and adrenal glands, so it was decided to carry out treatment with photodynamic therapy. Photodynamic therapy was carried out by the apparatus Latus 662 nm (ООО «Аткус», Russia) using of lightguide (diameter of 400 mcm) with output beam power of 1.4 W with 8 minutes of exposition (summed energy 150–180j) after injection of 0.35% solution of Radachlorin – photosensitizer – 20.0 ml i.v. (ООО «РаДа-фарма, Russia») in the amount of 1 mg/kg of body weight. Results and discussion: Marked persistent (9 months) stabilization of synchronous neoplastic process in the background system of monotherapy with etoposide and two-time endoscopic photodynamic therapy of tumors of the esophagus and bronchus. Thus, photodynamic therapy is the method of choice of palliative therapy to achieve a good survival rate at high quality of life.

P088

Rac1 and Bcl-2 expression changes in co-culture of mesenchymal stem cells and neuroblastoma cells after incubation with cisplatin

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Mesenchymal stem cells (MSCs) play an important role in tumorogenesis due to their pronounced tumor tropism, ability to mediate tumor growth, vascularization and development of chemoresistance. We investigated effect of cisplatin in co-culture of bone marrow derived MSCs and human neuroblastoma cells SH-SY5Y on Rac1 and Bcl2 mRNA expression using qPCR. SH-SY5Y cells were transduced with lentivirus expressing GFP. Co-cultures were established by mixing cells at 1:1 ratio and culturing for 72 h. Cisplatin was added and co-cultures incubated for additional 72 h. FACS was used to retrieve GFP expressing SH-SY5Y cells and non-labeled MSCs. Expression of Rac1 mRNA were increased 4-fold in MSCs cultured with SH-SY5Y. However, cisplatin induced 6-fold increase in Rac1 expression in MSC

treated with cisplatin, relative to untreated MSC controls. Expression of Rac1 only increased by 1.6-fold in cisplatin treated SH-SY5Y cells cultured in the absence of MSCs. In contrast, Bcl2 expression was induced 6-fold in control and cisplatin treated co-cultures, cisplatin treatment of MSC monocultures induced 50-fold decrease in Bcl2 expression relative to untreated MSCs. Bcl2 expression in co-cultured and cisplatin treatment of SH-SY5Y was increased by 6.5-fold and 8.5-fold respectively, whereas cisplatin induced 4.5-fold increase in Bcl2 expression in SH-SY5Y cells relative to controls. Increased Bcl-2 expression in SH-SY5Y in co-culture with MSCs could be a mechanism of bypassing pro-apoptotic effect of cisplatin.

P089

IL-2 genetically modified mesenchymal stem cells demonstrate increased VEGF, MMP2 and TGF-β1 genes expression

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Cell and gene therapy are emerging as promising approaches for cancer treatment. Mesenchymal stem cells (MSCs) are non-hematopoietic progenitor cells, which can be isolated from different types of adult tissues. Due to their tropism to the tumor niche, MSCs could be exploited to deliver various antitumor agents, including cytokines, with a view of preventing, delaying or reversing metastases. One example is interleukin-2 (IL2), an immunomodulating cytokine, which regulates the activities of white blood cells, and is one of the most established cancer immunotherapies. This study investigated the ability of over-expressing IL2 in MSCs and measured the consequence of IL2-overexpression of key cancer-related genes in MSCs. Human MSCs were isolated from adipose tissue and transduced with recombinant lentiviral vector expressing IL2 to generate MSC-IL2 cells. Expression levels of IL2, vascular endothelial growth factor (VEGF), matrix metalloproteinase-2 (MMP2) and transforming growth factor beta-1 (TGF-β1) mRNAs were determined by qPCR. Overexpression of IL2 in MSC-IL2 cells resulted in increased expression of VEGF (2.3 fold), MMP2 (2.3 fold) and TGF-β1 (1.6 fold) mRNAs relative to control MSCs. While it is well established that increased VEGF and MMP2 expression can stimulate blood vessel growth and metastases and thereby contribute to tumor progression, TGF-β1 can have both tumor suppressor or pro-oncogenic effects. However, given the increased expression of pro-metastatic factors in MSC-IL2, these potential tumor promoting effect requires additional investigation before further preclinical development.

P091

The results of treatment of patients with metastatic melanoma without a primary focus being detected

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The problem of treatment of patients with metastatic melanoma without primary tumor identified is an important and unresolved today. Therefore, the aim of our study is the statistical processing of the results of treatment of these patients Retrospective analysis of case histories and ambulatory cards of 581 patients was made from 1996 to 2008 in Tatarstan Cancer Center. In 36 patients on the basis of morphological studies of metastatic melanoma was diagnosed. The proportion of patients with metastatic melanoma revealed no primary lesion accounts for 6.2% of SAMPO. In this immunohistochemical study plays a leading role in the formulation of morphological diagnosis in these patients. Survival rates of patients with malignant melanoma without primary tumor identified comparable patients who had the same prevalence of metastases, but this was primary tumor. Five-year survival rate for stage IIIC was 18.2% and 18.4% (p = 0,042), to stage IV of 7.1% and 6% (p = 0,078), respectively. Staging when diagnosed with metastatic melanoma without a primary focus being detected is advantageously carried out according to the classification TNM-2002, and the data survival rates are comparable with the results obtained in patients with a known primary lesion. The absence of the primary tumor when metastatic melanoma is an unfavorable factor.

P093

RIG-I pathway stimulation polarizes neutrophils to anti-tumor type neutrophil and suppress tumor growth

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Recently, it has become clear the importance of tumor associated neutrophils (TAN) in the tumor microenvironment (TME). TANs are broadly classified into two types: anti-tumor type neutrophils (N1) and pro-tumor type neutrophils (N2). Neutrophils would polarize to either type by different stimulation and TME. TGF-beta has previously been shown to be a N2 polarization factor. N1 polarization factors are largely unknown, although several related factors such as TNF-alpha have been suggested. In our previous research, inactivated Sendai virus (HVJ-E) particles could stimulate N1 polarization directly. HVJ-E could also activate anti-tumor immunity via RIG-I/MAVS signaling pathway. So, we hypothesize that direct stimulation of RIG-I pathway may induce neutrophil activation and tumor suppression. To demonstrate whether RIG-I pathway can mediate anti-tumor neutrophil activation, we transfected low molecular weight (LMW) poly(I:C) into neutrophils to directly stimulate RIG-I pathway. LMW poly(I:C) transfected neutrophils were then intra-tumorally injected into mice bearing 4T1 mammary carcinoma tumors. After treatment, tumor growth suppression was observed. To confirm that RIG-I pathway induced neutrophil activation, we treated neutrophils with RIG-I inhibitor BX795 after LMW poly(I:C) transfection and injected these neutrophils into tumor bearing mice. BX795 treated neutrophil group showed decreased tumor growth suppression compared to untreated group. RIG-I stimulation also mediated anti-tumor immune cytokine release. Our research show that neutrophils released IFN-beta after RIG-I pathway stimulation and this IFN-beta release would be depleted by BX795 treatment. Taken together, our results showed that RIG-I pathway stimulated neutrophils could polarize neutrophils to anti-tumor type and suppress tumor growth.

P100

Transplantation of somatic cell-induced neural progenitors enhances functional recovery after stroke

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Induced pluripotent stem cell-derived neural progenitor cells (iPSC-NPCs) are a promising source of tailor-made cell therapy for neurological diseases. However, major obstacles, such as tumorigenic and spontaneous differentiation of iPSCs remains a concern for linical application. To circumvent complications related to iPSC-associated issues, we have previously established method to directly convert human fibroblast into neural progenitor-like population, namely induced neural progenitors (iNPs). We demonstrated that the iNP cells are able to give rise to various neural subpopulation including various neuronal subtypes, glial cells and oligodendrocytes *in vitro*. *In vivo* transplantation of iNP into normal rat brain showed that iNPs can integrate into adult brain tissue and differentiate into major neural cell types *in vivo*. Furthermore, implantation of iNPs epidurally over the peri-infarct cortex 7 days after permanent middle cerebral artery occlusion in adult rats resulted in improvements in paretic forelimb usage and grip strength from 10 days post-transplantation (dpt) onwards, as well as reductions in lesion volumes. This study demonstrates an alternative method to promote functional recovery after stroke.

P101

Repeated mesenchymal stromal cells treatment sustainably alleviates Machado-Joseph disease/spinocerebellar ataxia type-3

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Machado-Joseph disease (MJD)/Spinocerebellar ataxia type 3 (SCA-3) is caused by the over-repetition of a CAG repeat in ATXN3/MJD1 gene, which translates into a polyglutamine tract within ataxin-3 protein, causing neurodegeneration. There is no treatment for this fatal disorder. Exploratory clinical trials have shown that mesenchymal stromal cells (MSC) are safe and delay disease progression in SCAs. However, patients had unpredictably regressed to the status prior of treatment in a relatively short time. The objective of this work was to investigate the efficacy of repeated systemic MSC administrations in alleviating MJD phenotype and cerebellar neuropathology in a transgenic mouse model of MJD through motor behaviour, immunohistochemistry, western blot, neurospectroscopy and *in vivo* imaging analysis. Four intravenous administrations of MSC promoted sustained motor behaviour alleviation, whereas a single transplantation of MSC only produced transient effects, suggesting that MSC therapies should be re-designed to get sustained beneficial results in clinical practice. Interestingly, MSC injected systemically could reach the brain, but disappeared in 24-48 hours, corroborating with our/clinical results. Furthermore, MSC transplantation could promote preservation of the Purkinje cell number and the volume of cerebellar lobules. Importantly, an increased expression of GABA and glutamate correlated with motor improvements, indicating these metabolites may serve as valid neurospectroscopic biomarkers of treatment. The present study provides evidence that repeated intravenous transplantation of MSC is required to sustainably alleviate MJD phenotype and its efficacy can be monitored by the non-invasive 1H-MRS technique, thus providing novel contributions to the design of new clinical approaches for MJD and other SCAs.

P102

Generation of immunosuppressive iPSC-derived stromal cells for tissue regeneration

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Stromal cells are important components of all mammalian tissues contributing to vascular stability and organ integrity. Here, we aimed at developing stromal cells derived from induced pluripotent stem cells (iPSC) for organoid formation and organ regeneration. Healthy bone marrow and umbilical cord blood-derived stromal cells were used to generate iPSC (Sendai/OKSM), cultured under feeder-free conditions, followed by mesoderm induction and finally platelet-derived growth factor-induced stromal cell differentiation. Expandable and cryo-preservable CD73+/CD105+/CD90-/Tra-1-81- early iPS-derived stromal cells lacked immunosuppressive potential and showed different gene expression pattern compared to parental stromal cells. After successive passaging and maturation, a mature stromal phenotype developed including full immunosuppressive competence. CD90 surface expression, clonogenicity and gene expression comparable to parental stromal cells was acquired in animal serum-free stromal cell media conditions. Mature iPSC-derived stromal cells were able to form monotypic spheroids (mesenspheres) and maintained their clonogenicity upon 3D culture. Human 3D stem-cell-derived skin organoid formation using iPSC-derived stromal cells as dermal compartment, endothelial cells and adult interfollicular epidermal keratinocytes revealed organized structures of stromal-vascular aggregation with superficial anchorage of adult keratinocytes, indicating regenerative potential of iPS stromal cells. We present that sequential treatment of iPSC with mesenchymal induction and differentiation conditions resulted in mature stromal cells, featuring immunosuppressive, stem/progenitor and organ reconstitution potential.

P103

Impact of DNA damage response activation in haematopoietic stem and progenitor cells upon gene-targeting

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The activation of the DNA damage response (DDR) and its consequences in the hematopoietic stem and progenitor cells (HSPCs) are of interest for regenerative medicine and for genome engineering strategies of hematological disorders. However, little is known about the biological response to DNA double strand breaks (DSBs) induced by programmable nucleases in HSPCs. By employing optimized zinc finger nucleases and preassembled Cas9 ribonucleoproteins against distinct therapeutic loci, we observed transient DDR as detected by 53 binding protein 1 (53BP1) nuclear foci, with repair kinetics that depended on the chromatin context of the targeted locus and nuclease specificity. Remarkably, primitive cells exhibited slightly delayed kinetics of DSB rejoining compared to progenitors cells. When only one/two DDR foci per cell were detected immediately after nuclease-induced DSB, we observed a modest up-regulation of the cell cycle inhibitor p21 and only a transient growth arrest. However, sustained DDR due to delayed kinetics of DSB repair or nucleases with residual off-target activity, lead to higher induction of cell cycle inhibitors in HSPCs, a stable proliferative arrest and reduced clonogenic output. Gene expression analysis revealed an Ataxia-telangiectasia mutated kinase (ATM)-dependent pro-inflammatory program in edited cells that positively correlated with DSB severity and DDR persistency. Altogether, our findings provide mechanistic insights into the cellular response of HSPCs to single or multiple DSB, indicate that DDR, if not restricted in time, reduces the repopulating potential of HSPCs via cell-intrinsic and paracrine mechanisms, and highlight a strategy based on transient DDR modulation suitable for non-detrimental gene editing in HSPCs.

P104

Comparison of hematopoietic reconstitution dynamics of MPB- and BM-derived hematopoietic stem/progenitor cells gene therapy in Wiskott-Aldrich syndrome patients treated with lentiviral gene therapy

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Gene therapy (GT) for inborn errors is based on infusion of autologous Hematopoietic Stem/Progenitor Cells (HSPC) obtained from bone marrow (BM)- or mobilized peripheral blood (MPB). There is limited information on the kinetics of HSPC and hematopoietic reconstitution occurring after GT using these two sources. We performed a deep phenotyping of BM and peripheral blood (PB) composition at different time points after GT in 14 Wiskott-Aldrich syndrome patients treated with lentiviral transduced BM and/or MPB HSPC following reduced intensity conditioning. Five patients received BM, 8 MPB, and 1 patient received BM+MPB HSPC. MPB-GT patients showed a faster neutrophil engraftment (median: day +22 vs. 31) and platelet transfusion independence (75% vs. 0% at 1 month) as compared to BM-GT. Early hematopoietic recovery in BM-GT patients was accompanied by a progressive increase in primitive and committed progenitors followed by HSPC stabilization starting from 1-2 years post-GT. Conversely, in MPB treated individuals the HSPC compartment recovered already at 30 days and remained stable up to 2 years. We are collecting integration sites (IS) from 7 HSPC subpopulations and 13 mature BM and PB lineages at early and late reconstitution phases post-GT to compare reconstitution dynamics of the two HSPC sources. Preliminary data unveiled a higher number of IS in neutrophils at early time-points after MPB-GT, in line with faster hematopoietic recovery, and a higher level of gene correction in all compartments, including HSPC. These analyses will provide fundamental information on the contribution of distinct sources of HSPC to short-term and long-term engineered hematopoiesis.

P105

Chromosome transplantation in human iPS cells as a possible approach to treat disorders due to X chromosome abnormalities

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Many human genetic diseases are associated to gross mutations such as chromosome deletions, duplications or inversions. For these disorders conventional gene therapy, based on viral vectors or on the homologous recombination mediated by programmable nucleases, due to the limitation in the region size that must be corrected, is still unsatisfactory. At variance, chromosome transplantation (CT), defined as the perfect substitution of a defective chromosome with an exogenous normal one, could be applied to disorders characterized by chromosome abnormalities involving large DNA fragments. CT re-establishes a normal diploid cell, leaving no marker of the procedure, as we recently showed in mouse pluripotent stem cells. We demonstrate its feasibility in human pluripotent stem cells (hiPSCs), using cells reprogrammed from Lesch Nyhan disease (LND) patients, carrying mutations in the X-linked HPRT gene involved in the biosynthesis of purines. Corrected HPRT cells can be easily *in vitro*-selected making this pathology a good candidate for procedure set up. Accordingly, in the first step, we generated iPSCs from LND fibroblasts (46,XY) and inserted a HSV-TK transgene into the defective X-chromosome, by means of CRISPR/Cas9. Then, we introduced a normal exogenous X-chromosome, by retro-microcell mediated chromosome transfer, and selected 47,XXY LND-hiPSCs. Finally, we obtained corrected diploid cells (46,XY) where spontaneous mutated

X-chromosome loss was selected against HSV-TK. These results show for the first time that X-CT is applicable to hiPSCs, paving the way to the correction of several X-linked disorders associated to chromosome abnormalities.

P106

Improved haematopoietic engraftment due to the intrabone or intravenous co-transplantation of human haematopoietic stem cells and mesenchymal stromal cells in immunodeficient mice

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We have previously shown that the co-infusion of mesenchymal stromal cells (MSCs) with hematopoietic stem cells (HSCs) enhances the short- and the long-term hematopoietic reconstitution of transplanted mice, suggesting that MSCs facilitate the homing of HSCs to the bone marrow (BM) niches. Although conventional hematopoietic transplants are based on the intravenous (IV) infusion of HSCs, some studies propose the intrabone (IB) transplantation of HSCs constitutes an efficient alternative to improve HSCs homing. In this work, we have investigated the effect of the co-infusion of human adipose tissue derived MSCs (500,000 Ad-MSCs) in the engraftment of very low numbers of human CD34+ cells (4,000 cord blood CD34+ cells) either transplanted IV or IB in immunodeficient NSG mice. Our results firstly showed that IB-transplanted HSCs not only home in the transplanted BM, but also migrate to other BM niches although we could not confirm a higher hematopoietic engraftment as compared with IV-transplanted mice. Remarkably, Ad-MSC co-transplantation improved the hematopoietic reconstitution in NSG recipients regardless of the transplantation route, and in all instances, engrafted cells were able to differentiate towards the lymphoid and myeloid lineages. Taken together, our results demonstrate that Ad-MSC co-transplantation increases the engraftment of very low numbers of HSCs in immunodeficient mice, suggesting that this approach could be relevant in the clinical hematopoietic transplantation, particularly when low numbers of HSCs are available.

P107

***In vivo* generation of CAR-T cells by CD4- and CD8-targeted lentiviral vectors**

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T cells modified with CD19-specific chimeric antigen receptors (CARs) result in significant clinical benefit for leukemia patients. However, production of CAR T cells is time-consuming and expensive. Receptor-targeted lentiviral vectors (LV), which transfer genes selectively into particular types of lymphocytes may enable direct *in vivo* CAR gene delivery. Here, we assessed this strategy using CD4- and CD8-targeted LV, respectively. Cultivated hu-PBMC were transduced with the CD19-CAR gene by either CD4- or CD8-LV resulting in the generation of CAR-T cells. Both types of CAR T cells proliferated efficiently upon antigen exposure. For *in vivo* generation of CAR-T cells, NSG mice were i.p. injected with human PBMC followed by administration of CD4- or CD8-LV delivering the CD19-CAR. One week after vector injection mice were sacrificed, peritoneal cells, spleen and blood cells were analyzed by flow cytometry. Interestingly, vector treated mice contained substantially more CD4+ cells following CD4-LV treatment and substantially more CD8+ cells after CD8-LV injection. Notably, about 40-60% of CD4+ cells and 20-50% of CD8+ cells isolated from the peritoneum were CAR-positive, while no CAR cells could be detected in the CD4- and CD8-negative cell fractions, respectively. Moreover, control mice contained about 0.2-2.5% CD19+ cells floating in the peritoneal cavity, while these were absent in CD4-LV or CD8-LV treated mice, indicating that both types of *in vivo* generated CAR-T cells were functionally active. Our results show that distinct subtypes of CAR T cells can be generated *in vivo* using receptor-targeted LVs. # First two authors contributed equally to this work.

P108

A highly efficient and GMP-compliant protocol to manufacture CCR5-edited cells to treat HIV infection

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Targeted genome editing in blood and immune cells enable new therapeutic applications, especially for infectious diseases. We present a GMP-compliant protocol to manufacture CCR5-edited CD34+ hematopoietic stem and precursor cells (HSPCs) with the goal to cure patients suffering from chronic infection with human immunodeficiency virus type 1 (HIV1). We hypothesize that genetic disruption of the CCR5 gene, which encodes the major HIV1 co-receptor, in HSPCs will give rise to an HIV-resistant immune system after transplantation. We have developed engineered nucleases based on transcription activator-like effector nucleases (TALENs) targeting CCR5. Electroporation of CD4+ T-cells and CD34+ HSPCs with mRNAs encoding TALENs revealed disruption of up to 80% of CCR5 alleles in CD4+ T-cells and over 90% of alleles in HSPCs. The high gene editing frequencies in T-cells and HSPCs were confirmed by deep sequencing, and no cleavage activity above background levels were detected at the top 20 predicted off-target sites. CCR5-edited CD4+ cells preserved their proliferation capacity and their biological function. Importantly, these cells showed significantly reduced CCR5 expression and became resistant to infection with the R5-tropic HIV-1JR-FL virus. The CCR5-edited HSPCs maintained their proliferation potential and their capacity to differentiate into the various blood lineages *in vitro* and *in vivo*, and clonal analysis revealed bi-allelic CCR5

disruption in more than 75% of cells. In summary, our developed protocol enables highly efficient and GMP-compliant knockout of the CCR5 locus in clinically relevant cells, so forming the foundation for a planned phase I/II clinical study.

P109

Demonstration of immunomodulatory properties for human MuStem cell population, a promising candidate for cell therapy of muscular dystrophies

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Allogeneic cell transplantation protocols are highly limited by graft rejection. To overcome this issue, long-term immunosuppression (IS) is usually used, resulting in improved engraftment but also appearance of adverse effects. Pleiotropic immunomodulatory properties attributed to mesenchymal stem cells on many immune cells, through action on both paracrine secretion and cell contact. These features could increase their ability to engraft in allogeneic recipient despite the lack of IS. Also, immunomodulatory cell delivery may be beneficial in degenerative disorders to limit inflammation characterising tissues and interfering with repair. We showed that systemic delivery of allogeneic muscle-derived stem cells, named MuStem cells, to immunosuppressed dystrophic dogs lead to muscle regeneration and long-term clinical stabilization. Recently, human MuStem cells (hMuStem), exhibiting myogenic potential, were positioned as candidate for muscle-dedicated regenerative medicine. Interestingly, a transient IS was shown as sufficient to sustain their transplantation benefits and prevent host immunity response in allogeneic context, suggesting a possible immune privilege behaviour. Here, we explored modulation of immune effectors by hMuStem cells. Using *in vitro* assays, we examined the impact of hMuStem cells on T cell features and the complement system activation. Flow cytometry experiments and Elisa assays were performed to determine the immunophenotype of hMuStem cells. hMuStem cells secrete various immunomodulatory molecules and impact T cell proliferation and function through iNOS and PGE2 pathway. Also, they inhibit complement-mediated lysis through factor H secretion. Overall, we show that hMuStem cells modulate *in vitro* both T cell and complement functions, reinforcing their qualification as promising agent for clinical application.

P110

A phase 1/2 clinical trial for AAV8-mediated liver-directed gene therapy in adults with late-onset OTC deficiency

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Current therapy for ornithine transcarbamylase (OTC) deficiency is based upon restriction of dietary protein intake and nitrogen scavenging agents. Although this approach lowers the incidence of hyperammonemic episodes, risk of life-threatening hyperammonemic crises remains and neurocognitive outcomes are often suboptimal. Gene therapy aimed at restoring liver OTC activity represents a potential novel therapeutic strategy. DTX301 is a self-complementary recombinant AAV8 vector expressing a codon-optimized human OTC cDNA under the transcriptional control of a liver specific promoter. CAPtivate is a global multi-center open label Phase1/2 dose escalation trial evaluating the safety and preliminary efficacy of a single DTX301 IV infusion in adults with symptomatic late onset OTC deficiency. After this initial 52-week study period, subjects are followed for 5 years after dosing. Efficacy objectives include change in total ureagenesis capacity measured by *in vivo* stable isotope dilution assay and 24-hr NH3 AUC. Dosing of the first 3 patients at 2×10^12 GC/kg level was completed in November 2017. No infusion-related or serious adverse events have been reported to date. One patient achieved normalized ureagenesis rate sustained beyond 24-weeks post-dose and discontinued ammonia scavenger medications. After Data and Safety Monitoring Committee review in March 2018, the second cohort of 3 subjects dosed at 6×10^12 GC/kg is under enrollment. Early preliminary data from CAPtivate, an on-going Ph1/2 AAV gene transfer clinical trial, indicate that DTX301 has an initial benefit/risk profile that is favorable. DTX301 is a promising new therapeutic approach for OTC deficiency.

P111

Establishment of a tissue engineered product consisting of RPE derived from clinical grade human embryonic stem cell line cultured on human amniotic membrane for clinical applications

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Retinitis Pigmentosa (RP) is a group of inherited retinal disorder that can lead to blindness. The Retinal Pigment Epithelium (RPE) is a continuous monolayer of cuboidal epithelial cells, localized between the photoreceptors and fenestrated choroid capillaries. The RPE interacts with the photoreceptors for the maintenance of visual function. RP could be caused by degeneration or malfunction of the RPE cell layer. Two important features of human embryonic stem cells (hESC), self-renew and the ability to generate an unlimited number of RPE cells, make them very attractive tools in the cell therapy approach for retinal diseases. Maintaining the epithelial morphology of RPE cells is an essential parameter to consider in order restoring some visual function by cell therapy. For this reason, we have developed a tissue engineered product (TEP) that consists of RPE derived from hESC cultured for 4 weeks on denuded human amniotic membrane (hAM). We have optimized and transferred a differentiation protocol to generate RPE cell Banks from clinical

grade hESC line into a GMP facility. In parallel, we have established high quality standard to definite their characteristics, impurities and potency. This cells could be banked and maintain their phenotype and functionality when cultured on hAM. Indeed, when cultured for 1 month on denuded hAM, the RPE cells formed a typical retinal pigmented epithelium. Furthermore, hESC-derived RPE cells perform typical phagocytic activity and secrete VEGF in polarized manner. Finally, our TEP will be used for phase I/II clinical trials for the treatment of RP caused by a RPE defect.

P112

Comparative analysis of therapeutic efficacy of mesenchymal stromal cells isolated from different sources on rat model of thermal skin burn

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Wide range of cellular products consisting of different types of cells obtained from various sources are used today in the regenerative medicine. Each source has its advantages and disadvantages. Comparative studies of cells isolated from various sources, on a single model are practically absent. The rat model of thermal skin burn was used. Following cellular products were investigated: allogeneic placenta derived multipotent mesenchymal stromal cells (MSCs) (single injection 5 mln or two injections 5 mln each with 1 week interval), adipose-derived stromal-vascular fraction (SVF) (isolated from 1 ml of fat), SVF (isolated from 1 ml of fat) + allogeneic adipose-derived MSCs (adMSCs) (1, 5 or 10 mln), SVF (isolated from 1 ml of fat) + autologous adMSCs (5 or 10 mln), autologous adMSCs (1, 5 or 10 mln), allogeneic adMSCs (5mln), allogeneic gingiva-derived MSCs (1 or 5 mln). Investigated product was administered subcutaneously at 24 points along the circumference of burn and under the wound bottom. Cells were administered at day 3 (single injection) or days 3 and 10 (two injections) after burn modeling. Control group received equal volume of saline. Primary endpoint was the time to complete epithelialization of the wound. Secondary endpoints were - reduction of dermal wound area at days 21 and 30 and the degree of regeneration according to histological examination. Greater therapeutic efficacy of single injection of allogeneic placenta-derived MSCs compared to all other cells was demonstrated. Second injection of placental MSCs significantly improved results. The study was funded by Russian Science Foundation (project #17-75-30066).

P113

Myocardial infarct repair with human adult muscle-derived stem cells ''MuStem''

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Heart failure is a major public health with no effective cure. Cell-based therapy represents a promising strategy although none of the cells investigated until now fulfils all the expected requirements. We isolated a population of skeletal muscle-derived stem cells (MDSCs) from healthy dog, that we named MuStem cells, and established a proof of efficacy of its use in dystrophic dogs. Recently, the human counterpart (hMuStem cells) was isolated and characterized by contribution to fibre formation after injection into injured mice muscle and high secretory activity. These data placed it as a potential advanced therapy medicinal product. In the heart failure context, beneficial tissue remodelling and modulation of contractile function were reported following administration of murine MDSCs. Similar effects mainly attributed to trophic factors were described after mesenchymal stem cell delivery. Considering the regenerative capacity and paracrine effect of hMuStem cells, we investigated whether they could be an interesting alternative for myocardial infarction treatment. For this, coronary ligation and intra-myocardial administration of hMuStem cells were performed in a new Rag1 and Il2Rg KO immunodeficient rat model. As opposed to hMuStem-treated rats, control rats presented lung atelectasia, cardiac atrophy-dilatation and hepatic congestion. Moreover histological and molecular analyses showed that hMuStem cells were implanted into the host cardiac tissue without generating arrhythmia 3 weeks post-injection. Echographic analyses highlighted functional and structural improvements of the infarcted heart with an increase of the left ventricle ejection fraction. In conclusion, hMuStem cells are able to implant into infarcted hearts and generate beneficial functional impact.

P114

Cell sheets as a platform for therapeutic delivery and tissue modelling

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Cell sheets have gained attention as a tissue-engineering tool for delivery of living cells along with their extracellular matrix to stimulate regeneration and tissue repair. Within last decade we have focused on cell sheets from MSC and other postnatal stem cell types to treat vascular disease (limb ischemia and myocardial infarction) and cutaneous wound healing in corresponding animal models. We used different approaches - from viral delivery to enhance growth factor production and efficacy to fabrication of decellularized matrices to make a cell-free therapeutic product or a feasible matrix for cell seeding. Thus, we consider cells sheets as a versatile platform for minimally-engineered tissue constructs for numerous potential application in cell therapy. However, cell sheets seem to go beyond just a way to deliver cells in a feasible manner. Using time-lapse microscopy we found MSC-based cell sheets to self-organize in a heterogenous ''hills and valleys'' pattern. Furthermore, our data supports the fact that such behaviour of MSC may be due to their attempts to recapitulate a ''niche-like'' environment which was supported by our data of increased ''stemness factor'' expression in cell sheets compared to monolayer. Furthermore, we have found that within cells sheets proliferation occurs despite obvious ''contact inhibition'' of division due to high thickness.

Histology studies have shown that cell matrix composition is significantly modulated over time of culture and cell sheets may have a more ''tissue-like'' condition that expected. The study was funded by RFBR Grant #17-04-01452 and partly by RSF grant #16-15-00181 (histology and animal experiments)

P115

Application of combined gene and cell therapy within an implantable therapeutic device for the treatment of severe haemophilia A

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Haemophilia A (HA) is an X-linked bleeding disease due to factor VIII (FVIII) deficiency and new regenerative medicine approaches to treat/cure haemophilia A 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 the technologies for a novel *ex vivo* cell-based therapy to treat HA that should lead to improved patient quality of life. We isolated blood outgrowth endothelial cells (BOECs) from healthy and patients' blood. BOECs were efficiently transduced with a lentiviral vector carrying the B domain deleted form of human FVIII under the Vascular Endothelial Cadherin promoter (LV-VEC.hFVIII). BOECs were characterized for endothelial phenotype and the number of integrated LV copies/cell was ~ 3. By FACS, we demonstrated that FVIII was expressed by 80-90% in LV-VEC.hFVIII transduced cells, and FVIII activity was evaluated by aPTT and ELISA. Ten million LV-VEC.hFVIII-BOECs were transplanted intraperitoneally in association with cytodex® 3 microcarrier beads in NOD/SCID g-null HA mice (n = 6). BOECs survived and secreted FVIII at therapeutic levels (12%) for up to 18 weeks and ameliorated the bleeding phenotype of the transplanted mice. As next steps, LV-transduced HA patient BOECs will be transplanted into an implanted prevascularized, scalable medical device (Cell Pouch™, Sernova Corp.) and optimized for sustained secretion of therapeutic FVIII in the NOD/SCID g-null HA mice. This is in preparation for future human clinical testing within the device in HA patients by transplantation of GMP produced autologous gene corrected BOECs.

P116

Mitochondrial genome mutations in induced pluripotent stem cells

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Induced pluripotent stem cells (iPSCs) are important, potential sources for autologous cell replacement therapies to treat age-associated degenerative disease. However, it should be considered that somatic mitochondrial genome (mtDNA) mutations might be accumulated in human iPSCs from elderly individuals. In order to gain a further insight into the age-related progressive accumulation of mtDNA mutations in iPSCs, analysis of mtDNA mutation was performed with the iPSCs derived from young and elderly individuals, using Illumina MiSeq sequencer. We obtained 48 and 45 iPSCs lines from 31 elderly and 17 young subjects, respectively. The average number of mtDNA variants in an individual line was significantly higher in the group consisting of the elderly than that of a young group (1.90 ± 0.2 vs. 0.56 ± 0.1, $P < 0.0001$). Three percent of novel mutations were not reported in MitoMAP and 77% were non-synonymous or resided in RNA coding genes. Next, the selected iPSCs with certain mutations were differentiated into energy-demanding cells including hepatocytes, retinal pigment epithelium cells, and cardiomyocytes. The differentiated cells from mutant iPSCs displayed reduced mitochondrial functions. As demonstrated, the chance of occurrence of iPSCs carrying the pathogenic mtDNA mutations might be higher in the elderly subjects. Therefore, it is necessary to screen the mtDNA mutations in iPSC lines of such subjects prior to the application of cell therapy, disease modeling, or pharmacological screening.

P117

Generation of human iPSC-derived macrophages using a GMP-compliant process pipeline

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Macrophages are key components of the innate immune system with critical function in tissue homeostasis and host pathogen protection. Recently, transplantation of macrophages has been proposed as an effective and long-lasting therapy for different congenital disorders. Given the unique features of pluripotent stem cells, we aim to develop a scalable platform for the GMP-compliant generation of iPSC-derived macrophages. Considering our unique protocol to produce human iPSC-derived macrophages (iPSC-Mac) continuously, we established a suspension-based technique (4D), which is suitable for bioreactor mediated up-scaling. iPSC-Mac from the 4D-culture could be harvested continuously for 12 consecutive weeks. Of note, harvested cells showed a purity of >90% of CD45+ cells and stained positive for CD45+CD11b+CD14+CD163+. For future clinical application of iPSC-Mac, we propose to advance the protocol into a GMP-compatible ''all-in-one'' suspension cultivation and differentiation using stirred tank bioreactors. As a first step, we used GMP-compliant components to establish cultivation of iPSCs as pluripotent aggregates in suspension, which are suitable for bioreactor mediated up-scaling. After a phase of mesoderm priming for 7 days, aggregates were subjected to lineage instructive cytokines to induce the hematopoietic program. Production of iPSC-Mac started at day 14 of differentiation and continued for more than six-weeks with high efficiency. Generated iPSC-Mac exhibited classical

morphology, a surface marker profile of CD45+CD11b+CD14+ CD163+, and the ability to eradicate both pHrodo E. coli bioparticles and viable Staphylococcus aureus. Of note, differentiation of pluripotent aggregates derived from stirred tank bioreactors resulted also in the continuous production of iPSC-Mac, paving the way for highly innovative cell-therapies.

P118

MSC self-organization *in vitro* is concordant with elevation of regenerative potential and characteristics related to stem cell niche function

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Ability of mesenchymal stromal cells (MSCs) for proliferation and differentiation became a basis for therapeutic use. However, recent data on MSC nature suggested their regenerative potential is related to secretory activity of these cells. However, their role is not limited to paracrine function, but may be related to postnatal stem cells (SC) niche stromal component formation which suggests their important role in formation of SC-controlling microenvironment. We used cell sheets as a model system of cell-to-cell interaction and found that MSC in cell sheet culture reproduce characteristics of SC niche microenvironment: intercellular interactions, ECM and high local concentration of paracrine factors. MSC in cell sheets formed by the multilayered cells and ECM they produce are demonstrating self-organization into compacted conglomerates. We recorded the dynamics of formation of these conglomerates with the method of a time-lapse shooting for 14 days, the formation of conglomerates occurs by active migration of cells and the contraction of a group of cells joined together into arcs and rings. The most important observation was an increase in the expression and secretion of the factors VEGF and HGF by MSCs which were situated in these compact conglomerates. Our PCR-based assays showed increased expression of ''stemness factors'' (Oct-4, Nanog) in cell sheets compared to monolayer cultures. We will continue investigations in this area suggesting that through self-organization MSC exhibit their inherent capacity to participate in formation and support of SC niches. The study was funded by RFBR Grant #17-04-01452 and conducted using biomaterial collected under RSF grant #14-50-00029.

P119

Combining iPS cell-derived myogenic progenitors and human artificial chromosomes for genomic-integration-free, systemically deliverable cell and gene therapy of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene and primarily affects skeletal muscles, resulting in disability and premature death. Our study looks at different strategies to circumvent substantial obstacles in the development of novel therapies for this incurable disease. Although the limited availability of a large number of transplantable myogenic cells and the large size of the dystrophin gene (2.4Mb) could be tackled by combining human artificial chromosome (HAC)-based gene correction and pluripotent stem cell-mediated production of transplantable myogenic cells, another significant hurdle is posed by cell delivery, as skeletal muscle is the most abundant human tissue. Indeed the lack of efficacy in human primary myoblast-based clinical trials for DMD have been mainly ascribed to poor survival and migration of myoblasts. We hypothesised that the combination of key properties of myoblasts and perivascular cells may lead to the production of clinically-relevant induced pluripotent stem (iPS) cell-derived myogenic cells amenable to genomic-integration-free correction with HACs and, ideally, systemically deliverable. To achieve this aim, we differentiated healthy human and HAC-corrected DMD iPS cells into myogenic progenitors using and established small molecule-based protocol and then enhanced their migration ability by modulating the Notch and PDGF pathways. Treated cells acquired expression of perivascular markers and an improved migration capacity, confirmed also by RNAseq analyses and functional *in vitro* assays. Taken together our results lay the foundation for a small molecule-based strategy to enable systemic delivery of next-generation iPS cell-derived myogenic for autologous gene and cell therapy of DMD.

P120

Analysis of the threshold of corrected cells required for the phenotypic correction of erythrocyte pyruvate kinase deficiency

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Pyruvate kinase deficiency (PKD) is a rare autosomal recessive disease and the main cause of hemolytic non-spherocytic anemia. It is produced by mutations in the *PKLR* gene and can be fatal in some cases in early childhood. We have developed a gene therapy protocol based on the *ex vivo*correction of hematopoietic progenitors using a lentiviral vector (LV) carrying a codon-optimized version of the PKLR cDNA (coRPK). The vector has been tested in mice and has been designated as an Orphan Drug for the treatment of PKD by the European Medicines Agency (EMA) and United States Food and Drug Administration (US FDA). To investigate the minimal proportion of corrected cells required to achieve a therapeutic effect in PKD patients we conducted two different experimental preclinical approaches: In a first attempt, lethally irradiated PKD mice were transplanted with different proportions of wild-type and PKD cells (5 to 100%). 30% donor wild-type healthy cells were needed to achieve a full correction of the disease. In a second approach, mouse PKD hematopoietic progenitor cells were transduced with a GMP-like therapeutic lentiviral vector at different multiplicities of infection (MOI) that ranged from 0.3

to 50. Transduced cells were then transplanted into lethally-conditioned PKD recipients. Consistent with data obtained in the previous approach, 0.3 copies of the therapeutic vector per cell were sufficient to ameliorate the disease phenotype. These pre-clinical results offer new experimental evidence to define the conditions to be used in clinical gene therapy programs for the treatment of PKD patients.

P121

Clinical grade production of mesenchymal stromal cells from adipose tissue and foreskin

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The increasing use of mesenchymal stromal cells (MSCs) for clinical applications has prompted the search for feasible sources alternative to bone marrow (BM). Adipose tissue (AT) is an easily accessible and valuable source, as well as the recently described foreskin (FSK). The collection of both tissues doesn't raise any ethical controversy. The International Society for Cellular Therapy (ISCT) consensus has proposed several criteria for defining MSCs: adherence to plastic in standard culture conditions; specific phenotype; multipotency of differentiating *in vitro* into chondrocytes, osteoblasts and adipocytes. We standardised clinical-grade isolation and expansion of AT- and FSK-derived MSCs. Eleven AT samples were obtained after automated liposuction (7) and dermolipectomy (4) from different anatomical sites (breast, abdomen, arm, thighs). An average of 300 ml of adipose tissue was processed at each procedure; saline portion was processed where available. MSCs from 12 FSKs post-circumcision were concurrently isolated. The MSCs obtained from all the processed samples were properly characterised and differentiated, demonstrating fulfillment of ISCT criteria. In order to support their release as an off-the-shelf medicinal product, we validated an extended immunological panel in AT- and BM-MSC cell lines after interferon-γ priming, and tested it in 4 primary MSCs: CD274, HLA-DR, HLA-G, HLA-ABC, IDO-1, CD40, CD80, CD86 and CD54 can be used for selecting a more homogeneous immunosuppressive phenotype with potential greater clinical benefit in patients. Finally, the enrichment of progenitors (CD146+/CD271+) examined between day +10 and +13 after seeding further strengthens the releasing criteria of clinical grade MSCs from different sources.

P122

A novel non-integrative and autonomously replicating DNA vector system for the persistent genetic modification of stem cells and transgenesis

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The capability of stem cells (SCs) to differentiate into all cell types holds great promise for the future of gene therapy and regenerative medicine. Typically, the modification of SCs is done by using integrating viral vectors. Although unquestionably the most effective gene delivery systems in use today, their efficacy at gene transfer is tempered by their potential integrative mutagenesis and their typical silencing, either directly at the pluripotent stage or during differentiation. Here we demonstrate that a novel non-viral and non-integrating technology based on a Scaffold Matrix Attachment Region (S/MAR), delivers sustained therapeutic levels of transgene expression without compromising the viability of the host cell in any way. S/MAR DNA vectors can successfully genetically modify murine and human SCs without causing any molecular or genetic damage while sustaining high levels of transgene expression. For the first time, we demonstrate that non-viral episomal DNA vectors based on mammalian chromosomal elements can persistently genetically modify both murine and human SCs, robustly expressing the transgene during random or hematopoietic *in vitro* differentiation, whilst avoiding vector loss or differentiation-mediated transgene silencing. The vectors remained episomal and did not modify the stem cells' properties, as demonstrated by the expression of pluripotency markers and supported by microarray data showing minimal impact of the vector in the cells' transcriptome. As an ultimate demonstration, the vectors were challenged *in vivo* and were able to generate transgenic mice, which persistently expressed the vector in organs from different embryonic origins, including hematopoietic organs.

P123

Extracellular vesicles of placental expanded stromal cells have immunomodulatory capacity and pro-angiogenic function

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Peripheral artery disease affects >10% of the European elderly population resulting in increased risk for cardiovascular events and death. Critical limb ischemia (CLI) as its final stage often leads to amputation. Allogeneic placental expanded (PLX) stromal cells are currently evaluated in a clinical phase III trial as an advanced CLI therapy. In this study, we aim to identify the mode of action of this novel cell-based treatment. We hypothesized that PLX-derived extracellular vesicles (EVs) contribute to PLX effectiveness. PLX-derived EVs were purified by tangential flow filtration. Quantity, morphology and phenotype of EVs were characterized by tunable resistive pulse sensing (TRPS), transmission electron microscopy (TEM) and multiplex flow cytometry. Immune modulation capacity of EVs and their parental PLX cells was determined by the inhibition of mitogen-driven and allo-immunity-mediated T cell proliferation. Pro-angiogenic potency was evaluated comparing the relative contribution of PLX EVs and PLX-derived secreted soluble factors in Matrigel tube-like formation assays. PLX stromal cells were found to secrete mean 500 EVs per cell per 24h with a mean size of 120 nm as determined by TRPS and TEM. Surface marker profiling of tetraspanin positive EVs revealed a plurity of adhesion-mediating molecules. Both PLX cells and their EVs alone inhibited T cell proliferation. PLX-derived EVs also displayed 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 despite lack of engraftment of the transplanted allogeneic stromal cells.

P124

Pulmonary macrophage transplantation-based therapy for alpha-1 antitrypsin deficiency

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Alpha-1-antitrypsin (AAT) is an acute phase glycoprotein and an inhibitor of neutrophil elastase. AAT deficiency is a hereditary disease characterised by low serum levels of AAT protein, thus causing neutrophil elastase to progressively destroy lung tissue and induce early-onset emphysema. As described previously, *in vivo* transduction of alveolar macrophages (AMs) can provide AAT at levels sufficient to ameliorate emphysema progression. Based on existing data, here we investigate the pulmonary macrophage transplantation (PMT) employing AAT-overexpressing macrophages (AAT-MΦ) as a novel therapy for the pulmonary manifestations of AAT deficiency. For this purpose, human and murine hematopoietic stem and progenitor cells were transduced with 3rd generation SIN-lentiviral vectors expressing the human AAT and eGFP transgene under the control of different promoters. Upon differentiation, AAT-MΦ showed normal morphology, surface marker expression and functionality. Highest AAT expression levels were consistently observed from the constitutive CAG promoter as measured in cell lysates and supernatants. To study the potential of AAT-MΦ to engraft and secrete AAT *in vivo*, murine AAT-MΦ were intrapulmonary transplanted into Csf2rb-/- mice which lack AMs and thus represent ideal recipients. Two weeks after PMT donor-derived cells were detected by FACS in bronchoalveolar lavage fluid (BALF) and lung samples, and these cells presented a CD11c+/Siglec-F+ AM-specific phenotype. Moreover, human AAT was detected in the BALF of transplanted animals. In summary, AAT-MΦ present normal morphology as well as functionality, gain an AM phenotype and secrete AAT *in vivo*. In the near future we will investigate the capacity of AAT-MΦ to ameliorate emphysema in mouse models.

P125

Dissecting pancreatic exocrine heterogeneity to identify a bi-potent progenitor

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Type I diabetes is caused by a lack of insulin producing beta cells, which are destroyed by an auto-immune response. One possible treatment for these patients is beta-cell replacement therapy through transplantation of *in vitro* generated beta cells from alternative cell sources. We have previously shown that human adult primary exocrine can generate expandable 3D organoids with a ductal phenotype. These organoids can be expanded *in vitro* and can generate insulin producing cells *in vivo*, hinting at the presence of a bi-potent progenitor (with endocrine and exocrine capacity) within the culture. Our aim is to identify these putative bipotent progenitor cells within the freshly isolated exocrine tissue and in the derived organoid cultures by combining single cell RNA sequencing and FACS analysis. In order to increase the resolution of our analysis and to detect even rare subpopulation (<1%), we selected a combination of surface markers which based on preliminary results, allow to separate the different cell types of the exocrine tissue (ductal, acinar and centroacinar) and various subpopulations within organoid cultures. While early organoid cultures contain both acinar and ductal cell subpopulations, prolonged organoid cultures purely consist of ductal cells (as shown by single cell RNA sequencing analysis). We are comparing the gene expression profile of these different subpopulations. Finally, we are testing if organoid cultures have ductal functionality (carbonic anhydrase activity) and whether it is possible to differentiate them into endocrine and acinar cell types *in vitro*.

P126

Human mesenchymal stem cells genetically engineered to express alpha-1 anti-trypsin (apceth-201) confer a long-term survival benefit in lethal mouse models of graft-vs-host-disease

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Acute Graft-vs-Host Disease (aGvHD) is a frequent complication associated with allogeneic bone marrow transplantation (BMT). Immunosuppressants are used to manage aGvHD. However, steroid-refractory aGvHD develops in many cases and has an extremely poor prognosis. Therefore, new therapeutics are needed. Apceth has developed a mesenchymal stromal cell-based gene therapy product termed ''apceth-201''. This product consists of human allogeneic MSC, engineered by lentiviral transduction to express the proteinase inhibitor, alpha-1-antitrypsin to further augment the anti-inflammatory potential of the MSC. Using *in vitro* assays, we showed apceth-201 efficiently suppresses T cell proliferation and polarizes macrophages to an anti-inflammatory M2 type. To assess the *in vivo* efficacy of apceth-201 it was tested in two different mouse models for aGvHD. In a humanized model vehicle-treated control animals succumbed quickly to GvHD, whereas median survival was doubled in apceth-201 treated animals. Animals treated with apceth-201 showed significantly improved clinical scores and reduced levels of inflammatory markers while preserving bone marrow cellularity Next, the product was tested in a GvHD model system which mimics closely haploidentical BMT, now being evaluated for use in the clinic. Vehicle-treated control animals again succumbed quickly to GvHD whereastreatment with apceth-201 resulted in long term survival of 57% of the animals. Initially, all treated

animals showed clinical scores comparable to the control animals. Within a period of 25 days after the second injection, the clinical scores had returned to base line, indicating complete resolution of GvHD. This promising data has led to planning of a phase I/II study using apceth-201.

P127

Chimeric antigen receptor modified T cells using Sleeping Beauty system by electroporation and combination with co-culture expansion has cytotoxic activity *in vitro* and *in vivo*

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Chimeric Antigen Receptor (CAR) based therapies require T cell expansion to reach optimal cell numbers. We have herein explored the cut-and-paste mechanism of the Transposon Sleeping Beauty (SB) system along with gene delivery by electroporation to generate CAR+T cells. The T cells were expanded by co-culture with the Epstein-Bar virus transformed allogeneic lymphoblast cell LAZ388 (L388). Peripheral blood mononuclear cells were isolated using Ficoll and electroporated using the Nucleofector II combined with plasmids enconding 19BBz CAR in the pT3 SB transposon backbone and SB100x transposase following stimulation up to 3 times with irradiated L388 cells. The phenotype and cytotoxic activity *in vitro* and *in vivo* was evaluated. 19BBz+ and mock cells showed 28 and 15-fold expansion respectively compared to d+1 values with frequencies of memory cell subpopulations differing between these conditions. NK cell depletion favored the expansion of CD8+CD62L-CCR7+ cells. LAZ388 stimulation favored CAR+ cell expansion, yielding up to 60% of 19BBz+ T cells on first cycle (14-20 days). Baseline d+1 CAR expression ranged from 2,5% to 37%. CAR+T cells eliminated $CD19^+$ cell lines such as Nalm-6, K562, L388 and RS4;11 cells while the CD19 negative K562 cell line showed reduced lyisis. In NSG mice engrafted with the B cell leukemia RS4;11, $19BBz^+$ T cells were able to significantly improve survival. These results indicate that 19BBz CAR gene transfer by combining the SB system, electroporation and co-culture with a L388 is a simple and straightforward method for inducing long term CAR expression and target specific cytotoxicity.

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Development of testing methodologies to detect residual host genomic DNA for lentivirus vector-based gene therapy products

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To ensure the safety of biological therapeutic products, the WHO has recommended that residual host genomic DNA levels be less than 10 ng/dose, meaning that the development and establishment of fast, sensitive, and reliable testing methodologies is necessary for the validation of lentivirus vector-based gene therapy products. When 293T cells are used as the cell substrate, the specific pieces of residual host genomic DNA that must be detected are the 18S rRNA gene of the most abundant genes and the genes for E1a and SV40 large T-antigen (LTA), both potential oncogenes. By utilising quantitative real-time polymerase chain reaction (qPCR), we developed and validated a test method that adheres to ICHQ2(R1) and the European Pharmacopoeia 8.0. Here, we show that the quantitation limit, the lowest concentration of residual 293T host genomic DNA where the calculated unknown is within ±2 folds of the associated standard value, was 5 pg, whereas the linearity of R2 for standard curve and the range were higher than 0.99 and 100 ng – 1 pg, respectively. Additionally, the detection limits of E1a and SV40 LTA, the lowest concentrations of host residual genomic DNA which demonstrate 95% positive cut-off, were 50 and 100 copies, respectively. Taken together, our results demonstrate that a qPCR-based technique can be employed for the rapid, accurate, and reliable detection of host residual genomic DNA of 293T cells in a highly gene-specific manner.

P129

The effect of Toll-like receptor 3 (TLR3) on regenerative properties of human periodontal ligament stem cells (PDLSCs)

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Periodontal ligament stem cells (PDLSCs) are somatic stem cells resided in periodontal ligament which connect the tooth root with alveolar bone. Toll-like receptor 3 (TLR3) play important roles in innate immune responses activated by double stranded RNA (dsRNA) released from viral pathogens. TLR3 has been known to regulate several immunomodulatory factors in several cell types. However, the effect of TLR3 activation on immunomodulation and differentiation capacity of PDLSCs has not been investigated. In this study, we investigated the role of TLR3 in immunomodulation and its role in osteogenic differentiation of PDLSCs. TLR3 signaling was stimulated in PDLSCs using TLR3 agonist, polyinosinic-polycytidylic acid [poly(I:C)]. The immunomodulatory expression levels including IFNγ, IDO, IL-6, COX-2 and HLA-G was evaluated. The osteogenic differentiation efficiency of PDLSCs was determined under poly(I:C) treatment. Our results showed that Poly(I:C) markedly promoted the expression of IFNγ, IDO, IL-6, COX-2 and HLA-G in PDLSCs. Upon osteogenic differentiation, poly(I:C) significantly increased the expression of osterix (OSX) and calcium deposition. mRNA expression level of IFNγ and IDO of differentiated cells were markedly higher than untreated cells. Moreover, the inhibition of TLR3 in TLR3-activated cells using siRNA suppressed OSX and WNT3A mRNA levels compared with the control. Our finding displays the advantages of poly(-I:C) on immunomodulation and osteogenic differentiation capability of PDLSCs. We suggested that activation of TLR3 may enhance the regenerative properties of PDLSCs which may raise the successful of stem cell therapy by increasing the osteogenic differentiation potential with retardation of host immune response.

P130

Promotion effects of LPA on the osteogenic differentiation of human umbilical cord blood-derived mesenchymal stem cells

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Lysophosphatidic acid (LPA) is a phospholipid derivative that can act as a signaling molecule to activate G-protein-coupled receptors known to regulate cell proliferation and migration. Recent experiments suggested that the Hippo-YAP/TAZ signaling pathway is a downstream target of LPA for regulating cell proliferation and migration. It has been shown that LPA inhibits LATS kinase, a major core-kinase of Hippo-YAP/TAZ pathway, resulting in up-regulation of YAP/TAZ transcription co-activator and its down-stream target genes expression. The novel role of Hippo-YAP/TAZ signaling pathway has recently been demonstrated in osteogenesis as the up-regulation of YAP promotes osteogenic differentiation. However, it is still unclear whether LPA could enhance oeteogenic differentiation through the activation of YAP/TAZ. In this study, we aim to determine the effect of LPA in osteogenic differentiation. Human umbilical cord blood-derived mesenchymal stem cells were harvested and cultured in osteogenic differentiation medium supplemented with LPA for inducing YAP/TAZ activity. We find that LPA significantly enhances osteogenic differentiation when compared with control as shown by more mineralized nodules formation and Alizarin red-S staining and expression of osteogenic differentiation markers. To confirm the effect of LPA, inhibition of YAP/TAZ activity by Dobutamine (DH) was served as a negative control. In contrast to LPA treatment, we find that DH diminished osteogenic differentiation. From these results, we proposed LPA as an activating molecule for osteogenic differentiation of human mesenchymal stem cell.

P131

Generation and transplantation of patient-specific iPSC-derived neuroepithelial stem cells for Machado-Joseph disease treatment

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Machado-Joseph disease (MJD) is a neurodegenerative disease caused by an expanded polyglutamine tract within the Ataxin-3 protein. This mutant Ataxin-3 protein causes neuronal degeneration in specific brain regions, such as the cerebellum. We previously demonstrated that murine neural stem cells (NSC) transplantation in MJD mice promote neuropathology and motor impairments improvement. Nevertheless, most available human NSC sources are associated with ethical and immunological problems, potentially overcome by induced-pluripotent stem cells (iPSC)-derived neural progenitors. The goal of this work was to evaluate whether it is possible to generate iPSC-derived neuroepithelial stem cells (NESC) from fibroblasts of MJD-patients, adequate for cerebellar transplantation. Human iPSC-derived NESC were obtained by reprogramming fibroblasts of Control and MJD-patients, with lentivirus encoding for Oct-4, Klf4, c-Myc and Sox-2, into iPSC, which were induced to NESC. The obtained cells upon *in vitro* differentiation originated heterogeneous cultures composed by cells positive for glial, neuronal, functional excitatory and inhibitory synapse markers and, by cells responding to potassium but not to histamine stimulation, consistent with functional neurons profile. Subsequently, the NESC were transplanted into the cerebellum of NOD/SCID mice. Two months after transplantation it was observed that cells survived, migrated out of the transplantation site and differentiated into neurons and glial cells. No major neuroinflammation was detected and a minor co-localization between graft-derived cells and cell death markers was observed. Overall, our results indicate that it is possible to generate iPSC-derived NESC from fibroblasts of MJD-patients with the potential to be used as a cell source for neuroregeneration and neuroprotection.

P132

Analyzing effect of process changes on gene expression in regulatory macrophages using RNAseq profiling

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Robust and reproducible process is fundamental for manufacturing cell therapy products. Accurate cell characterization is pivotal to standardizing production parameters and ensure consistency, because even small process changes can lead to shifts in cell function potentially affecting safety or efficacy. In this study, we used gene expression profiling to identify alterations in macrophage characteristics due to different manufacturing processes. Gene expression plasticity is central for macrophages' timely responses to cues from microenvironment permitting phenotypic adaptation from pro-inflammatory (M1) to wound healing and tissue-regenerative (M2). A distinct class of macrophages, regulatory macrophages (Mreg), dampen inflammatory responses and prevent immunopathology associated with prolonged inflammation. Immunomodulatory properties of Mregs make them an attractive candidate for cell-based therapies where immunosuppression might be therapeutically beneficial. Macrophages mature from monocytes and several manufacturing protocols, differing in medium, length of differentiation, cytokines and production vessel, are currently used. Generally, a limited set of markers is used to characterize the phenotype of manufactured Mregs or to assess their secretion profile. However, for a subset of markers gene expression levels varied with different production methods. To assess the global effect of manufacturing conditions on Mreg expression profiles, RNAseq analysis was performed. In parallel, comparator phenotype macrophages, M1, M0, and M2a, and a clinically relevant monocyte derivative, were produced from monocytes and their expression profiles were analyzed alongside. Using this approach a novel constellation of process specific biomarkers were identified. These data will support product development and define the critical quality attributes, relevant to the mode of action and safety.

P133

Enhanced generation of human embryonic stem cells with minimized trophoblast cell proliferation for clinical application

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Embryonic stem cells are unlimited source of cell that can be propagated and maintained in pluripotent state. They hold great promises for disease modeling, drug discovery and clinical application as a potential source of cells for tissue engineering and regenerative medicine. Many techniques including mechanical ICM isolation, laser dissection, and whole embryo culture have been used to derive hESC lines. However, the hESC derivation efficiency remains low, usually less than 50%, and it requires a large number of human embryos to derive a significant number of hESC lines. Due to a shortage of and restricted access to human embryos, a novel approach with better hESC derivation efficiency is badly needed to decrease the number of embryos used. We hypothesized that the low hESC derivation efficiency might be due to extensive proliferation of trophoblast (TE) cells which could interfere with ICM proliferation. We therefore developed a methodology to minimize TE cell proliferation by culturing ICM in a feeder-free system for 2-3 days before transferring them onto feeder cells. This minimized trophoblast cell proliferation (MTP) technique could be successfully used to derive hESCs from normal, abnormal, and frozen–thawed embryos with better derivation efficiency of more than 50% (range 50–100%; median 73%). This methodology can be effectively used to derive hESCs from both normal and abnormal embryos under feeder-free conditions with higher efficiency when compared with other methodologies. With this methodology, large-scale production of clinical-grade hESCs is feasible.

P134

Induction of myogenic differentiation in spheroids from oral mucosa derived mesenchymal stromal cells

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Availability of autologous cell sources with high myogenic potential remains one of the key problems of musculoskeletal disorders cell therapy. Aim of work was to study spontaneous myogenic differentiation of mesenchymal stromal cells (MSC) from alveolar mucosa (MSC-amc) and attached gingiva (MSC-ag) in 2D (monolayer) and 3D (spheroids) culture. In 2D culture MSC-ag were not able to differentiate spontaneously in myogenic direction, whereas in 40% of MSC-amc spontaneous differentiation took place at passage 3-4. Cells formed multinucleated myotubes and expressed MyoD. Expression of sarcomeric alfa-actin either was absent or a small amount of protein was distributed evenly in cytoplasm. In 3D culture MSC-ag and MSC-amc formed compact spheroids during the first 24 hours. We did not observe spontaneous myogenic differentiation in spheroids from MSC-ag. At the same time, in all spheroids obtained from MSC-amc by day 7 there were hallmarks of spontaneous myogenic differentiation with the formation of muscle tubes. These differentiated spheroids did not contain early progenitor cells, there was no expression of MyoD, but we observed not single myotubes but more differentiated well-formed myofibrils with characteristic nuclei peripheral arrangement and cross-striation, marked by antibodies against sarcomeric alfa-actin. Oral and gingival mucosa differ in ontogenetic origin and structure, which could determine differences in myogenic potential of cells from these two areas. In summary, 3D culturing of MSC-amc stimulates effective spontaneous myogenic differentiation with myotubes fusing into myofibrils. Spheroids from MSC-amc can become an alternative source of myogenic cells. The study was financially supported by Russian Science Foundation (grant #17-75-30066).

P135

Reduced activity of the complement system through elevation of complement factor I using AAV mediated gene delivery to the liver

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This study aims to down-regulate the complement system through AAV gene delivery to minimise inflammatory associated damage. The complement system is involved with clearance of pathogens and damaged cells and its dysregulation has been involved in the progression of pathological inflammation in disorders such as aged-related macular degeneration, glomerulopathies, haemolytic syndromes and autoimmune disease. Complement inhibition is therefore a potential treatment for a broad range of disorders. Factor I is a complement regulatory protein, produced and secreted by hepatocytes into the circulatory system. Its primary role is to down-regulate the complement system through breakdown of C3b, a protein central to multiple complement pathways. We aimed to identify the effect of Factor I over-expression in mice using AAV mediated gene therapy. We have shown that delivery of the murine factor I coding sequence to the liver enhances serum Factor I levels in mice by an average of 4-5-fold. *in vitro* functional assays have shown this induces a 50% reduction in complement activity. Vector efficacy was tested *in vivo* using a mouse model of ischemia reperfusion injury (IRI), however, prior delivery of the AAV-Factor I vector does not significantly protect from kidney tubular injury as indicated by creatinine and urea levels as well as microscopic assessment of renal architecture. The conclusion from these experiments is that elevated Factor I by AAV gene delivery down-regulates complement activity but is insufficient to prevent injury in this acute model of renal damage. Further models of chronic complement-mediated damage are to be explored.

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Therapeutic effect of mesenchymal stem cells derived from the human umbilical cord in a rabbit temporomandibular joint model of osteoarthritis

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Osteoarthritis (OA) is a common degenerative condition of the temporomandibular joint (TMJ) characterised by chronic inflammation and damage to the joint structures. However, owing to the complexity of TMJ-OA, only symptomatic treatments are currently available. Recent reports have shown that stem cells may play a role in exerting anti-inflammatory effects and in promoting defective tissue regeneration. Human mesenchymal stem cells (MSCs) were isolated from the umbilical cord matrix (UCM), a discarded tissue, after birth and have excellent potency of differentiation and proliferation. We investigated the cartilage regeneration and anti-inflammatory effects of human UCM-MSCs (hUCM-MSCs) for the treatment of TMJ-OA. After selecting a suitable cell line, we examined different therapeutic concentrations of hUCM-MSCs (1×105, 5×105 and 1×106 cells) for the treatment of monosodium iodoacetate-induced TMJ-OA in a rabbit model. The regenerative and anti-inflammatory effects of hUCM-MSCs were confirmed at all concentrations tested in the TMJ-OA model. The median dose of hUCM-MSCs showed the most prominent effect on cartilage regeneration via the upregulation of growth factors, extracellular matrix markers, anti-inflammation cytokines and reduction of pro-inflammatory cytokines. The anti-inflammatory effect of hUCM-MSCs was comparable with that of dexamethasone (DM). However, the DM has no cartilage regeneration effect on the model. In conclusion, our results suggest that hUCM-MSCs may be significantly effective, warranting a novel treatment option in treating human TMJ-OA in the future.

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Optimization of mesenchymal stromal cells culture in serum-free media using cell-free extracellular matrix biomaterial

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Multipotent mesenchymal stromal cells (MSC) obtained from various adult tissues showed a great promise for regenerative cell therapy. The translation of cell therapy approaches into clinical practice implies the abandonment of animal additives usage for the isolation and cultivation of cells, thus stimulating the manufacturers to develop serum-free media. Maintenance of culture growth requires to use supplements that mimic the microenvironment of cultured cells within the tissue in situ. One of the critical component of such microenvironment is extracellular matrix (ECM). We supposed that decellularized ECM produced by MSC could improve MSC culture in serum-free media. Our results showed that cultivation of human MSC in serum-containing media (specialized medium to support the growth of undifferentiated stromal cells AdvanceSTEM™ (HyClone, USA) or DMEM (HyClone, USA)) supported cell proliferation, stromal morphology, immunophenotype and ability to differentiate into osteogenic, chondrogenic and adipogenic directions. The transition to serum-free media (NutriStem®, (BioIndustries, Israel) and STEMPRO® MSC SFM (Thermo Fisher Scientific, USA)) could significantly increase the rate of MSC proliferation, but these media were not suitable for the effective isolation of primary MSC from adipose tissue. Using our developed protocol to produce decellularized biomaterial based on ECM secreted by MSC cell sheets we applied it as a substrate for MSC cultured in serum-free media and significantly improved the effectiveness of isolation and culture, preserving cell morphology and proliferation rate as well as preventing cell senescence.

P138

Influence of mesenchymal stem cell-derived microvesicles on skin regeneration

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Regenerative potential of adipose derived stem cells (ADSCs) cytochalasin B induced microvesicles (CIMV) was studied using mouse skin photoaging model. CIMV are able to interact with tissues and cells *in vivo*, stimulate angiogenesis and can be used in the treatment of skin lesions. White mice were divided into groups (intact, UV and UV+CIMV). UV and UV+ CIMV groups were regularly subjected to UV-irradiation for 6 weeks. CIMV were administered 6 weeks later intradermally in an amount equal to the biomass of 1 million ADSCs in 500 μl of PBS. Four weeks later, density of the capillary network was assessed using Easy-LDI laser Doppler and dorsal skin was examined histologically. In the UV+ CIMV group, blood microcirculation increased compared to the control (UV-group 111.52 ± 14.56 apu and UV+CIMV group 146.73 ± 10.66 apu). Furthermore, in UV+CIMV group, the thickness of the epidermis increased in comparison with UV-group (UV-group 25.24 ± 7.66 μm, UV+CIMV group 28.95 ± 13.53 μm). The thickness of the dermis in the UV-group increased in comparison with the intact animals, as well as in UV+CIMV group compared to UV-group (intact 242.73 ± 41.45 μm, UV-group 329.85 ± 48.68 μm, UV+CIMV group 456.06 ± 71.34 μm). Thus, the use of CIMV promotes skin regeneration and can represent a promising strategy for developing a new acellular therapeutic approach that could overcome the barriers and risks associated with the use of live stem cells.

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Cell mediated gene therapy for neurodegenerative diseases, neurotrauma and stroke

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Limited regeneration in the CNS and a lack of available and effective methods for stimulation of neuroregeneration propose searching for the strategies designed to overcome the neural cells death at pathologic conditions, such as neurodegenerative diseases, neurotrauma and stroke. Earlier, we hypothesized that cell-mediated triple gene therapy based on umbilical cord blood mononuclear cells (UCB-MC) transduced with adenoviral vectors carrying vascular endothelial growth factor (VEGF), glial cell line-derived neurotrophic factor (GDNF) and neural cell adhesion molecule (NCAM) is the promising therapeutic approach for increasing survivability of affected neurons. In this gene-cell construct the UCB-MC are cells with a highly prominent natural secretory potential serve as «bioreactors» for production of the therapeutic molecules (VEGF, GDNF and NCAM) with well-known neuroprotective function. The role of VEGF in preventing the consequences of neurodegeneration may be beneficial for restoring the microcirculation as well. The recombinant NCAM helps UCB-MC to survive and migrate to the site of neurodegeneration. Importantly, cell-mediated gene delivery makes the viral antigens inside the *ex vivo* transduced UCB-MC invisible to the recipient immune system. In animal models of amyotrophic lateral sclerosis, spinal cord injury and ischemic stroke we obtained the evidence of the therapeutic efficacy of genetically engineered UCB-MC overexpressing VEGF, GDNF and NCAM.

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In vivo enhanced anti-inflammatory effects of human mesenchymal stromal cells transfected with CXCR4 and IL10 mRNAs

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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 based on 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 (2G-MSC) by means of the transient expression of CXCR4 and IL10 using monocistronic and bicistronic mRNAs. 2G-MSCs maintained the typical immunophenotype and differentiation capacity of MSCs. Functional *in vitro* assays showed an increased migration capacity and improved immunosuppression as compared to unmodified MSCs. Moreover, *in vivo* experiments demonstrated the increased anti-inflammatory potential of 2G-MSCs, revealed by a reduction in both the local inflammation and leukocytes infiltration in a LPS-induced inflamed pad mouse model. Biodistribution assays showed the presence of MSCs in these target inflamed pads, revealing an improvement in site-directed migration of 2G-MSCs. Taken together, our results demonstrate that the transient expression of homing and anti-inflammatory molecules enhances the therapeutic effect of Ad-MSCs in an inflammatory mouse model.

P141

Baboon retrovirus envelope pseudotyped lentivectors permit robust transduction of NK cells and represent and improved tool for cancer immunotherapy

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Natural killer cells (NKs), with their intrinsic ability to recognize and kill tumor cells represent an interesting tool for immunotherapy, even in an allogenic setting. Although infusions of activated NKs are promising for immunotherapy, the genetic modification of NKs could strongly improve their functions. However, NKs are quite resistant to transduction with classical VSVG pseudotyped lentivectors (LVs). We hypothesized that alternative lentiviral pseudotypes might result in more efficient transduction. Freshly isolated NKs or obtained from the NK Activation and Expansion System (NKAES) were transduced with a GFP-reporter gene using different lentiviral envelopes: baboon endogenous retrovirus (BaEV-), RD114-, MV-LV (LVs pseudotyped with measles virus envelope) and VSV-G-LVs. NKs were expanded using the NKAES system for 14-21 days and transduction was assessed. BaEV-, RD114- and MV-receptor expressions were evaluated by RT-PCR and flow cytometry under different stimulations. VSV-G-LVs resulted in poor transduction rate of freshly isolated NKs (0,28 ± 0,12%) while RD114- and MV-LVs performed better (21 ± 2,8% and 13 ± 4,7%, respectively). The use of BaEV-LVs outperformed them all with a transduction rate mean of 30 ± 2,2% in freshly isolated NKs and 87 ± 6.7% in NKAES, even at low vector doses. BaEV-LV transduction efficacy could be attributed to the BaEV receptors' expression patterns. NKs were transduced with BaEV-LVs encoding chimeric antigen receptors (CAR) against CD22 or GD2. Sorted and re-expanded transduced NKs kept their CAR-expression and retained their cytotoxic functions against cancer cells. In summary, these BaEV-LVs represent a NK-transduction technique that will allow the development of efficient NK-based immunotherapies such as CAR-NK cells or enhanced NKs.

P142

GMP-grade production of tIPE, a cell-based gene therapy product to treat neovascular age-related macular degeneration (nvAMD) developed in the TargetAMD project

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Transferring Gene Therapy Medicinal Products (GTMP) from laboratory to GMP-compliant manufacturing is critical for implementation of advanced therapies. In preparation for a phase Ib/IIa clinical trial for the treatment of nvAMD, the pre-clinical studies for the cell based GTMP, tIPE, have been completed and GMP-validation is ongoing. In an 1 hour-long procedure, autologous IPE cells will be isolated, transfected with the gene encoding pigment epithelium-derived factor using the non-viral SB100X transposon system cloned in plasmids free of antibiotic resistance markers and transplanted subretinally. The Swiss regulatory authority, Swissmedic, defined the PEDF-transfected IPE cells as the GTMP, which must be produced under GMP guidelines; due to product-inherent limitations for in-process quality controls, Swissmedic requested that validation is realised by completing ≥80 tIPE productions from human donor eyes meeting the specifications. These were defined as: ≤0 CFU microorganisms, ≤2 EU/ml endotoxins, ≥70% viable cells, ≥80% DNA delivered, ≥5,000 isolated cells, ≤5 min transport time from GMP laboratory to operating room and 0-30°C temperature during transport. In parallel to product quality, the process, equipment (Cliniporator model T-AMD and microcuvettes), transport and personnel are validated/qualified for tIPE manufacturing under conditions simulating the proposed clinical trial and meeting the specifications of sterility (≤0 CFU microorganisms), cell viability (98.71 ± 0.01%), DNA delivered (80%), number of cells isolated (22,500 ± 7,500), transport time (2 min) and temperature during transport. The GTMP manufactured under GMP guidelines so far confirm that the tIPE meets the specifications and therefore, is ready to be administered to patients once clinical trial approval is obtained.

P143

Analysis of the interaction of mesenchymal stem cells with artificial human neuroblastoma cell-derived microvesicles using imaging flow cytometry

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Currently there is an increasing interest in studying the role of extracellular vesicles (EVs), and in particular microvesicles (MVs), acting as multi-signal messengers of the tumor stroma, in tumor development and progression. Tumor cell-derived EVs are considered as a promising vector for targeted delivery of antitumor agents due to tropism and ability to release their contents into the cytoplasm of recipient cells. Mesenchymal stem cells (MSCs) are known to have tropism toward tumor niches. Presumably, with the accumulation of MSCs at tumor sites, these cells differentiate into pericytes or tumor-associated fibroblasts, thereby forming a supporting tumor growth microenvironment. However, besides the ability to promote tumor progression, MSCs can also suppress tumor growth by inhibiting of proliferation and cell cycle progression. We studied interaction of neuroblastoma SH-SY5Y cytochalasin B induced MVs (CIMVs) with human bone marrow-derived MSCs using Imagestream X Mark II (Amnis-EMD-Millipore) imaging flow cytometry. MSCs and SH-SY5Y-CIMVs were stained with DiO and DiD fluorescent dyes, respectively, and were co-cultured for 24 hours. It was shown that 6% of MSCs in the single cell suspension contained a large number of SH-SY5Y-CIMVs per cell, while 42% of MSCs contained a few SH-SY5Y-CIMVs per cell. Thus we demonstrated internalization of SH-SY5Y-CIMVs by MSCs, however, further studies are required to determine the effect of SH-SY5Y-CIMVs on pro- or anti-oncogenic phenotypes and function of MSCs.

P144

Comparison of scale down model performance to at-scale to facilitate biopharmaceutical process development

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Cell-based therapies have the potential to transform the treatment of challenging diseases, such as cancer, and bring about a new era of medicine to address currently unmet patient care needs. Effective manufacture of these products is crucial to fully realise this potential. Scale down of the at-scale operation allows for the identification of critical process parameters and supports process development, characterisation, optimisation and validation of the process, avoiding the limitations of conducting experimental studies at-scale. We have developed a scale down model of the in-house at-scale T cell manufacturing process. In order to demonstrate this model as an appropriate representation of the at-scale manufacturing process, a comparison of model performance to at-scale was performed using T cells taken at a range of time points throughout each process. Key cell characteristics were assessed, including; cell growth dynamics, immunophenotype and function, the processes. Cells from both processes exhibit equivalent viability, growth dynamics and expansion capacity, indicating preliminary trends in growth dynamics between the two processes. Here we have shown the development of our scale down process to model the at-scale manufacturing process. In particular, key areas where the scale down process most accurately models the at-scale product quality attributes, and potential limitations, were highlighted in order to demonstrate the appropriateness and applicability of the model as a tool for developing and characterizing the at-scale manufacturing process.

P145

Development of novel antifibrotic therapy based on human MSC secretome

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Mesenchymal stromal cell (MSC)-based therapy was able to inhibit fibrosis in different tissues, and this effect was mostly mediated by MSC secreted factors. We aimed to develop a novel antifibrotic drug, which included components of MSC secretome - extracellular vesicles (EV) or soluble factors (SF). The fractions enriched by EV or SF were isolated from 2 days conditioned medium (CM) of human MSC (hTERT MSC, ATCC) or human dermal fibroblasts by ultrafiltration. The model of

TGFβ-induced differentiation of fibroblasts to myofibroblasts was used to study the antifibrotic activity. The effects of MSC-CM fractions were analyzed after 4 days of fibroblast incubation with EV or SF by expression of α-smooth muscle actin (αSMA) and collagen type I, vinculin redistribution and collagen gel contraction assay. We showed that MSC-EV fraction inhibited the differentiation of fibroblasts into myofibroblasts and induced dedifferentiation of myofibroblasts on morphological and functional levels. We also found that MSC-SF fraction had similar, but less stable and reproducible effects. In the range of different concentrations (up to 50x) 5x and 10x concentrations of both fractions were the most effective. By contrast to MSC, fibroblasts secretome fractions didn't demonstrate antifibrotic activity indicating the specificity of MSC secretome effects. Taken together, different components of MSC secretome could be used as potential therapy for fibrotic diseases. Our data indicate that MSC-EV fraction is more promising for drug development. The study was funded by RFBR grant #18-015-00525 and conducted using biomaterial collected under RSF grant #14-50-00029.

P146

Membrane vesicles as biocompatible vectors for bioactive molecules and drugs delivery

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There is a trend in regenerative medicine from cell to cell-free therapy based on extracellular membrane vesicles (MVs). MVs mediate intercellular communication and deliver bioactive molecules such as cytokines, chemokines, growth factors, mRNA, miRNAs and siRNA. Cytochalasin B-induced membrane vesicles (CIMVs) were shown to deliver fluorescent dye to recipient cells (Mao et al., 2011), as well as an antitumor drug (doxorubicin) *in vivo* (Peng et al., 2015). However, factors affecting efficiency of substances delivery by CIMVs to recipient cells has not been investigated. The aim of our work was to evaluate the impact of concentration of loaded drug and amount of applied CIMVs on the efficiency of delivery to target cells. CIMVs were obtained from HEK293FT donor cells and stained with CFDA SE dye (2.5μM or 10μM). HEK293FT recipient cells were treated with CIMVs at four different concentrations (15.62μg/ml, 62.5μg/ml, 125μg/ml, 250 μg/ml). The percentage of CFDA-positive recipient cells (CFDA+ cells) was determined by flow cytometry. We determined that efficiency of substances delivery by CIMVs depends on the amount of applied CIMVs to recipient cells. CIMVs (loaded with 10μM CFDA SE) delivered the dye dose-dependently to recipient cells (36.1 ± 4.4; 50.8 ± 3.9; 73.3 ± 7.74; 89.81 ± 0.6% CFDA+ cells). We also found that efficiency of substances delivery by CIMVs is directly proportional to the concentration of loaded substances (fluorescent dye CFDA SE).

P147

Influence of magnetic nanoparticles stabilized with polyelectrolytes on 2D and 3D cell cultures formation

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In this work 2D and 3D cell cultures were obtained from cells modified with magnetic nanoparticles stabilized with various polyelectrolytes. In our work, we: cultivated A549 cells and formed from them 3D spheroids (by the ''hanging drop'' method); synthesized magnetic nanoparticles (by the method of reducing iron salts) and stabilized them with various polyelectrolytes: PAH (Poly(allylamine hydrochloride), 15 и 70 kDa), PDADMAC (Poly(diallyldimethylammonium chloride), PEI (Poly(ethyleneimine)) («layer-by-layer» method). The A549 cells were functionalized with the resulting magnetic nanoparticles previously stabilized with polycations. In addition, cell samples were analyzed using different types of microscopy (light, dark-field, confocal, atomic force etc.), as well as the measurement of charge and size of magnetic nanoparticles by dynamic light scattering and the electrophoretic mobility of particles using the Doppler effect was done. We obtained the 2D and the 3D cell cultures from A549 cells functionalized with magnetic nanoparticles. The charge of magnetic nanoparticles after stabilization by polyelectrolytes has changed from negative (-24.5 mV) to positive (PAH 15 kDa: +58.9; PAH 70 kDa: +67; PDADMAC 20%: +55; PDADMAC 35%: +19; PEI: +31.5 mV). We also obtained images by different types of microscopy for analyzing the distribution of nanoparticles inside the 2D and 3D cell cultures. The obtained magnetic nanoparticles can become a promising material for use in biomedical research and tissue engineering, as well as for drug delivery. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and funded by RFBR according to the research project No 18-34-00306.

P148

Gene modification of fibroblasts with FGF2 increases the efficiency of the cell therapy of thermal skin burn in rabbits

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Skin burn is one of the biggest problem in veterinary medicine. The treatment of skin burn using cultured fibroblasts is considered promising approach. Gene modification of fibroblasts is used to enhance their regenerative potential and represent the next step in tissue engineering of the skin. In our study, we cultured and genetically modified dermal fibroblasts (DFs) to express human fibroblast growth factor (FGF2). DF-FGF2 were used to stimulate regeneration in an experimental model of skin burn (third degree) in rabbits. DFs were obtained by explantation from biopsy specimens of rabbit skin. After that DFs were transduced with recombinant lentivirus carrying FGF2 cDNA. Animals were divided into 2 groups (control and experimental). Native or genetically modified fibroblasts (1000000 cells/animal) were applied in 1ml of Tissucol (fibrin glue). In the control group final healing and scar formation occurred on the 24th-25th day of the study, and in the experimental group on 22nd-23rd day. Despite the minimal time differences in epithelialization of wounds in both groups, histological studies showed that the skin of rabbits from experimental group was at a later stage of repair compared to control group. Our results demonstrated more effective burn wound regenerated using genetically modified DFs compared to native fibroblasts.

P149

"Smart" nanostructured scaffolds for osteoregeneration in veterinary

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Background Modern advances in stem cell biology are opening the way for new approaches to the treatment of a number of diseases, including cardiovascular diseases, neurodegenerative diseases, musculoskeletal diseases, diabetes and cancer. These areas, involving a personalized approach to therapy and surgery, may include cellular substitution therapy, drug treatment, or a combination thereof. The main idea is to stimulate the body's own regenerative abilities, by promoting survival, proper migration, proliferation and differentiation of endogenous progenitor stem cells. Cells can be introduced from the outside to increase the pool of progenitor cells. Results Presented study describes the use of ''smart'' nanostructured scaffolds based on chitosan, agarose and gelatin with and without halloysite clay nanotubes, to restore bone defects. We evaluated the possibility of adhesion, proliferation and differentiation of stem cells on matrices using the different microscopy methods. In addition, distribution of cells in the matrix and the adhesive properties of functional composites were studied. Biopolymer scaffolds in combination with stem cells were suitable for the regeneration of bone in dogs together with standard surgery. The absence of inflammatory reactions on biopolymer matrices and allogenic stem cells was revealed even in the case of an animal prone to inflammatory and allergic reactions with the standard method of fracture regeneration. Positive dynamics in the fusion of even chronic bone defects with biopolymer matrices in combination with mesenchimal stem cells was noted. Acknowledgments: the work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and RFBR 18-415-160010 grant.

P150

Optimization of transfection conditions and reagents to improve the transfection efficiency of dCas9-Activator plasmid into human cells

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Activation of endogenic master transcription factor genes is one of the strategies to reprogram a cell type into another one. Deactivated Cas9 (dCas9) linked activator complexes have been demonstrated to be used for this purposes. Transient expression of dCas9-activator bearing vectors is enough to drive cell reprogramming. It is because the activated endogenic master transcription factor genes usually positively regulate the expression of themselves along with their target genes. Hence, instead of viral vectors, mammalian expression plasmids are widely used for expression of dCas9-activator complexes. However unlike viral vectors, expression plasmids can be transferred into the cells either through electroporation or transfection reagents. The overall efficiency of cellular reprogramming primarily depends on the success of plasmid transfection efficiency into the host cells. In this study we compared the different factors to optimize the transfection protocol for dCas9-VP64-GFP bearing plasmid which will be used in our further reprogramming studies. To begin with we compared the efficiency of two most common cationic lipid based transfection reagents to transfer the plasmids into HEK cells by following the manufacturer's protocols. Then we determined the most suitable plasmid concentration to reach out the highest amount of transfected cell number with less toxicity. Different serum free buffers were also compared to optimize the plasmid/transfection reagent complex ligation. Finally and most importantly we compared the effect of pH during preparation of transfection mixture. As the result here we demonstrated the most suitable conditions and reagents for transfection of dCas9-Activator plasmids into HEK cells for efficient cell reprogramming.

P151

Micellar formulation of methylprednisolone succinate for local therapy of spinal cord injury in rats

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Glucocorticoids therapy is a common approach in acute spinal cord injury (SCI) treatment. Carboxylated trifunctional block copolymer (TBC) of ethylene oxide and propylene oxide was previously identified as an effective penetration enhancer in neuronal tissues and cells. In situ self-assembled nanoformulation of the TBC with methylprednisolone succinate (MPS) was recently developed. The formulation is based on uniform mixed micelles (TBC-MPS) with a size <20 nm, which are characterized by increased MPS/TBC ratio and intracellular uptake. The application of the following agents based on carboxymethyl cellulose (CMC): CMC-MPS (15,6 mg \ ml) and TBC-MPS (2.5:15,6 mg \ ml) and infusion of MPS (30 mg/kg) were applied within 3 hours after SCI. Epidural electrical stimulation (L2) with EMG of motor evoked potentials (MEPs) of m. soleus and m. gastrocnemius was conducted in all groups. MPS infusion had a inhibitory effect on the early component of MEPs related to activation of the motor axons. The CMC-MPS application showed effects similar to MPS infusion, indicating the lack of the effect of CMC alone. The administration of TBC-MPS in contrast to MPS resulted in facilitation of early and middle components of MEPs, presumably as a result of delivery of MPS to the spinal cord neurons and fibers and masking negative effect of the MPS. Our results suggest that local MPS therapy of SCI by application of TBC-MPS had a facilitation effect. To clarify TBC mechanisms of action further investigation is demanded. Supported by RFBR, research project No 18-315-00267

P152

Analysis of secretome of umbilical cord blood mononuclear cells after gene modification

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Stem cell-based gene therapy is a novel therapeutic approach for treatment of various human diseases. Therapeutic effect of transplanted cells is believed to be associated with secretion of various pro- and anti-inflammatory cytokines and anti-apoptotic factors. However, little is known about secretome of genetically modified stem cells, which is essential for their therapeutic application. We analyzed changes in secretome profile of human umbilical cord blood mononuclear cells (UCB-MC) which were genetically modified (GM) using recombinant adenoviruses (Ad5-FGF2, Ad5-VEGF и Ad5-SDF1) encoding various pro-angiogenic factors. Un-transduced and Ad5-EGFP transduced UCB-MC served as controls. Analysis of released cytokines, chemokines and growth factors in supernatants of GM cells was performed using xMAP Luminex Technology. Similar secretome profile was demonstrated in untreated and genetically modified UCB-MC and characterized by wide range of constitutively expressed interleukins, growth factors, eotaxin, G-CSF, GM-CSF, IFN-g, IP-10, MCP-1, MIP-1a, PDGFbb, MIP-1b, RANTES, TNF-a, CTACK, GROa, HGF, IFN-a2, LIF, MCP-3, M-CSF, MIF, MIG, SCF, SCGF-b, TNF-b, TRAIL. UCB-MC simultaneously transduced with Ad5-FGF2, Ad5-VEGF or Ad5-SDF1 showed expression of encoded recombinant proteins. Increased production of IL-8, IL-12, FGF-2, GM-CSF, IP-10, IL-2Ra, IL-3, IL-16, IL-18, CTACK, GROa, IFN-a2, LIF, MCP-3, MIF, b-NGF, TRAIL was found which was higher than in controls. Our results demonstrate that GM UCB-MC with adenoviruses increases expression of pro-angiogenic factors and induces secretion of anti/pro inflammatory mediators. Thus, application of various genes and their combinations modulates secretome profile of stem cells.

P153

Therapeutic potential of iPSCs derived from a patient with mitochondria disease

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POLG plays an important role in mtDNA replication and proof leading and its mutations are related to the development of mtDNA defects. We induced iPSCs from a paternally inherited heterozygous POLG mutation family; two parents, and three children including a patient carrying 4,395 bp mtDNA deletion (mt8921-mt13316) with 24% heteroplasmy in blood, diagnosed as Pearson syndrome. iPSCs lines were generated from skin fibroblasts of the patient and his family and analyzed mtDNA deletion and point mutation using quantified PCR and Miseq, respectively. Three iPSCs lines out of 13 were free of the deletion and heteroplasmy levels of the remaining 10 iPSCs lines ranged from 7% to 53% in the patient. Whereas no iPSCs lines from the other family members carried the mtDNA deletion. The heteroplasmy level was gradually decreased during *in vitro* culture. The number of mtDNA point mutations in iPSCs was similar regardless of the mutant POLG, however, was higher in the parents than the children. Our results suggested that the culture of iPSCs can eliminate mtDNA deletion and the mutant POLG does not affect point mutation in iPSCs. Thus, iPSCs derived from a Pearson syndrome patient can be a useful source for autologous stem cell therapy.

P154

The study of spectroscopic signature of human pluripotent stem cell-derived hepatocytes using synchroton FTIR

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Human pluripotent stem cells (hPSCs), such as human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), hold great promise for regenerative medicine due to their unique properties of self-renewal and multilineage differentiation. Although various methods have been used to induce the hepatic differentiation of hPSCs, those hPSC-derived hepatocyte-like cells generally lack important metabolic functions of mature hepatocytes and therefore unsuitable for most research and clinical applications. To improve the efficiency of hepatic differentiation, fast and extensive characterization of hPSC-derived hepatocytes obtained from various culture systems. The present study demonstrated that the FTIR microspectroscopic analysis was superior than other conventional assays, such as morphological examination and detection of hepatic gene and protein expression, for characterizing hPSC-derived hepatocyte-like cells. This technique can also be used to identify and distinguish various stages of hepatic differentiation of hPSCs which other conventional assays failed to accomplish. We therefore believe that the FTIR microspectroscopy can effectively be used to compare the efficiency of various hepatic induction protocols and lead to the establishment of a better procedure for generating hPSC-derived hepatocytes in the future.

P155

Ultrastructure of human adipose-derived multipotent mesenchymal stromal cells loaded with synthetic microcapsules

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Cell-based targeted drug delivery has potential to transfer encapsulated bioactive substances to specific organs and tissues.

Human adipose-derived multipotent mesenchymal stromal cells (hAMMSCs) represent an appropriate type of potential cargo cells due to their phagocytic function. Microcapsules (2 microns) were fabricated using alternating adsorption layers of polyelectrolytes onto calcium carbonate micro-particles and capsule shells were coated with BSA. hAMMSCs were isolated from healthy subjects, cultured and incubated with plain FITC-labeled microcapsules in RPMI at various ratios for 16 hours at 37°C. After incubation, the cells were further cultured for 72 hours and then analyzed with transmission electron microscopy. Control untreated hAMMSCs had elongated shape and intact plasma membrane with filopodia and blebs. The cells had granular cytoplasm that contained mitochondria, endoplasmic reticulum, lipid droplets, vacuoles of various size and large nuclei with a wrinkled surface and invaginations. hAMMSCs incubated with microcapsules at a 1:10 ratio, demonstrated minimal ultrastructural changes relative to control despite uptake of 1-5 microcapsules per cell. hAMMSCs incubated with microcapsules at a 1:45 ratio were stuffed with intact or damaged microcapsules. These cells had irregular shape, damaged plasma membrane, degranulated cytoplasm, condensed nuclei and swollen mitochondria with ruptured cristae. Thus, the ultrastructural integrity of hAMMSCs depended on the number of microcapsules internalized, where a mixture at the 1:10 cell/capsule ratio being optimal for cell loading.

P156

Rapid high sensitivity detection of extra-cellular vesicles by PATfix™ HPLC equipped with multi-angle light scattering (MALS)

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1: BIA Separations d.o.o.

Chromatographic detection of extracellular vesicles (EVs) in biological samples is challenging because they are present in very small concentrations in comparison to contaminants. Detection by UV absorbance is so heavily dominated by proteins and DNA that vesicle populations cannot be discriminated without subsequent off-line testing of fractions. This poster shows several examples in which MALS, coupled to an analytical HPLC system enables sensitive on-the-fly detection of vesicles separated by a hydrogen bonding monolith.

P157

Chromosome transplantation to correct the chronic granulomatous disease defect in mouse pluripotent stem cells

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In spite of the progress in gene editing achieved in recent years, a subset of genetic diseases involving structural chromosome abnormalities, including aneuploidies, large deletions and complex rearrangements, cannot be treated with conventional gene therapy approaches. We have previously conceived the Chromosomal Transplantation (CT) strategy to replace an endogenous mutated chromosome with an exogenous normal one. As a proof of principle, to test the feasibility of this approach, we focused on Chronic Granulomatous Disease (CGD), a severe X-linked immunodeficiency due to abnormalities in CYBB (GP91) gene leading to alteration of granulocytes function, that in 10 to 15% of patients is due to large deletions. Here we show that the gene defect can be corrected by CT in induced pluripotent stem cells (iPSCs) from a CGD male mouse model. The Hprt gene of the endogenous defective X chromosome was inactivated by CRISPR/Cas9 technology and a normal donor X chromosome was introduced by microcell-mediated chromosome transfer (MMCT), exploiting the HAT selection system. X-transplanted clones (XXY) were obtained, and diploid XY clones, which have spontaneously lost the endogenous X chromosome, were isolated. These cells were differentiated toward the myeloid lineage, and functional granulocytes producing the normal GP91 protein were obtained. Therefore, we propose the CT approach as innovative strategy to correct cells from patients affected by X-linked diseases with large deletions, such as the Duchenne Dystrophy, or complex mutations, such as Fragile X disease, for which available treatment is still unsatisfactory.

P158

Development of advanced *in vitro* assays for biological products to detect adventitious bovine viruses utilizing TCID50 and next-generation sequencing

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In order to control viral contamination during the GMP manufacture of biological products using animal-sourced raw materials, it is necessary to develop advanced, reliable, and highly sensitive *in vitro* assays to detect potential adventitious contaminants. This is because biological products are manufactured in cells and any introduction of adventitious agents during contaminates the products. For products manufactured using bovine-sourced materials in Vero and BT cell lines, the detection limit of *in vitro* assays for bovine herpesvirus, reovirus-3, bovine parainfluenza, and bovine viral diarrhoea virus is 1 TCID50. However, broadness is as important a factor as sensitivity and reliability for these screens so that unexpected adventitious viral agents are able to be identified, as vesiviruses, polyomaviruses, circoviruses, epizootic haemorrhagic disease virus, bluetongue virus, bornaviruses, and Cache Valley virus, in addition to the viruses above, have been detected and identified as viral contaminants derived from bovine serum since 2003. Here, we describe using next generation sequencing (NGS) with deep coverage to augment conventional screens by addressing the detection limits of and the potential detection of unknown contaminants by currently-used methods, which was validated experimentally using bovine herpesvirus only or along with spikes of human cytomegalovirus and Kaposi's sarcoma-associated herpesvirus in 293T master cell banks. Our results support the notion that NGS-based detection assays can be used as a complementary tool for identifying potential adventitious agents, with the high sensitivity of current screens and the broad method shown here combining to detect and identify known and unknown viral contaminants in various biological products.

P159

Immunoexpression of PSD95 and synaptophysin in the motononeurons of the lumbosacral spinal segments of mouse during experimental hypogravity

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The study of the adaptive and compensatory reorganisation of motor system in condition of weightlessness is a critical problem of space biology. In this work we investigated the level of functional activity of motoneurons in the lumbosacral spinal cord of the mouse after simulating of hypogravity on the model of antiorthostatic hanging of the hindlimb. The experiments were performed on 10 mature male mice of c57black/6 line with weight 28 ± 8g. The level of functional activity of spinal neurons was evaluated based on degree of expression of the marker proteins: synaptophysin on the presynaptic membrane and the PSD95 on the postsynaptic membrane. The analysis of the immunohistochemical reaction against these two marker proteins demonstrated a decrease in the level of immune expression of both proteins in motoneuronal bodies in the experimental animals after 30 days of antiorthostatic hanging. At the same time, the immune expression of PSD95 was decreased more significantly compare to immune expression of synaptophysin (decreased by 36% vs. by 25%) from the level of immune expression of these proteins in the mice of the control group. Thus, these results support that the development of the motor dysfunctions during hypogravity can be associated with changes in the motoneurons of the lumbar spinal cord. This work was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities, No 17.9783.2017/8.9.

P160

Decellularized extracellular matrix of human mesenchymal stromal cells as a novel biomaterial for regenerative medicine

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Multiple bioactive products secreted by stem and progenitor cells, in particular extracellular matrix (ECM) proteins, are actively involved in the modulation of tissue repair and regeneration. Mesenchymal stromal cells (MSC), a promising source of multipotent adult stem and progenitor cells for cell therapy and tissue engineering, produce a wide range of bioactive molecules including ECM components thus regulating the regeneration processes. The aim of our study was to develop a novel biomaterial based on decellularized ECM produced by human MSC cultured in cell sheets. Several agents for decellularization were selected based on the conservation of ECM structure and effectiveness of DNA removal: CHAPS, sodium deoxycholate, DNAse I and apoptosis inductor (rotenone). Optimal protocols for decellularization of ECM produced by MSC (immortalized human MSC, ATCC) cell sheets were developed. It was shown by different methods that the structure of obtained biomaterial was meshy and branched. Structural ECM proteins (collagen I type, fibronectin and laminin) were preserved after decellularization. The viability and proliferation of MSC and human umbilical vein endothelial cells (HUVEC) cultured on decellularized biomaterial were retained, and cells aligned the biomaterial and remodeled it. CFU test showed that MSC formed denser colonies on decellularized biomaterial compared to plastic. Our results demonstrate the biological activity of ECM components produced by MSC and provide the basis for developing biomedical cell-free products for regenerative medicine. The study was funded by the Russian Ministry of Education and Science grant #MK-2422.2017.7 and conducted using biomaterial collected under RSF grant #14-50-00029.

P161

How to find the right dose for successful transition of ATMPs from the nonclinical to the clinical development stage

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Transition from nonclinical to clinical stage is an important step in drug development. Finding the right dose for the first in human study can be a challenging undertaking for biopharmaceuticals. For Advanced Therapy Medicinal Products (ATMPs) this is even more challenging, as candidates of this product category rarely follow common principles such absorption, distribution, metabolization and excretion. Moreover, a classical dose-response relationship does not apply. For example, cell-based products can proliferate, differentiate and even persist lifelong in a different phenotype than initially administered. Furthermore, virus-based ATMPs such as oncolytic viruses can replicate in target tissues. With this even Paracelsus' toxicology principle ''only the dose makes the poison'' is in question, which was applicable for more than five centuries. As the ''acting dose'' can be very different from the administered dose the dose finding exercise for ATMPs is often about finding answers to the question: How to dose a living drug? Based on real-life case studies, this contribution will address how dosing approaches can be established and which key questions need to be answered for a tailored dose-finding exercise.

P163

Assessment of bystander effects of mesenchymal stem cells carrying a cytosine deaminase suicide gene on glioma cells

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Glioblastoma multiform (GBM) is the most severe cancer in the nervous system. Despite the progress has been made in treating GBM, the most effective therapy including combination of chemotherapy and radiotherapy has remained as a palliative cure. Recently, stem cells carrying suicide gene have emerged as the therapeutic candidates for the bystander effects on

neighboring cancer cells while limiting cytotoxicity to other cells. Here, we report a method to accurately measure the bystander effects in co-culture system. Stable transduction of reporter genes such as green fluorescent protein (GFP) did not alter the sensitivity of glioma cells to 5-fluorouracil, product converted from a nontoxic prodrug, 5-fluorocytosine (5-FC) by a suicide gene, cytosine deaminase (CD). Unlike conventional mitochondrial enzyme-based assays, image analysis of fluorescence signals from GFP-labeled glioma cells allowed separation of surviving glioma cells from co-cultured therapeutic stem cells expressing CD. The results indicate that our experimental approach to determine the bystander effect of therapeutic stem cells against glioma cells in the presence of 5-FC. Furthermore, our method can be expanded as an *in vitro* assay for screening anti-cancer drugs or for determining effectiveness of candidate drugs before advancing into animal studies.

P164

Differentiation of non-human primate pluripotent stem cells into functional keratinocytes

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Epidermal grafting using cells derived from pluripotent stem cells will change the face of this side of regenerative cutaneous medicine. To date, the safety of the graft would be the major unmet deal in order to implement long term skin grafting. In this context, experiments on large animals appear unavoidable to assess this question and possible rejection. Cellular tools for large animal models should be constructed. In this study, we have generated monkey pluripotent stem cells-derived keratinocytes and evaluated their capacities to reconstruct an epidermis, *in vitro* as well as *in vivo*. Monkey pluripotent stem cells were efficiently differentiated into keratinocytes able to reconstruct fully epidermis presenting low level of Major Histocompatibility Complex Class-I antigens opening the way for autologous or allogeneic epidermal long term grafting. Functional keratinocytes generated from non-human primate ESC and iPSC reproduce an *in vitro* and *in vivo* stratified epidermis. These monkey skin grafts will be considered to model autologous or allogeneic epidermal grafting using either ESC or iPSC. This graft model will allow us to further investigate the safety, efficacy and immunogenicity of non-human primate PSC-derived epidermis in the perspective of human skin cell therapy.

P165

Non-hematopoietic umbilical cord blood stem cells exert a neuroprotective effect via reduced numbers of infiltrating immune cells in chronic stroke and traumatic brain injury

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Acute therapies for stroke and traumatic brain injury (TBI) are limited. Within the first 24-48 hours following both stroke and TBI, a rapid influx of peripherally derived immune cells is observed. These cells cross the damaged blood brain barrier leading to an inflammatory microenvironment that results in additional death of neurons within the penumbra of the lesion. Stem cell therapies offer an avenue to address the acute neuroinflammatory response in stroke and TBI. We have identified a line of non-hematopoietic umbilical cord blood stem cells (nh-UCBSC) which can be delivered systemically, 48-hours following middle cerebral artery occlusion or controlled cortical impact in adult rats. Using flow cytometry, we observed a normalization of immune cells within the damaged hemisphere of nh-UCBSC treated animals, relative to injured controls 7-days following treatment. Transcriptomic analysis of the infarcted hemisphere of nh-UCBSC treated rats confirmed these findings. We also identified a normalization of genes related to blood-brain barrier function, cytokine signaling, and apoptosis. Furthermore, behavioral deficits were reduced following treatment. Interestingly, no human cells were identified within the brains of injured animals suggesting nh-UCBSC therapy is acting on secondary lymphoid organs, dampening the extravasation of immune cells. Future studies will examine the interaction of the spleen with nh-UCBSC therapy in these animal models of stroke and TBI.

P166

Hematopoietic stem cell gene therapy for Ifnγr1 deficiency protects mice from mycobacterial infections and paves way for macrophage transplantation therapy

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Mendelian Susceptibility to Mycobacterial Disease (MSMD) is a rare primary immunodeficiency, characterized by recurrent severe infections by otherwise only weakly virulent mycobacteria. MSMD is caused by mutations in genes connected to the IL12-Interferon gamma (IFNγ)-loop leading to an impaired activation of macrophages (MΦ). Specifically, mutations in the IFNγ-receptor-1/2 (IFNγR1/2) genes result in a life-threatening disease phenotype. Here we show the first hematopoietic stem

cell (HSC) gene therapy approach for IFNγR1-deficiency utilizing SIN lentiviral vectors. Transplantation of HSCs transduced with either constitutive or myeloid-specific constructs into Ifngr1-/- mice prior to infection with BCG lead to a reduced mycobacterial burden both in lung and spleen as well as a significantly increased survival of transplanted mice. Genetically corrected MΦ were highly enriched in the lung of transplanted animals, despite low gene marking in HSCs. This highlights the importance of MΦ in the pathophysiology of MSMD and was further supported by the myeloid specific promotor being as efficient as the constitutive promotor. Thus, we decided to establish a macrophage transplantation strategy to harness the therapeutic potential of these cells. To open the niche and facilitate an engraftment of transplanted cells we utilized clodronate liposomes to deplete alveolar MΦ. This led to robust engraftment of transplanted MΦ, which was not observed in non-pre-treated animals, where MΦ fail to engraft and mature. In summary, we demonstrate a successful gene therapy approach for IFNγR1-deficiency and show a novel MΦ transplantation approach that could pave the way for new therapeutic interventions for MSMD and possibly also other diseases.

P167

Retroviral UNC13D transfer restores cytotoxic function in T cells derived from familial hemophagocytic lymphohistiocytosis type 3 patients

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Hemophagocytic lymphohistiocytosis (HLH) is an immunological disorder which can manifest at any stage of life. It arises from either a genetic predisposition (primary HLH) or is acquired (secondary HLH). Familial HLH type 3 (FHL-3) is the most abundant variant of primary HLH in Germany and accounts worldwide for about 30% of all FHL cases. It is characterized by mutations in the UNC13D gene, resulting in functionally impaired or absent Munc13-4 protein. As a consequence, cytotoxic vesicles in T and NK cells are not processed properly, which compromises T and NK cell mediated killing of antigen-presenting cells. This in turn triggers a rapid chain of events leading to hyperinflammation, multiple organ failure and a potentially fatal outcome if left untreated. To validate our approach for clinical translation, we set out to correct the FHL-3 phenotype in patient derived T cells by delivering a codon-optimized UNC13D cDNA by either alpha- or gamma-retroviral gene transfer. Our results based on flow cytometry, Western blot and digital droplet PCR, show that the established gene transfer protocols can be applied to FHL-3 patient cells with various genetic backgrounds and that the genetic correction restores the degranulation capacity of cytotoxic T cells without compromising the phenotype of the transduced T cells. Furthermore, we demonstrate that the co-introduction of the GMP compliant dLNGFR receptor enables us to enrich for the transduced cells for further propagation. In summary, this study lays the foundation for a future adoptive immunotherapy approach to stabilize FHL-3 patients with autologous, immune competent T cells.

P168

Enchanced dark-field microscopy for histological detection of nanostructured scaffolds after implantation into bone defects

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Bone is a hard tissue containing organic matrix, minerals and water. Despite its relative strength, bone tissue is prone to destruction as a result of trauma or genetically determined pathological brittleness due to a deficiency of organic components. Clinically used devices for fixation and splicing of bones are mainly made of metals and often their using requires an additional operation to remove them from the body. In this regard, biodegradable natural materials are more preferable. Biopolymers are one of the main and common biomaterials used in tissue engineering, including bone regeneration due to their high resorption rates and the possibility to control the rate of degradation. We use the biopolymeric nanostructured hydrogel porous scaffolds with clay nanoparticles which accelerate mechanical strength of material and mesenchymal stem cells for bone restoration in veterinary practice. This combination allowed to accelerate the regenerative processes in the dog's bone after trauma. We evaluated the distribution of nanoparticles in the matrix material and destruction of scaffold after implantation to the site of the defect using enhanced dark-field microscopy and spectral analysis (CytoViva®). This method allowed to visualize and distinguish native bone tissue, scaffold and nanoparticles without specific staining. A study of a series of histological sections of bone showed that halloysite nanotubes are integrated into the newly formed bone tissue and do not cause a pronounced inflammatory reaction in the surrounding tissues. Acknowledgments: the work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and RFBR 18-415-160010 grant.

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Migration ability of human polymorphonuclear leukocytes loaded with synthetic microcapsules

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Targeted drug delivery to brain is a hot topic because most substances are unable to cross the blood-brain barrier. Polymorphonuclear leukocytes (neutrophils), inflammatory blood cells, have been investigated as potential carriers of encapsulated therapeutic agents to areas of acute inflammation. Microcapsules about 2 microns in size were fabricated using alternating adsorption layers of polyelectrolytes onto calcium carbonate micro-particles used as a sacrificial template. Human neutrophils

were isolated from the blood of healthy donors and then incubated with the plain FITC-labeled microcapsules at a cell to capsule ratio 1:1 for 20 minutes at 37°C in RPMI-1640. Immediately after incubation, the cells were washed, and internalization of microcapsules was confirmed with transmission electron microscopy. The migration ability of the cells through a 3-μm-pore polycarbonate membrane was assessed using a HTS Transwell plate with IL-8 as a chemoattractant. Cells were allowed to migrate for 1 hour at 37°C and the fraction of migrated cells were quantified by flow cytometry. Experiments performed with neutrophils isolated from 5 independent donors demonstrated that on average $\sim$10% of the cells loaded with microcapsules maintained their migration ability. These data comprise a proof-of-principle that supports the possibility of using patient-derived neutrophils as a potential vehicle for microencapsulated drug delivery. This may be a promising strategy for targeted therapies in human diseases in which PMNLs play a role, such as stroke and traumatic brain injury.

P170

Gene therapy for adenosine deaminase 2 deficiency

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Adenosine deaminase 2 deficiency (DADA2) caused by loss-of-function mutations in the CECR1 gene is characterized by vasculitis, ischemic strokes, intracranial hemorrhages, hematological abnormalities and immunodeficiency mainly affecting B cells. Hematopoietic stem cells (HSCs) transplantation restored ADA2 activity and improved the inflammatory and hematological manifestations in transplanted DADA2 patients, indicating that ADA2 correction in HSCs may represent an alternative treatment for patients lacking a compatible donor. We studied two twin patients with severely diminished ADA2 activity in plasma due to the missense variant p.L188P and an intronic deletion resulting in decreased allele transcription. Sib1 exhibited a more severe neutropenia, immunodeficiency and vasculitis as compared to Sib2. Both presented reduced proportion of memory/switched B cells, abnormal CD4/CD8 T-cell ratio and decreased T-cell proliferation to nominal antigens. We found in healthy donors' HSC sources that CECR1 is expressed in CD34+ cells from umbilical cord blood (UCB), and is absent in mobilized peripheral blood (MPB) CD34+ cells, while a low expression is observed in BM-derived CD34+ cells. Transduction of MPB and UCB CD34+ cells with a PGK-ADA2 lentiviral vector (LV) efficiently restored ADA2 expression and no toxic effect was observed. Studies on immunological reconstitution in NSGW41 immunocompromized mice and on the efficacy of transduction in patient's CD34+ HSCs and monocytic cell lines are ongoing. In conclusion, biallelic CECR1 mutations in twin patients caused a distinct phenotype, suggesting that other factors may contribute to DADA2 pathophysiology. The ADA2-LVs represent a valid tool to assess the safety and efficacy of a gene therapy approach for DADA2.

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Patient-specific iPSC-derived endothelial cells provide long-term phenotypic correction of hemophilia A

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Hemophilia A (HA) is an X-linked bleeding disorder caused by factor VIII (FVIII) gene mutations. Somatic cells can be reprogrammed to generate autologous, disease-free iPSCs and differentiated into cells relevant for gene and cell therapy. We generated patient-specific-disease-free iPSCs from peripheral blood CD34+ cells and differentiated them into functional endothelial cells (ECs) secreting FVIII for gene and cell therapy approaches. CD34+ cells were isolated from healthy and HA donors and reprogrammed with a Cre-Lox LV carrying OCT4-SOX2-KLF4 and miRNA302/367. iPSCs were characterized for stem cell markers, telomeres length and karyotype analysis. Germ layers markers expression and differentiation potential was assessed on embryoid bodies. iPSCs were differentiated in EC with an optimized protocol. Obtained cells acquired endothelial-like morphology, expressed ECs markers and were able to form tubules when cultured in matrigel. Moreover, the differentiation protocol allowed to obtain iPSCs-derived-ECs that did not express lymphatic ECs markers but specific markers (Ets1-2, Gata2-3, Sem3A, CDH11, NRCAM) demonstrating the acquired phenotype of microvascular ECs. Finally, HA-iPSCs were transduced with a lentiviral vector carrying FVIII driven by an endothelial-specific promoter and differentiated into ECs. FVIII-corrected EC were intraportally transplanted in monocrotalin-conditioned NOD/SCID-gnull HA mice. Transplanted cells engrafted and proliferated in the liver along the sinusoids, for long term showing a stable therapeutic FVIII activity (over 6%). These results demonstrate that the hemophilic phenotype can be rescued by transplantation of ECs derived from HA FVIII-corrected iPSCs, confirming the feasibility of cell reprogramming strategy in patient derived cells as an approach for HA gene and cell therapy.

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Effect of genetic modification with reporter genes on mesenchymal stem cell differential ability into osteogenic, adipogenic and chondrogenic lineages

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Mesenchymal stem cells (MSCs) demonstrate tropism to tumor sites and injury. This selective tropism has been attributed to high levels of inflammatory mediators produced in associated microenvironment. Targeted delivery of anticancer drugs using MSCs will allow to increase the efficiency of therapy and to reduce side effects. In our study murine MSCs were isolated from adipose tissue by enzymatic disaggregation with collagenase. Cells were largely positive for mesenchymal stem cell surface markers including CD44, CD90, CD29, CD105, CD73 and Sca-1 and negative for hematopoietic stem cell surface markers. Recombinant lentiviruses LV-ffLuc and LV-GFP were produced using HEK293T packing cell line. MSCs were transduced with recombinant lentiviral vectors encoding ffLuc (MSCs-ffLuc) or gfp gene (MSCs-GFP). Murine MSCs-ffLuc and MSCs-GFP were differentiated into osteogenic, adipogenic and chondrogenic lineages using StemPro osteogenesis/chondrogenesis or adipogenesis differentiation kits (Thermo Fisher Scientific) in accordance with the manufacturer's protocol. Differentiation potential was assessed by histological staining, i.e. Von Kossa staining for osteogenic differentiation; oil-red-O staining for adipogenic differentiation and Alcian Blue staining for chondrogenic differentiation at day 28 of differentiation induction. Genetic modification of MSCs with LV-ffLuc and LV-GFP had no significant effect on MSC ability to differentiate into osteogenic, adipogenic and chondrogenic lineages. Distribution of the cells after injection in animal models will be further investigated. Resulting data will be used for the development of new anti-cancer therapy approaches based on modified MSCs.

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Isolation and characterization of CSF-NSCs from premature infants with intraventricular haemorrhage

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Intraventricular haemorrhage (IVH) is a common cause of morbidity and mortality in premature infants. The incidence of premature infants with IVH has declined in recent years, but remains a significant problem in infants with very low birth weight and extremely low birth weight. The rupture of the germinal matrix entails loss of neural stem cells and disturbs the normal cytoarchitecture of the ventricular zone compromising the organization and function of the ependymal lining and cortical neurogenesis. Premature infants with severe IVH present a higher risk to develop post-haemorrhage hydrocephalus and exhibit long-term neurological deficits with cognitive and psychomotor disabilities. Here we demonstrate that stem cells can be easily and robustly isolated from the haemorrhagic cerebrospinal fluid (CSF) obtained during therapeutic neuroendoscopic lavage to seal the injured germinal matrix and reduce the intracerebral pressure. These CSF cells grow in neurospheres, are positive for CD133 and CD24 and negative for CD45, express Sox2 and nestin, can be expanded, and differentiate into neurons, astrocytes and oligodendrocytes upon withdrawal of mitogens. They are therefore, very similar to fetal neural stem cells (NSC). Notwithstanding, these CSF-NSCs present several distinctive hallmarks such as an increased expression of podocalyxin (PODXL) and IL1RAP that is maintained upon purification of the CD133+ fraction, as well as upregulation of MHC type II proteins. *In vivo* studies to demonstrate engraftment in the subependymal zone and absence of tumorigenesis are underway. These CSF-NSCs pose no ethical concerns and could be useful for the development of an autologous therapy for infants with IVH.

P178

Intravenous administration of engineered AAV gene therapy capsid demonstrates improved CNS transduction in adult mice

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Adeno-associated viral (AAV) vectors exhibit several favorable attributes for delivery of gene-based therapies to the central nervous system (CNS), including safe and sustained transgene expression within neurons. Historically, CNS gene transfer has been achieved by direct injection of AAV vectors into the brain parenchyma or cerebrospinal fluid. Recently, intravenous (IV) AAV administration has been increasingly employed to leverage the extensive brain vasculature to facilitate distribution to central neurons and glia. However, the limited ability of most natural AAVs to traverse the blood-brain barrier has impeded the use of systemic AAV dosing paradigms for CNS targeting. Here we report the evaluation of a barcoded library containing engineered vectors that were screened for CNS delivery following IV administration in adult mice. Compared to the parental capsid, many variants demonstrated over 100-fold greater CNS levels based on DNA and RNA barcodes. Most notably, the use of the novel variant VoyC01 resulted in significant enhancement of biodistribution and transduction in the brain over a previously described, highly neurotropic VOY101 variant in mice. At the cellular level, VoyC01 successfully transduced both neurons and astrocytes within multiple brain regions. In summary, the VoyC01 vector demonstrated enhanced CNS gene transfer properties and low peripheral exposure in adult mice. Furthermore, its increased CNS transduction relative to VOY101 demonstrates the opportunity for continued improvements by using engineered capsids for IV delivery of gene therapy to the brain.

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A novel AAV design improving efficiency and safety in a gene therapy approach for Rett syndrome in a mouse model

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Rett syndrome (RTT) is severe neurological disorder caused mainly by loss-of-function mutations in MECP2 gene. Despite the severity of the disease, recent studies have provided a strong

proof-of-concept that the reactivation of Mecp2 in mutant mice results in a robust reversal of phenotype. Given this prospective, a gene therapy approach aiming to restore Mecp2 expression through viral-based transduction *in vivo* has become a promising strategy to treat RTT. Recently the Adeno-Associated Virus (AAV) serotype 2/9 has been exploited to this purpose into Mecp2-null mice. Unfortunately, this strain upon intravenous injection poorly transduces the brain compared to liver. For these reasons, the introduction of novel strains is necessary to strengthen the potential of the gene therapy. In this project we have exploited the AAV-PHP.eB, a new enhanced variant of the AAV-PHP.B generated to efficiently transduce the mouse brain after intravenous delivery. Thus, we have tested three different viral doses (1×1011-13 vg/mouse) of the Mecp2 expressing AAV-PHP.eB by tail injection in adult Mecp2-null mice observing severe adverse effects only with the higher dosage. Both the other two doses induced a significant beneficial effect on the disease progression with no adverse effects in wild-type mice. Indeed we have confirmed by immunofluoresence and Western Blot analysis a wide transfer of MeCP2 in the brain respect to the liver. In this study we demonstrated how global-scale targeting of the brain can be achieved by a single systemic injection and this can contribute to safer and more efficient gene therapy approach for Rett syndrome.

P180

An miRNA-based gene therapy approach to target mutated SOD1 in key cell types in amyotrophic lateral sclerosis (ALS)

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by progressive death of motor neurons (MNs) leading to fatal paralysis. ALS cases are grouped into two categories: sporadic ALS (sALS) and familial ALS (fALS). 20% of the latter are caused by mutations in the superoxide dismutase 1 (SOD1) gene. Therefore, one sound approach for the treatment of SOD1-related ALS is to downregulate the expression of mutated SOD1 in cells that are vulnerable to the disease using RNA interference (RNAi) as a potential gene therapy. We have developed a cell-specific bicistronic adeno-associated viral (AAV) vector cassette that can selectively express an artificial murine-miRNA (mmu-miRhSOD1) in MNs and astrocytes. We found that when administered into the cerebrospinal fluid (CSF) of SOD1G93A ALS mice, AAV9 can effectively target MN and astrocytes in the spinal cord and allows for rescue of motor function. With the aim of translation to clinics, we have designed 7 artificial human-miRNAs targeting hSOD1 (hu-miRhSOD1) that could be expressed via our bicistronic vector and their efficiency tested in HEK293T. The silencing potential of the hu-miRhSOD1 sequences demonstrated equal or superior efficiency *in vitro* compared to the original mmu-miRhSOD1. Processing accuracy of the miRNAs, assessed using next-generation sequencing, showed that 99% of the hu-miRhSOD1 produced contained the predicted seed sequence and 5 out of 7 hu-miRhSOD1 showed minimal expression of the complementary passenger strand, therefore limiting the risk of potential off-target effects. These results support the use of our novel bicistronic vector in preclinical studies in view of translation to clinics.

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AAV-mediated protein trans-splicing in the retina

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Retinal gene therapy with AAV vectors is safe and effective, yet it is limited by AAV cargo capacity of about 5 kb. To overcome this limitation we used intein-mediated protein trans-splicing to reconstitute large proteins in the retina. Inteins work as independent peptides fused to the C- and N- termini of two host proteins (i.e. the two halves of a large protein) and mediate their association in a multistep autocatalytic process. To test protein trans-splicing in the retina, we generated two AAV vectors separately encoding each of the two halves of either EGFP or large therapeutic proteins flanked by split-inteins. These include ABCA4 and CEP290, respectively defective in Stargardt disease (STGD1) and Leber congenital amaurosis 10, two severe and common inherited blinding diseases. We identified in each protein optimal splitting points for the generation of AAV-intein constructs which take into account both amino acid residue requirements for trans-splicing to occur, as well as the preservation of the native protein domains. Upon co-administration of both AAV split-intein vectors, full-length proteins were reconstituted in the mouse and pig retina as well as in human retinal organoids derived from iPSCs. Importantly, the levels of large protein reconstitution that have been achieved by protein trans-splicing reduce lipofuscin accumulation in the retina of a STGD1 mouse model. Our data support the use of intein-mediated protein trans-splicing in combination with AAV subretinal delivery for gene therapy of inherited blindness due to mutations in large genes.

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Genome editing to generate a pig model of Stargardt disease type 1

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Stargardt disease type 1 (STGD1), due to mutations in the ABCA4 gene, is the most common form of inherited macular degeneration. Abca4-/- knockout (KO) mice are currently used as animal models of STGD1, however they recapitulate only some of the features of the disease. This might be due to the structure of the mouse retina, which largely differs from that of humans. The porcine eye, instead, shares many similarities with the human eye both in terms of size and retinal architecture. Thus, we have planned to generate a pig model of STGD1, by exploring either: i. somatic cell nuclear transfer (SCNT) from primary fibroblasts edited using CRISPR/Cas9 technology, to generate ABCA4 KO pigs; or ii. photoreceptor somatic gene transfer of CRISPR/Cas9 with adeno-associated viral (AAV) vectors in adult pigs. We achieved effective editing of the ABCA4 gene in both ABCA4 KO pigs and in retinas of adult pigs 6 months after subretinal delivery of AAV vectors encoding for Cas9 and the corresponding gRNAs. Notably, ABCA4 gene modification resulted in undetectable levels of ABCA4 protein production in ABCA4 KO pigs and significant, although variable, reduction in the ABCA4 levels in AAV-Cas9-injected pigs. Interestingly, we found increased levels of lipofuscin in the retinas of both ABCA4 KO pigs and AAV-Cas9-injected pigs. Further characterization of STGD1 phenotype development in both animal models is in progress. If successful, the generation of an animal model of STGD1 will provide unique tools to both better understand STGD1 mechanism as well as testing new therapies.

P183

Let-7 microRNA overexpression mediated by lentivirus alleviates neuropathology, balance and motor deficits in Machado-Joseph disease mouse models

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Machado-Joseph disease or spinocerebellar ataxia type 3 (MJD/SCA3) is a genetic neurodegenerative disorder associated with expansion of the number of CAGs in the coding region of the ATXN3/MJD1 gene, which translates into an expanded polyglutamine tract within ataxin-3 protein. MJD patients have severe clinical manifestations and premature death and there is no treatment for this fatal disorder. We and others provided evidence that autophagy impairments contribute to MJD pathogenesis. Recently, we also brought evidence that the let-7 microRNA is a key regulator of autophagy with particular relevance in polyglutamine disorders. In this work we aimed at investigating let-7 potential as a new therapeutic approach in a lentiviral-based and in a transgenic mouse model of MJD. Injection of lentivirus encoding for let-7 into the striatum of a lentiviral MJD mouse model resulted in a 20% increase of let-7 levels and increased levels of LC3-II. A robust and significant let-7-mediated reduction in the number of ubiquitin-positive inclusions and neuronal dysfunction were observed. Balance and motor coordination were assessed in transgenic MJD mice up to 12 weeks post-injection of LV encoding let-7 into the cerebella. A better performance in rotarod, swimming pool, and beam walking tests was observed in let-7-treated Tg mice. In agreement, the treated group exhibited significantly larger cerebellar layers thickness, suggesting prevention of neurodegeneration. In conclusion, let-7 was shown to activate autophagy, reduce neuronal dysfunction and aggregation in the rodent brain and ameliorate motor deficits. Therefore, lentiviral mediating overexpression of let-7 may represent a new therapeutic approach for MJD.

P185

Robust SOD1 knockdown in large mammal spinal cord using a novel delivery paradigm with AAV gene therapy targeting SOD1 for the treatment of SOD1-ALS

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Mutations in superoxide dismutase 1 (SOD1) result in progressive motor neuron loss and cause up to 20% of familial ALS. Studies using transgenic mice expressing SOD1 mutations have demonstrated reduced neuropathology, improved motor behavior and extension of survival with lowering of SOD1. Here, we report results from a series of *in vitro* and *in vivo* studies to select an AAV gene therapy targeting SOD1 with RNAi, and from studies in large mammals to optimize delivery paradigms to motor neurons throughout the spinal cord. To select a RNAi sequence targeting hSOD1, synthetic siRNA duplexes were first screened in Hela cells. Potent RNAi sequences were cloned into different pri-miRNA cassettes, then screened in multiple human cell lines. The best candidates were used to generate AAV vectors for *in vivo* studies in transgenic mice expressing human wild-type SOD1. SOD1 suppression was assessed by RT-qPCR and precision and efficiency of miRNA processing by deep sequencing. The top pri-miRNA cassette/RNAi sequence was then evaluated in pigs using a novel dosing paradigm. SOD1 knockdown was evaluated with RT-qPCR on laser captured motor neurons, by branched DNA assay on tissue punches, and by in situ hybridization. With the novel dosing paradigm, the lead candidate robustly and safely suppressed SOD1 in spinal cord motor neurons in pigs, notably including the cervical levels critical for respiratory function. Our findings support the use of AAV gene therapy targeting SOD1 with RNAi as a potential approach for treatment of SOD1-ALS with a novel delivery paradigm.

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Intravitreal injection of AAV expressing soluble VEGF receptor-1 variant induces anti-VEGF activity and suppresses choroidal neovascularisation

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Choroidal neovascularisation (CNV) is the defining feature of the wet subtype of age-related macular degeneration (AMD), a leading cause of irreversible blindness worldwide. Current anti-VEGF-based treatments for wet AMD require frequent injections, which is often burdensome to both patients and healthcare providers, with gene therapy possibly offering a better alternative. Here, we investigate the therapeutic potential of a recombinant adeno-associated virus 2 expressing a soluble variant of VEGF receptor-1 (rAAV2-sVEGFRv-1) in a laser-induced CNV model. C57/B6 mice were intravitreally administered with rAAV2-sVEGFRv-1, rAAV2-GFP, or bevacizumab, an off-label wet AMD treatment, after CNV lesions were induced via laser photocoagulation. Immunostaining was performed with phalloidin and CD31 to measure CNV extensiveness, F/80 and CD11b for inflammatory cell infiltration, and pan-cytokeratin to visualise fibrotic progression. Our results show that rAAV2-sVEGFRv-1 (5.0x107 v.g.) possesses anti-angiogenic, anti-inflammatory, and anti-fibrotic properties. rAAV2-sVEGFRv-1 was demonstrated to significantly decrease retinal CNV lesion size (1336 ± 186) when compared to rAAV2-GFP-treated (2949 ± 437, $p = 0.0043$), mock-treated (3075 ± 265, $p = 0.0013$), and bevacizumab-treated models (995 ± 234). Infiltration by inflammatory cells significantly decreased with rAAV2-sVEGFRv-1 administration, whereas groups treated with rAAV2-GFP did not. Additionally, anti-apoptotic activity was observed via TUNEL assay in rAAV2-sVEGFRv-1- (16.0 ± 3.6) and rAAV2-GFP-treated (46.0 ± 7.5, $p = 0.003$) mice. Overall, the effectiveness of the rAAV2-sVEGFRv-1 viral vector compares positively to bevacizumab in addressing the major symptoms of wet AMD. Taken together, these results demonstrate the ability of a low dose of rAAV2-sVEGFR-1 to exert a therapeutically relevant anti-VEGF effect in a CNV model, and strongly suggest gene therapy as an effective and convenient treatment for sustained VEGF suppression.

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Sustained mutant Huntingtin lowering in the brain and cerebrospinal fluid of Huntington disease minipigs mediated by AAV5-miHTT gene therapy

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HTT-lowering therapies hold great promise to slow-down or halt neurodegeneration in Huntington disease (HD). We have developed an engineered microRNA targeting human huntingtin (HTT), delivered via adeno-associated viral vector serotype 5 (AAV5-miHTT), leading to efficient HTT-lowering *in vitro* and *in vivo* in rodent models. Here, we have used transgenic HD (tgHD) minipigs to assess the translatability of our approach in a large animal model. Animals were injected with AAV5-miHTT (1.2x1013 gc/brain), bilaterally into striatum (caudate and putamen) and sacrificed 6 months post-treatment. Widespread brain biodistribution of vector DNA was observed, with the highest levels in target (striatal) regions but also in thalamus and cortical regions, in both grey and white matter. Expression of miHTT was highly correlated with vector DNA in all brain areas. Corresponding to the vector DNA and miHTT expression, a reduction of mutant HTT (mHTT) mRNA and protein was observed in AAV5-miHTT treated animals with respect to controls. mHTT protein lowering was on average more than 75% in the injected areas, and between 30-50% in most of the distal regions. Translational pharmacokinetic and pharmacodynamic measures in the cerebrospinal fluid (CSF) were in line with the effects observed in the brain. We detected miHTT in the CSF, and CSF mHTT protein lowering up to 50% at 3 and 70% at 6 months post-dosing. This study demonstrates widespread biodistribution and durable efficiency of AAV5-miHTT in disease-relevant regions in a large brain, and the potential of CSF translational measures to follow-up efficacy.

P188

Zero incidence of adeno-associated virus serotype 9 (AAV9) antibodies in a cohort of spinal muscular atrophy (SMA) type 1 patients screened in STR1VE, a pivotal phase 3 study of AVXS-101

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Spinal muscular atrophy (SMA) type 1 is a rapidly progressing and debilitating neurodegenerative disease characterized by mutations in the survival motor neuron 1 (SMN1) gene. Onasemnogene abeparvovec (AVXS-101) is a recombinant, replication-incompetent AAV9-based gene replacement therapy (GRT) containing the human SMN1 gene. In a phase 1 trial (NCT02122952), AVXS-101 showed improved survival, motor function, and motor milestone achievement in patients with SMA type 1. No formal studies assessing AAV9 antibody prevalence rates in infants with SMA have been performed, but antibodies against AAV9 are thought to be rare in infants. STR1VE is a phase 3, pivotal study of AVXS-101 in symptomatic SMA type 1 patients <6 months of age at the time of dosing (NCT03306277). For eligibility, antibody titres against AAV9 were measured by Enzyme-Linked Immunosorbent Assay; per protocol, patients with antibody titres >1:50 were excluded. Amongst 27 infants (median age 3 months, range <1 month to 5.6 months) who were screened for elevated antibody titres, none manifested exclusionary antibody titres. Consistent with the experience from the AXVS-101 phase 1 trial (NCT02122952), in which only 1 of 16 patients were excluded from the study due to elevated AAV9 antibody titres, screening results from the STR1VE study suggest that elevated antibody titres against AAV9 appear to be a rare event that should not impact the ability of the vast majority of infants with SMA to receive treatment with an AAV9-based GRT.

P189

Adipose stem cells and their paracrine factors are therapeutic for early retinal complications of diabetes in the Ins2Akita mouse

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Early-stage diabetic retinopathy (DR) is characterised by neurovascular defects. In this study, we hypothesized that human CD140b-positive adipose-derived stem cells (ASCs) or their secreted paracrine factors could therapeutically rescue early stage DR features in an Ins2Akita mouse model. Ins2Akita mice at 24 weeks of age received intravitreal injections of CD140b-positive ASCs (1000 cells/1uL) or 20X conditioned media from cytokine primed ASCs (ASC-CM, 1uL). Three weeks post-injection, Ins2Akita mice receiving ASCs had ameliorated decreased b-wave amplitudes and vascular leakage but failed to improve visual acuity, whereas Ins2Akita mice receiving ASC-CM demonstrated amelioration of all aforementioned visual deficits. The ASC-CM group demonstrated partial amelioration of retinal GFAP immunoreactivity and DR related gene expression but the ASC group did not. While Ins2Akita mice receiving ASCs exhibited occasional (1 in 8) hemorrhagic retinas, mice receiving ASC-CM had no adverse complications. *in vitro*, ASC-CM not only suppressed microglial activation, but also protected against TNFα induced endothelial permeability as measured by transendothelial electrical resistance. Biochemical and molecular analyses demonstrated several anti-inflammatory proteins including TSG-6 being highly expressed in cytokine primed ASC-CM. Altogether, ASCs or their secreted factors mitigate retinal complications of diabetes in the Ins2Akita model. Further investigation is warranted to determine whether ASCs or their secreted factors are safe and effective therapeutic modalities long-term as current locally delivered therapies fail to effectively mitigate the progression of early-stage DR. Nonetheless, our study sheds new light on the therapeutic mechanisms of adult stem cells, with implications for assessing relative risks/benefits of experimental regenerative therapies for vision loss.

P190

Robust Huntingtin knockdown in cortex and putamen in large mammals using a novel dosing paradigm with VY-HTT01, an AAV gene therapy targeting Huntington for the treatment of Huntington's disease

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Huntington's disease (HD) is a fatal, inherited neurodegenerative disease characterized by progressive motor, neuropsychiatric, and cognitive impairment. The disease is caused by an expanded trinucleotide repeat in the huntingtin gene (HTT), which results in a toxic gain-of-function of mutant huntingtin protein. As partial suppression of HTT in the brain is both safe and effective in animal models of HD, we are pursuing a therapeutic strategy focused on lowering HTT. VY-HTT01 is a potent AAV gene therapy encoding a primary miRNA targeting human HTT mRNA selectively for knockdown. Here, we describe dosing optimization studies in nonhuman primate to achieve HTT knockdown in both the putamen and cortex. A novel dosing paradigm with MRI-guided convection-enhanced delivery facilitated distribution of VY-HTT01 to the cortex, and resulted in safe suppression of HTT in cortical neurons, notably in the primary motor and somatosensory cortices. Robust HTT knockdown was observed in laser captured cortical neurons with RT-qPCR and in tissue punches from the cortex with the branched DNA assay, which was supported by in situ hybridization (ISH) for HTT mRNA. Vector genome (VG) measurement by ddPCR and ISH for VG demonstrated broad distribution in brain regions that included the infusion sites and the cortex, correlating with HTT mRNA knockdown in these regions. Furthermore, histopathological analysis of the brain showed good tolerability 5 weeks after dosing. These findings support the use of VY-HTT01, an AAV gene therapy targeting HTT with RNAi, with a novel dosing paradigm as a potential approach for the treatment of Huntington's disease.

P191

Allele-specific gene editing for Huntington's disease mediated by a self-inactivating CRISPR/Cas9 system

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Huntington's disease (HD) is a fatal neurodegenerative disorder caused by CAG expansion in the huntingtin (HTT) gene. Considering that the mutation is a toxic gain-of-function, a promising approach would be to decrease the expression level of the mutant HTT. This can be achieved with the recently characterized CRISPR/Cas9 system. In a previous work, we described the kamiCas9, a self-inactivating CRISPR/Cas9 system designed for the transient expression of the Cas9 protein. We demonstrated the high editing efficiency of WT and mutant HTT *in vitro* and *in vivo* with an important reduction of the off-target frequency. However, a selective editing of mutant HTT, using an allele-specific approach, represents the safest way to preserve WT HTT expression and functions. We thus developed more complex strategies to discriminate mutant and wild-type HTT genes by using single-guide RNA targeting sequences containing Single Nucleotide Polymorphism (SNP) in the HTT gene. A first *in vitro* screening, allow us to discriminate the two best candidates to trigger the cleavage of the mutant HTT respectively in the promoter and intron 1. Through this approach the exon 1 of the mutant HTT, which is the region containing the CAG expansion, could be selectively removed. This strategy has then been validated in human embryonic kidney 293T (HEK- 293T) cells and is currently tested in HD mouse models. These results demonstrate the potential of the self-inactivating CRISPR/Cas9 editing for applications in the context of neurodegenerative diseases and a proof of principle of allele-specific disruption of the human HTT gene.

P192

Viral reprogramming of reactive astrocytes into neurons as a regenerative therapy in stroke

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Direct reprogramming of astrocytes to neurons following injury is an alternative approach to cell transplantation in regenerative medicine. In this method, viral vectors force expression of neurogenic transcription factors on an astrocyte specific promoter. In previous studies, neuronal reprogramming of reactive astrocytes was observed using retroviral or lentriviral vectors. Adeno-associated viruses (AAV), however, represent a safer delivery vector. In the current study, we interrogate the reprogramming efficiency of human and canine astrocytes, *in vitro*, as well as in animal models of cerebral ischemia. We observed both canine and human astrocyte cultures are readily infected with multiple AAV serotypes, with AAVrh10 demonstrating the highest transduction efficiency. We also observed canine and human astrocytes expressing immature neuron marker DCX following transduction with AAVrh10 expressing either Ascl1 or Ngn2 on Gfap or Ng2 promoters. Further analyses are ongoing to identify molecular changes during reprogramming. In our preliminary *in vivo* studies, ischemic mice were injected with control rhAAV10 expressing GFP on a CAG promoter. We observed robust GFP expression throughout the ischemic zone, with both NeuN+ neurons and GFAP+ astrocytes co-expressing GFP. We are currently investigating the therapeutic use of AAV vectors to reprogram astrocytes following cerebral ischemia in rats.

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dCas9-based Scn1a gene activation restores inhibitory interneuron excitability and attenuates seizures in Dravet syndrome mice

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Dravet syndrome (DS) is a severe epileptic encephalopathy caused by heterozygous loss-of- function mutations of the SCN1A gene, indicating haploinsufficiency as the pathogenic mechanism. Here, we tested whether catalytically dead Cas9 (dCas9)-mediated Scn1a gene activation can rescue Scn1a haploinsufficiency in a mouse DS model and restore physiological levels of its gene product, the Nav1.1 voltage-gated sodium channel. We screened single guide RNAs (sgRNAs) for their ability to stimulate Scn1a transcription in association with the dCas9 activation system. We identified a specific sgRNA that could increase Scn1a gene expression levels in cell lines and primary neurons with high specificity. Nav1.1 protein levels were augmented, as was the ability of wild-type immature GABAergic interneurons to fire action potentials. A similar enhancement of Scn1a transcription was achieved in mature DS interneurons, thus rescuing their ability to fire. To test the therapeutic potential of this approach, we delivered the Scn1a- dCas9 activation system to DS pups using adeno-associated viruses. Febrile seizures were significantly attenuated. Our results pave the way for exploiting dCas9-based gene activation as an effective and targeted approach in DS and other disorders resulting from altered gene dosage.

P194

Characterization and transplantation of CD73-positive photoreceptors isolated from human iPS cell-derived retinal organoids

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Photoreceptor degenerative diseases are a major cause of blindness. For stem cell-based therapy using human induced pluripotent stem cells (hiPSCs) is crucial to obtain a homogenous photoreceptor cell population. We previously showed that the cell surface antigen CD73 is specifically expressed in photoreceptors in hiPSC-derived retinal organoids. Flow cytometry analysis in dissociated cells from retinal organoids indicated that the percentage of CD73+ cells increased with organoid maturation, with CD73+ cells representing more than 60% of cells at day 180 of differentiation. Targeting of CD73 by Magnetic-Activated Cell Sorting (MACS), led to enrichment to 90% of CD73+ cells in the positive sorted fraction. RT-qPCR analysis on sorted CD73+ cells showed over-expression of both cone and rod-specific genes compared to dissociated retinal cells before MACS. We confirmed that CD73 targeting by MACS is an effective strategy to separate a homogenous population of photoreceptors by using a fluorescent cone rod homeobox (crx) reporter hiPSC line. Freeze-thawing of whole retinal organoids resulted in a source of viable cells and did not affect MACS effectiveness.

Subretinal transplantation in NUDE rats, suggested that both unsorted retinal cells and sorted CD73-sorted photoreceptor precursors from D120 organoids are safe, with no proliferation of human cells. Finally, transplantation studies demonstrated the capacity of CD73-sorted cells to survive and mature in close proximity to host inner retina of a model of photoreceptor degeneration during several weeks. These data demonstrate that CD73+ photoreceptor precursors hold great promise for a future safe clinical translation.

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Effect of neutralizing anti-AAV antibodies on vector transduction following intravitreal administration of AAV in non-human primates

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Pre-existing immunity (PEI) remains a challenge for many adeno-associated virus (AAV) gene therapies due to high prevalence of natural AAV in the general population. A natural AAV infection induces an immune response leading to generation of neutralizing antibodies (NAbs) that bind to and neutralize the virus, thus preventing subsequent infections. Even low serum anti-AAV NAb titers have been shown to have a negative impact on AAV transduction in humans using systemic vector delivery approaches. Ocular gene therapies appear less impeded by NAbs due to the partial immune privilege status of the eye. Some degree of vector neutralization by NAbs is however thought to take place when the vector is injected intravitreally, although its significance in the clinic has not been extensively characterized. In this study we wanted to establish a correlation between NAb titers in serum and vitreous humor, and to study the effect of PEI on vector neutralization after intravitreal delivery in non-human primates. Naïve animals with both low and high endogenous serum AAV NAb titers as well as animals pre-immunized against AAV were injected intravitreally with AAV vectors carrying a reporter transgene. Serum and vitreous samples were collected prior to intravitreal delivery and throughout the study to be assayed for AAV NAbs. Reporter transgene expression levels in the retina were monitored as an indicator of transduction efficiency. The results are expected to provide better predictability of NAb screens, to help validate gene therapy candidate exclusion criteria, and to evaluate parameters for feasibility of repeat vector administrations.

P196

Induced pluripotent stem cell-based endothelial cell models of the human blood-brain barrier to screen gene and other therapies for CNS entry

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Attempts to develop therapeutics for diseases of the central nervous system (CNS) have been hindered by the lack of predictive cell culture models of the blood-brain barrier (BBB). Advances in induced pluripotent stem cell (iPSC) technology and availability of reproducible differentiation protocols into endothelial cells have facilitated progress. In this study we present efficient differentiation of three different wild-type iPSC lines into brain microvascular endothelial cells (BMEC). The differentiated cells display several membrane markers of BMEC, exclude paracellular crossing tracers and exhibit significant barrier tightness as measured by trans-endothelial electrical resistance (TEER), ranging from 1,500 to >6,000 Ωcm^2. TEER values at the higher end of this range have only been previously reported in BBB models involving co-culture of different cell types. This study demonstrates that barrier tightness comparable to *in vivo*and co-culture systems can be achieved by single-cell BMEC models and that barrier tightness depends on the iPSC clone from which the endothelial cells are generated. Further, we demonstrate significant barrier crossing by doxycycline as a positive control for penetrance, and by adeno-associated virus (AAV) vector serotype 9, but only very limited crossing by a cell penetrating peptide-conjugated antisense oligonucleotide. In conclusion, selected iPSC-based models of the human BBB display robust phenotypes and can be used to screen drugs for CNS penetration in culture. We now intend to develop a medium to high-throughput screening system for CNS entry based on the clone with the most robust BBB phenotype.

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Long-term uninterrupted high dose treatment reduces the beneficial effects of GDNF gene delivery

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GDNF and Neurturin protect nigro-striatal dopaminergic neurons and reduce motor symptoms when applied terminally in toxin-induced Parkinson's disease models. However clinical trials based on intraputaminal GDNF protein administration or rAAV-mediated Neurturingene delivery have demonstrated only modest clinical benefits. In addition to its neurotrophic properties, GDNF also interferes with dopamine homeostasis via time and dose-dependent neurochemical effects such as e.g inhibition of tyrosine hydroxylase transcription and inhibition of dopamine transporter activity (1) (2). Our hypothesis is that depending on the delivery parameters, these neurochemical effects could be deleterious and reduce GDNF beneficial effect. We have described a doxycycline (dox)-regulated vector (AAV-DoxON-GDNF) allowing to adjust GDNF dose and period of administration at clinically-acceptable antibiotics doses (3). In the present study, we have created a unilateral partial dopamine deficit by injecting 6-hydroxydopamine, in the right rat striatum, which provokes an asymmetrical behavior. We have then injected a high dose of AAV-DoxON-GDNF in order to mimic conditions of potential GDNF overdosing. Three modalities of dox administration were applied: no treatment, continuous (4 months) treatment or 4 intermittent 2-weeks treatments. The ''no dox'' and continuous treatments resulted in a respectively 4-fold and 20-fold increased GDNF levels as compared to endogenous striatal

concentration. At 16 weeks post-vector injection, low-dose or intermittent high-dose GDNF treatments resulted in recovery of the motor symptoms whereas a long-term high-dose treatment did not provide significant benefits. In future clinical trials, it will be important to regulate GDNF administration in order to avoid overdosing potentially reducing the clinical benefits.

P198

AAV-GBA1 gene therapy for Parkinson's disease

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Glucocerebrosidase (GCase) is an enzyme involved in the lysosomal ceramide synthesis pathway. Heterozygous mutations in Gba1, the gene encoding GCase, are one of the strongest risk factors for developing Parkinson's disease (PD). Sporadic PD patients not expressing Gba1 mutations also have decreased GCase activity. Data from preclinical studies demonstrate that AAV-GBA1 treatment suppresses α-synuclein accumulation, extends survival, and improves cognitive outcomes in GBA mutant and PD mouse models. Although these studies provide a proof of concept for AAV-GBA1 gene therapy in preclinical models, they utilize serotypes/promoters that primarily target neurons. However, recent human and mouse RNA-seq data demonstrate that GBA1 is highly enriched in astrocytes. Given these findings, we propose to assess the effects of AAV-GBA1 expression when restricted to astrocytes vs. neurons only and compare these findings to ubiquitous expression. Here we present data comparing the effects of cell-type specific vs non-selective AAV-GBA1 expression on GCase activity and localization in primary cortical cultures, containing both neurons and astrocytes. We also show data assessing the correlation between GBA protein levels and GCase activity following AAV-GBA1 treatment *in vitro*. Moreover, we present data from preliminary *in vivo* studies assessing the effects of non-selective AAV-GBA1 expression on GCase function (i.e. GCase activity and GBA1 substrate levels) along with transcriptional changes. These findings will help determine the best approach for AAV-GBA1 treatment to produce significant functional changes and will inform on the potential for AAV-GBA1 gene therapy in PD.

P199

Using integration-deficient lentiviral vectors as a therapeutic strategy for spinal muscular atrophy

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Spinal muscular atrophy (SMA), the most common genetic disorder resulting in infantile death, is caused by defective production of Survival Motor Neuron (SMN) protein encoded by SMN1, leading to degeneration of motor neurons (MNs) and neuromuscular dysfunction. Integration-deficient lentiviral vectors (IDLVs) are highly efficient tools for delivering therapeutic genes. We have developed IDLVs expressing a codon-optimised version of SMN1 under three different transcriptional controls. E15 primary cortical and MNs transduced with IDLVs show significantly increased SMN intensity following immunofluorescence. Similarly, fibroblasts obtained from severe SMA patients show significantly increased SMN protein following transduction with IDLVs under all three transcriptional controls (CMV, hSYN, hPGK). Significant increases in gem number (a functional endpoint representing increased SMN1 expression) has been shown in two neuronal cell lines, Neuro2a and SH-SY5Y, as well as severe SMA fibroblasts. Since MNs are the primary pathological target of SMA, the use of our vectors in induced pluripotent stem cell (iPSC)-derived MNs has been tested and shown up to 70% of cells can successfully be transduced leading to a 23-fold increase in SMN protein levels. Finally, pre-symptomatic delivery of treatment may prevent development of the SMA phenotype and the irreversible damage that accompanies this. Therefore, we have delivered our IDLVs *in utero* at E16 assessing biodistribution of eGFP expression showing complete transduction of the spinal cord and MNs. Delivery of the murine Smn gene increased Smn protein levels at all levels of the spinal cord. Taken together, these results suggest that IDLVs are efficient tools for SMA gene therapy.

P200

SINEUP for GDNF rescues motor deficits and neurodegeneration in a mouse model of Parkinson's disease

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Glial cell-derived neurotrophic factor (GDNF) is a protein with a potent action in promoting the survival of dopamine (DA) neurons. Several studies indicate that increasing the GDNF levels may be beneficial for the treatment of Parkinson's Disease (PD), by reducing the neurodegeneration of DA neurons that is the cause of this disease. Despite a plethora of preclinical studies showing GDNF efficacy in PD animal models, efficacy in humans is questionable and still there are open questions such as delivery and safety. We took advantage of the discovery of a new class of long non-coding RNA (lncRNA) called SINEUPs that are able to increase selectively the expression of the target mRNA at the post-transcriptional level to increase in a physiological range the endogenous GDNF protein. Our SINEUP-GDNF was active *in vitro* to increase selectively the GDNF protein of about two-fold. AAV9-mediated delivery of SINEUP-GDNF in the striatum of WT mice led to an increase of GDNF protein, the stable expression of our SINEUP-GDNF and to a potentiation of DA system functions. Moreover, this physiological increase of GDNF was able to ameliorates motor deficits and neurodegeneration of DA neurons in a PD mouse model. Our data indicate that SINEUP-GDNF could represents a new way to increase in a more specific and physiological way the GDNF protein levels and to reduce DA neurodegeneration.

P201

Prosavin®, a dopamine gene therapy for advanced Parkinson's disease: 5 years phase I/II clinical update

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Parkinson's disease (PD) is caused by the progressive degeneration of dopaminergic neurons. The primary standard of care for PD is oral dopaminergic based therapies; these are highly efficacious but their long term use is complicated by motor fluctuations from intermittent stimulation of dopamine receptors and off-target effects. Therefore, a therapy that provides a more continuous and local supply of dopamine offers a potential therapy for these patients. ProSavin® is a gene therapy product that utilises a lentiviral vector to transfer three genes that are critical for de novo dopamine biosynthesis in the striatum, the area of the brain that is depleted of dopamine in PD. Fifteen advanced PD patients have received ProSavin® in three dose cohorts. ProSavin® has been demonstrated to be safe and well tolerated at all doses evaluated to date. No serious adverse events related to the study drug or surgical procedures were observed. ProSavin® continued to be safe and well tolerated in patients with PD. Improvements in motor behaviour over baseline continued to be reported in the majority of patients who could still be evaluated up to 5 years of follow up. To increase the efficacy further we have developed Axo-Lenti-PD, a re-engineered version of ProSavin®, that expresses the same enzymes but with an increased dopamine production per genetically modified cell. A Phase I/II clinical trial of Axo-Lenti-PD in the UK and France in PD patients is in planning. The project has been supported by the UK Technology Strategy Board (Innovate UK).

P202

Effects of mesenchymal stromal cells and neural progenitor cells derived from them by direct reprogramming in experimental ischemic stroke

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Cell–based therapies have emerged as a potential strategy for ischemic stroke treatment. Transplantation of several cell types proved to be safe and effective in enhancing recovery in animal models. Further research is needed to identify the most active cell types, optimal administration routes and mechanisms underlying the beneficial action of cell transplantation. We amended the frequently used rat middle cerbral artery occlusion (MCAO) model of ischemic stroke by improving the operation technique and developing an MRI-based method of quality control. The amended model was used to compare short- and middle-term effects of intra-arterial injection of three kinds of human cells - mesenchymal stromal cells isolated from placenta (pMSCs) or dental pulp (dMSCs), and neural progenitor cells derived by direct reprogramming of bone marrow MSCs. All types of cells penetrated the blood-brain barrier and either were homed around blood vessels or traveled towards the injury site and brain neurogenic zones. Both pMSCs and dMSCs significantly enhanced functional recovery by the 7th day after MCAO, while injection of drNPCs – after 14 days. Only pMSCs improved the survival rate during the first post-operation month and only drNPCs caused rapid reduction of the volume of the infarction zone. All cell types stimulated neurogenesis in brain neurogenic zones. Our results demonstrate parallels and variation in the therapeutic effects of different cell types delivered to the close proximity of brain tissue ischemic injury and provide insights into the mechanisms of action of transplanted cells.

P203

Effects of VEGF and FGF2 gene therapy on sciatic nerve regeneration in rats

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We investigated gene therapy of sciatic nerve injury using plasmid pBud-VEGF-FGF2 encoding both vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2). Experiments were performed on male Wistar rats. 5mm diastasis was formed on left sciatic nerve, afterwards ends of nerve were joined through nerve graft. All animals were randomly divided into two groups (VFGF-FGF2 or 6.4% NaCl). Treated rats were injected in the sciatic nerve with 10μl of plasmid DNA solution at three sites (conc. 30μg/μl), whereas control animals were injected with NaCl. Sciatic function index (SFI) test post injury showed higher results in treated group compared to control. Plasmid DNA injection stimulated vascularization of sciatic nerve's distal segment as showed by laser Doppler EasyLDI (Aimago, Switzerland). Absolute perfusion units (apu) in treated group on 30th day post injury (dpi) was 264.26% ($p < 0.05$) higher and on 60th dpi 57.25% ($p < 0,05$) higher compared to control group. Furthermore, the number of myelinated fibers in distal segment between 30 and 60 dpi increased by 32% ($p < 0.05$), which is 15.1% ($p < 0.05$) higher compared to control group. Finally, number of neurons in L5 DRG was 22.32% ($p < 0.05$) higher on 30th dpi and 28.41% higher ($p < 0.05$) on 60th dpi compared to control group. Thus, pBud-VEGF-FGF2 plasmid injection during acute period of sciatic nerve trauma stimulated regeneration of motor function, nerve vascularization, axon growth, and supported neurons survival in L5 DRG. Study was supported by Program of Competitive Growth of KFU and funded by state assignment 20.5175.2017/6.7.

P204

Tubulation of rat's sciatic nerve and stimulation of vascularisation using VEGF and FGF2 encoding gene therapy plasmid

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In the left sciatic nerve, 5mm diastase was formed through dissection at mid-thigh level by two parallel incisions. The ends of nerve were joined using 7mm long NeuraGen tube (2.2mm diameter, INTEGRA, USA) in a way that 1mm of nerve stumps were inside tube with 5mm diastasis between distal and proximal ends. Tube and nerve ends were anastomosed via 4 epineurial interrupted sutures. In treatment group, distal and proximal ends of the nerve were injected with pBud-VEGF-FGF2 plasmid at concentration 30μg/μl in 2 points, 15μl to each site, control group was injected with the same volume of 6.4% NaCl. Vascularization in nerve was assessed using laser Doppler EasyLDI (Aimago, Switzerland) at 30 and 60 days post operation. Animals were anesthetized with chloral hydrate (400mg/kg in 6.4% NaCl solution) and then access to sciatic nerve was made. The laser ray was focused on distal part of nerve and parameters of microcirculation were analyzed in real-time. Vascularization of the distal nerve after tubulation was reduced in comparison with intact animals, whereas in VEGF-FGF2 group vascularization was higher than in intact animals by 83.52% ($p<0.05$) at 30th day and by 76.23% ($p<0.05$) at 60th day post operation. In VEGF-FGF2 group parameters of vascularization were higher than in NaCl group by 105.37% ($p<0.05$) at 30th day and by 97.2% ($p<0.05$) at 60th day post operation. Thus, injection of pBud-VEGF-FGF2 plasmid stimulates vascularization process in sciatic nerves. Study was supported by Program of Competitive Growth of KFU and funded by state assignment 20.5175.2017/6.7.

P205

Non-viral transfections with the neuroprotective factors PEDF and GM-CSF reduces oxidative stress in human pigment epithelium cells *in vitro* offering a promising approach to treat avascular age-related macular degeneration

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Oxidative stress, inflammation and possible reduction of neuroprotective factors may be significant events leading to retinal degeneration in avascular age-related macular degeneration (aAMD). We hypothesize that creating a neurogenic and neuroprotective retinal environment by transplanting transfected pigment epithelial cells that overexpress PEDF and GM-CSF would prevent retinal pigment epithelial cell degeneration, the development and progression of aAMD. To determine whether overexpression of PEDF and GM-CSG are protective, the anti-oxidant potential of the proteins was determined in human pigment epithelial (PE) cells transfected with the PEDF and/or GM-CSF genes. The constructs pT2-CMV-PEDF-His, pT2-CMV-GMCSF-His and pCMV(CAT)T7-SB100X, where SB100X is the hyperactive Sleeping Beauty transposase, were delivered to PE cells by electroporation (Neon®, ThermoScientific). Gene and protein expression were determined by RT-qPCR, Western Blot, immunofluorescence and ELISA at 1 to 8 weeks post-transfection. At 10 weeks cells were exposed to H2O2 (250-500 μM) for 24 h and the anti-oxidant glutathione levels determined. Transfected cells showed increased PEDF and GM-CSF gene expression (PEDF = +900-fold; GM-CSF = +2x105-fold) and protein secretion (PEDF = +500-fold; GM-CSF = +200-fold) compared to non-transfected cells at all time points (ANOVA, $p<0.0001$). 1.00 ± 0.04 PEDF and 5.00 ± 1.00 GM-CSF gene copies were integrated into the cells' genome. Transfection was confirmed immunohistochemically. After H2O2 treatment, glutathione in transfected cells increased significantly compared to non-transfected cells (PEDF = 1.58-fold; GM-CSF = 1.55-fold; PEDF+GM-CSF = 1.80-fold) (ANOVA, $p<0.0001$). The results presented here 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 retinal milieu to prevent cell degeneration in aAMD.

P206

Evaluation of gene therapy in spinal cord injury using of neurotrophic factor BDNF and regulation with miR-9/124 in SCI rat model

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SCI is one of the most significant neurological disorders. Despite disabilities and psycho-socio-economic outcomes in societies, there is no effective treatment yet. In recent years application of gene therapy is taken into consideration by neuroscientists. BDNF is an essential neurotrophic factor in CNS. MicroRNAs are small non-coding RNAs regulating gene expression at post-transcriptional level. miR-124 and miR-9 are two of the most abundant miRs in the mammalian CNS which have important functions in neuronal development. Synergic regulatory effects of these miRs has proven in previous studies. The present study was designed to investigate the effects of BDNF with and without regulation of miR-9 /124 in SCI rat model. For this purpose, Sprague-Dawley adult rats underwent T8-9 spinal cord compression injury. We used an intrathecal injection of lenti-BDNF and lenti-miR-9/124 in SCI rat models. BDNF and miR-9/124 coding sequences were cloned into a pCDH lentiviral vector. BDNF and miR-9/124 were successfully expressed as evidenced by RT-PCR and Western blot assays. The therapeutic effects were evaluated by standard BBB test and results were compared in studied groups. In conclusion, we found that the function of BDNF gene in presence of high-level expression of miR -9/124 could be promoted. Furthermore, increasing effectiveness of BDNF neurotrophin with high level expression of miR-9/124 may enhance neuroprotective effects and contribute to the locomotor functional recovery after SCI.

P207

Application of autologous peripheral blood mononuclear cells into the area of spinal cord injury of pigs in subacute period

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Cell therapy is a promising approach for spinal cord injury (SCI) treatment. Peripheral blood mononuclear cells (PBMCs) contain different progenitor cell populations, including endothelial progenitor cells, hematopoietic stem cells and mesenchymal stem cells. We investigated the efficiency of application of PBMCs enclosed into a fibrin matrix (Tissucol, Baxter) into the SCI area in a subacute period in pigs. Female adult potbellied pigs (n = 10, 9-12 kg weight, 3-4-month-old) were used. The dosed contused SCI was performed under anesthesia by weight-drop device. The impactor was centered above Th11 and the impact rod (50 g) dropped from 20 cm height on the spinal cord with the subsequent compression by the same weight for 10 minutes. Six weeks after the surgery 8 million of autologous PBMCs enclosed in Tissucol (150 μL) were applied on top of the SCI area. The control animals received Tissucol application only. The animals were in the experiment for 4 months after application and reoperation. Porcine Thoracic Injury Behavior Scale (PTIBS) index, electrophysiological studies and histological assessment were performed to assess the efficiency of therapy. Our results demonstrated that application of PBMCs in fibrin matrix onto the area of SCI contributes to partial restoration of conduction in the posterior columns of the spinal cord and tissue retention at the site of injury.

P208

Genetically engineered umbilical cord blood mononuclear cells for therapy of spinal cord injury in combination with epidural stimulation

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Spinal cord injury (SCI) continues to be a social problem and has a significant impact on government burden since the absence of the safe and effective therapies to improve quality of life and increase life expectancy in patients. The electrotherapy is a well-known technique and has long been used for neurorehabilitation in patients with SCI. Recently, gene therapy has been introduced in treatment of spinal cord injury as a successful approach. Here for the first time, we employed combined cell-mediated triple-gene therapy with epidural stimulation in rat with SCI model. We used intrathecal administration of genetically modified UCB-MC overexpressing therapeutic genes VEGF, GDNF, and NCAM. Epidural electrical stimulation was performed simultaneously above the injury epicenter to support the axons regeneration and below the neurotrauma to maintain motoneurons deprived of connections with the upper neural cells. The electrophysiological investigation of hindlimb skeletal muscles, behavioral tests, morphometric analysis of grey and white matter sparing and immunofluorescent study of the molecular and cellular changes in spinal cord demonstrated higher efficiency of combined therapy (epidural stimulation with *ex vivo* triple gene therapy) than electrotherapy along. Our results suggest that intrathecal injection of genetically modified UCB-MC overexpressing recombinant neurotrophic factors and neuronal cell adhesion molecule with dual epidural stimulation represent a novel potentially successful approach for SCI treatment.

P209

Development of an AAV-based microRNA gene therapy to treat spinocerebellar ataxia type 3

ABSTRACT WITHDRAWN

P210

Exploring the role of miRNAs on microglia activation

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Chronic inflammation leads to the release of increased levels of cytokines into circulation, which can ultimately access the brain and permanently activate microglia, potentiating neuroinflammation and neuronal damage. Chronic microglia overactivation has been lately associated with neurodegenerative or psychiatric diseases, including Depression. miRNAs have different molecular targets and can regulate distinct disease-associated pathways. The goal of this study is to explore the role of miRNAs on microglia activation driven by inflammatory cytokines. Primary microglia was obtained from P1 or P2 Wistar Han rat pups. Each pup was euthanized for brain isolation and dissection. Mixed glial cells were cultured in poly D-lysine coated flasks for 30 days, and microglia was obtained every 10 days of culture, by shaking at 37°C for 2h at 200 rpm. Isolated microglia were stimulated for 6h with TNF-α (20 ng/mL), IL-1β (50 ng/mL) or IL-4 (20 ng/mL). RNA from activated microglia was extracted for gene and miRNA expression analysis. Microglia CD11bhigh/CD45low expression signature revealed a cell purity of >90%. TNF-α significantly induced the overexpression of pro-inflammatory genes NOS2, IL-6 and TNF-α, through activation of the NF-κB pathway. Oppositely IL-4 stimulation significantly inhibited NOS2 expression. miRNA microarray revealed 24 down and 24 upregulated miRNAs (>1.2 fold) after TNF-α microglia activation. Interestingly, expression of let-7 family members was reduced upon stimulation. Therefore, the role of these miRNAs is being further explored to unravel new therapeutic mechanisms. Acknowledgments Project NORTE-01-0145-FEDER-000012 (NORTE 2020, PORTUGAL 2020, ERDF). JPB (BiotechHealth PhD Programme) and MIA were supported by FCT.

P211

Assessing cerebellar neurometabolic biomarkers by *in vivo* magnetic resonance spectroscopy in a transgenic mouse model of Machado-Joseph disease

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Machado-Joseph disease (MJD) or Spinocerebellar ataxia type 3 is the most common form of dominant SCA. It is associated with the over-repetition of CAGs in the MJD1/ATXN3 gene, which translates into an expanded ataxin-3, conferring toxic properties to this protein and resulting in severe clinical features. Currently there is no therapy and no biomarkers are known. Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) have been very useful for the non-invasive diagnostic and follow-up of diseases, but they can also be used to extrapolate for the success of therapies. However, this kind of assessments in mouse models is rare, which hampers an efficient translation of therapeutics tested at the pre-clinical level into the clinics. The aim of this study was to perform a longitudinal assessment of neurochemical profiles in the cerebellum of both MJD transgenic mice and controls using a 9.4 Tesla scanner and investigate if the MRS data correlates with motor performance. Five neurochemicals were significantly different between MJD and wild-type mice in the cerebellum: N-acetylasparate (NAA), NAA+N-acetylaspartylglutamate (totalNAA), Glutamate, Taurine and myo-Inositol (Ins). Neurochemical ratios NAA/Ins and NAA/total Choline, previously correlated with clinical status in SCA patients, were also reflected in this animal model. Moreover, alterations in levels of NAA, tNAA, Glutamate, Taurine, Ins and NAA/tCho in the cerebellum of MJD mice were significantly correlated with motor performance, as assessed by rotarod. In conclusion, neurochemical biomarkers can be used to monitor promising therapies during preclinical trials in MJD mice and subsequently be translated to human clinical trials.

P212

Gene and cell therapy for brain disorders: the case of the polyglutamine Machado-Joseph disease

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Tremendous progress of gene therapy and its tools - viral and non-viral vectors for gene therapy have been achieved. Unfortunately, issues related to the efficacy and safety of vectors, as well as insufficient knowledge from mechanisms of disease, lack of reliable models and biomarkers to monitor disease progression and therapeutic efficacy, prevented gene therapy from rapidly reaching results in line with initial expectations for many diseases with unmet medical needs including brain disorders. However, the scenario is changing due to progress in identifying causative gene mutations, common mechanisms of disease, such as autophagy impairments, proteolysis defects or others, from which promising therapeutic approaches are emerging. Furthermore, gene editing as well as induced pluripotent stem cell technologies have been enabling the generation of new cell and animal models of disease potentially more informative and more reliable as well as new cell sources for regenerative medicine. The polyglutamine Machado-Joseph disease is a model brain disorder for whose treatment, over the last 2 decades we have been developing promising gene and cell therapy technologies including gene addition (beclin-1, calpastatin, sirtuin-1, NPY), gene silencing (siRNAs, microRNAs and shRNAs for ataxin-3 and A2A receptor), gene editing (Talen and CRISPR), mesenchymal, neural and induced pluripotent stem cells transplantation, which will be highlighted in this talk. It is expected that our pre-clinical results will contribute to the current acceleration in the discovery of effective therapies for this and other brain disorders with unmet medical needs.

P213

AAV.PHP.eB-mediated OPA1 gene expression in a mouse model of Parkinson's disease as a valuable strategy for neuroprotection

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Parkinson's disease (PD) has been linked to defects in the mitochondrial function; indeed, reduced activity in the mitochondrial complex I impairs mitochondrial respiration and is associated with the degeneration of the dopaminergic neurons of the Substantia Nigra. More recently, the mitochondrial involvement in PD has been extended to perturbations to fusion/fission. OPtic Atrophy protein (OPA1), a dynamin-related GTPase of the inner mitochondrial membrane, participates in mitochondrial fusion and apoptotic mitochondrial cristae remodeling. Complex I inhibition leads to the disruption of OPA1 oligomeric complexes that are crucial for healthy mitochondria. Similar deficiencies have been observed in postmortem PD Substantia Nigra samples. Strikingly, these mitochondrial changes can be reverted if the levels of OPA1 are increased. These observations are prompting us to speculate whether the increase of OPA1 activity might affect alpha-synuclein accumulation and have beneficial effects in terms of neurodegeneration. To answer this question, we investigated whether the overexpression of OPA1 is a viable therapeutic option against the neurodegenerative process in murine model of PD. Thus, we delivered OPA1 in the substantia nigra, using an AAV-PHP.eB vector in order to increase the expression of OPA1. Consequently, we injected AAV-PHP.B-SCNA-A53T, to trigger neurodegeneration and alpha-synuclein accumulation. Mice were checked for motor function, neuronal survival, a-Syn accumulation, gliosis and astrocytosis. To modulate OPA1 gene expression levels, two promoters with different expressivity were cloned upstream the OPA1 gene and compared for their neuroprotection efficacy. With this project we aim at discovering a potential novel target for gene therapy to prevent neurodegeneration and disease progression.

P214

Developing gene therapy for FAM161A associated retinitis pigmentosa in a murine model

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Loss-of-function mutation in the FAM161A gene causes autosomal recessive retinitis pigmentosa (RP28) and is regarded as a retinal ciliopathy. Fortunately, the disease progression is slow and retinal structure in patient is relatively preserved, which enables the development of a potential cure with gene augmentation therapy to replenish the affected region with wildtype gene products. We have generated several adeno associated virus (AAV) based gene therapy vectors containing various photoreceptor specific promoters and human FAM161A mRNA isoforms (Long or Short). Administration of these vectors into Fam161a -/- mice through subretinal injection resulted in transient but not persistent improvements in full field electroretinogram (ERG). To maximize therapeutic efficacy, we have cloned three murine Fam161a mRNA isoforms (L, S and S1) from mouse retina which all exploit a non-conserved alternative translation initiation site. Unlike their human counterparts, *in vitro* expressed murine FAM161A proteins failed to assemble themselves into the cytoskeleton network, but tended to form aggregates within or around the nucleus. Murine isoforms were then packaged into AAV2/8 vectors and delivered *in vivo* by subretinal injection. Transgene expression and retinal functionality are currently evaluated and compared with human FAM161A vectors. Our study provides a valuable ground to validate the gene therapy of FAM161A associated RP and highlights the possible species difference that may diminish therapeutic efficacy in animal model.

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Gene therapy in Rho-/- mice, a model to validate rod-specific vectors and to identify biomarkers of irreversible function restoration

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Targeting specifically the diseased cell is an important factor to improve gene therapy. Not only does this specificity avoid adverse effects due to irrelevant cellular expression but it also allows to limit the amount of administrated vector and thus preserves the immune system from an overload of vector particles. In retinal diseases, one main target cell for therapy is the rod photoreceptor, mostly affected by genetic defects in retinitis pigmentosa. In order to test future specific vectors targeting rods, we decided to perform gene replacement in the Rho-/- mouse model of rod deficiency. After amplification of the mouse rhodopsin mRNA from C57Bl6 retina extract, we cloned it downstream either of the CMV promoter for *in vitro* studies or of the human Rho promoter (Alloca et al. (2007), J. Virol) for *in vivo* studies. We produced an AAV8-pseudotyped vector with this latter cassette and subretinally injected the vector into Rho-/- mice. We validated expression of the Rhodopsin protein by transfection in 293T cells. Animals injected at 1 month of age, when 70% of photoreceptor layer thickness remains, didn't improve their visual function as measured by ERG. As Palfi et al. (2015) demonstrated visual restoration of Rho-/- mice after injection of an AAV8 vector at P3-P4, further work is needed to precisely define the therapeutic window in regard to the degeneration progression and to identify biomarkers of irreversible function restoration.

P216

Genetic modification of freshly isolated primary human pigment epithelial cells to treat nvAMD

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Neovascular age-related macular degeneration (nvAMD) causes severe vision loss and is characterised by choroidal blood vessels growing into the subretinal space, triggered by overexpression of the vascular endothelial growth factor and decreased expression of the pigment epithelium-derived factor (PEDF). In recent years, we have developed an approach involving the subretinal transplantation of genetically modified pigment epithelial cells that stably overexpress PEDF. All methods were established using research-grade materials. However, the transfer to the clinic requires safe plasmids free of antibiotic resistance markers (pFAR) and a certified electroporation device (CliniporatorTM). Immediately after isolation, iris (IPE) and retinal (RPE) pigment epithelial cells from human donor eyes were transfected with two pFAR4-derivatives encoding PEDF and the enhanced Sleeping Beauty (SB100X) transposase. Furthermore, a Luciferase-encoding pFAR4 was used that allowed for a more rapid detection of successful gene transfer. Effective transfection was demonstrated in independent experiments using 10,000-50,000 cells. Transfection using the pFAR4-Luciferase resulted in efficiencies of 23.5-38.0% (IPE) and 41.0-48.2% (RPE). PEDF-transfected cells showed an up to 5-fold (IPE) and 18-fold (RPE) increase in PEDF secretion. SB100X-mediated transfection of freshly isolated pigment epithelial cells resulted in elevated levels of PEDF. The pFAR technology allows for plasmid propagation in the absence of antibiotics, which is a crucial safety issue for plasmid use in human. The transfection of freshly isolated cells using the certified CliniporatorTM is an important step towards the efficient and safe delivery of the PEDF transgene to autologous cells *ex vivo*, followed by their transplantation to the subretinal space of nvAMD patients.

P217

AAV-mediated RP2 replacement in a patient-derived *in vitro* disease model

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Mutations in RP2, encoding a ubiquitously expressed protein involved in ciliary trafficking, are responsible for approximately 15% of X-linked retinitis pigmentosa cases. A patient harbouring the most common nonsense RP2 mutation, R120X, was identified (Target 5000). The aims of this study were to generate and characterise a patient-derived R120X primary fibroblast disease model of RP2, and to analyse the effects of AAV-delivered RP2 replacement. First, AAV tropism for fibroblasts was determined employing CAG-EGFP delivered using three different AAV serotypes (MOI = 1x105 vg/cell); AAV2/2 was found most efficient. In untreated R120X fibroblasts, the expression level of RP2 mRNA was 7.5 ± 3.2 fold lower while RP2 protein was absent compared to wild type fibroblasts. The R120X cell line displayed a distinctive phenotype including Golgi fragmentation, measured by distribution of GM130 (a Golgi marker protein) and mislocalisation of IFT20 (an intraflagellar trafficking protein). The areas of GM130 and IFT20 immunolabeling were significantly greater in R120X compared to wild-type fibroblasts ($87.58 \pm 58.27\ \mu m^2$ vs $31.18 \pm 19.35\ \mu m^2$, and $71.30 \pm 77.28\ \mu m^2$ vs $17.29 \pm 12.06\ \mu m^2$, respectively; $n = 90$-120 cells, $p < 0.0001$). Transduction of R120X fibroblasts with AAV2/2-CAG-RP2 resulted in $119 \pm 67\%$ expression (compared to wild-type) of RP2 protein and a significantly reduced GM130-positive area ($58.1 \pm 40.79\ \mu m^2$, $n = 120$ cells, $p < 0.0001$). RP2 replacement therapy was thus demonstrated to provide benefit using cellular assays in a patient-derived *in vitro* disease model. Further assays to evaluate efficacy *in vitro*, and *in vivo* studies to assess efficacy and safety aspects of the treatment are in progress.

P218

Cytokine profile in blood serum and cerebrospinal fluid in human traumatic spinal cord injury patients

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Spinal cord injury (SCI) is characterized by damage to the blood-brain barrier (BBB), which separates brain tissue from circulating blood and extracellular fluid. Damage to BBB together with inflammation leads to tissue infiltration with activated leukocytes. The main function of these cells is to remove the debris and prevent infection; however, they also can promote inflammation and trigger the autoimmunity. The leukocyte migration regulated by cytokines, which are produced by damaged cells. We investigated serum and cerebrospinal fluid (CSF) cytokine levels in patients ($n = 100$) with SCI at different stages (acute, subacute, intermediate, recovery and late periods) of the disease using Bio-Plex Human Cytokine 27-plex Assay and 21-plex Assay (Bio-Rad). Increased serum level of TNF-beta in SCI ($\sim$2,8 fold) was found during 5 years after the injury when compared to control group. At the early stages of SCI (8-14 days), only serum level of TNF-beta was found upregulates. Cerebrospinal fluid of patients with SCI (8-30 days post-injury) was characterized by increased levels of cytokines/chemokines due to the neurotrauma thus none of studied analytes decreased. Levels of TNF-beta, CTACK and GRO-alfa in CSF of SCI patients remained elevated 30 days post-injury as compared to control group. Our results emphasize the complex cytokine imbalance in SCI patients, which could hold the key to understand the mechanisms of immune response and inflammation and help to develop gene and cell therapy treatments of neurotrauma.

P221

Submacular injection without vitrectomy of a photoreceptor-targeted AAV5 in cynomolgus macaques

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Vitrectomy with submacular delivery AAV is standard in some gene therapy clinical trials. We proposed submacular injections could be successfully performed in macaques with intact vitreous. Four macaques injected bilaterally with 1e12 vg/ml AAV5.hGRK1.eGFP. Small volume induced foveal detachment, aqueous paracentesis, then bleb expansion. In two animals, vehicle used for foveal detachment, 100μL AAV5 for bleb expansion (Group 1). In two animals, 100μL AAV5 used for both steps (Group 2). Serum neutralizing antibodies (Nab) analyzed pretest and 6-week termination. Optical coherence tomography (OCT) and confocal scanning laser ophthalmoscopy (cSLO)

regularly performed. Submacular delivery accomplished in all 8 eyes. One Group 2 eye received only 50μL AAV5 after retinal tension developed. One Group 1 eye developed macular hole after air bubble inadvertently introduced during bleb expansion; hole resolved after 1 week, but subfoveal detachment persisted through termination. In remaining eyes transient bleb detachment resolved at 1 week with ellipsoid zone and foveal bulge return in 5/7 eyes by termination. cSLO showed faint GFP fluorescence at 1 week, progressively stronger through termination. Mild uveitis evident in animal with hole at 1 week, resolved by week 2 with anti-inflammatories. Both Group 2 animals developed minimal intermediate uveitis weeks 1 through 6. NAb negative all animals pretest. One Group 2 animal developed strong anti-AAV5 response at 6 weeks; responses all others were modest. Three-step submacular injection without vitrectomy is effective for delivery of AAV in macaques. Use of vehicle creating initial foveal detachment may decrease subsequent vitreal inflammation and reduce NAb formation.

P222

Non-invasive allele-specific silencing therapy and biomarkers for Machado-Joseph disease

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Machado-Joseph disease (MJD) is the most common dominantly-inherited ataxia. Although there is no cure, our group showed that RNA interference holds great promise for its treatment. However, previous experiments involved craniotomy and in situ injection in the brain. In the present study we aimed at developing an adeno-associated viral vector serotype 9 (AAV9)-based system that enables: non-invasive delivery of RNA interference-based treatments to the brain, specific silencing of mutATAX3 and alleviation of MJD by intravenous (iv) injection. Firstly, AAV9 vectors encoding an artificial microRNA that targets the mutant form of ataxin-3 mRNA (AAV9-mirATAX3) were generated. Their efficacy and specificity were tested in neuronal cells and the therapeutic potential was then evaluated in a severely-impaired transgenic mouse model. Mice were iv-injected at postnatal day one (PN1); were submitted to behavioural tests at three different ages (PN35, 55, and 85) and sacrificed at PN95. At PN75, animals underwent Magnetic resonance imaging and spectroscopy (MRI/MRS) to evaluate morphological and metabolic changes of cerebellum. AAV9-mirATAX3's treatment reduced the number of protein aggregates and cerebellar neuropathology, leading to significant improvements in all behavioural tests. Moreover, MRI/MRS data indicated that mirATXN3 treatment ameliorates the levels of a specific set of neurometabolites, which can be used in the future as therapeutic biomarkers. Overall, this study provides compelling evidence that a single iv injection of AAV9-mirATAX3 is able to transpose the BBB, silence mutant ataxin-3 and alleviate MJD. This is the first time that a non-invasive and allele-specific silencing approach produces a positive impact in a neurodegenerative disorder.

P223

Long-term aflibercept expression levels in non-human primates following intravitreal administration of ADVM-022, a potential gene therapy for wet age-related macular degeneration

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Inhibition of VEGF is the mode of action of several standard-of-care therapies for wAMD, including aflibercept. Gene therapy could provide sustained anti-VEGF levels in the retina following a single injection, providing clinical benefits while drastically reducing treatment burden. In this study, we report the expression of aflibercept driven by ADVM-022, a recombinant AAV vector, following a single intravitreal (IVT) administration to non-human primates and compare them to levels resulting from a single IVT dose of aflibercept protein. Aflibercept levels in aqueous and vitreous humor, retina, and choroid 56 days following IVT ADVM-022 were equivalent to those in aflibercept-injected animals at Day 21-28, well within the therapeutic window (8 weeks) of the aflibercept standard of care. A trend towards increased aflibercept levels with increasing doses of ADVM-022 was observed. Expression levels in vitreous humor at the highest dose (2E12 vg/eye) in the presence of prophylactic prednisone were approximately 2-fold less compared to 2E12 vg/eye ADVM-022 alone. This difference did not reach statistical significance and was in the range of efficacious levels observed in laser-induced choroidal neovascularization model in NHP studies. Ocular tissue expression levels at doses evaluated (2E11-2E12 vg/eye) 56 days post-dose were within the range previously observed 16 months post-dose. Ongoing studies show that vitreous humor aflibercept levels 22.5 months post injection are similar to levels 1 month post dose. In conclusion, this study demonstrates the potential for a single IVT injection to deliver long-term efficacious treatment for wAMD.

P226

CRISPR/Cas9 gene editing in human pluripotent stem cell-cardiomyocytes provides a platform for modeling hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is a prevalent and complex cardiovascular genetic disease. Mutations in sarcomeric genes play a key role, leading to left ventricle thickening and heart dysfunction. We aimed to model HCM *in vitro* using genome-edited human pluripotent stem cell- derived cardiomyocytes (hPSC-CM), in order to overcome the limitations of animal models and genetically unrelated healthy controls. Herein, we have engineered 11 genetic variants of the HCM-causing mutation c.C9123T-MYH7 (p.R453C-βMHC) using CRISPR/Cas9 genome editing, in 3 independent hPSC lines of origin. Isogenic sets were differentiated to hPSC-CMs for high-throughput molecular assays and functional assessment. Gene-edited hPSC-CMs cultured as 2D monolayers or as 3D engineered heart tissues exhibited the main molecular hallmarks of HCM (hypertrophy, multi-nucleation, hypertrophic marker expression and sarcomeric disarray). Functional evaluation of the generated lines supported the energy depletion model due to higher metabolic respiration activity, accompanied by abnormalities in calcium handling and contraction force. Partial phenotypic rescue was achieved with ranolazine but not omecamtiv mecarbil, while RNAseq highlighted potentially novel molecular targets for therapy, such as long non-coding RNAs that are currently under investigation. Altogether, the genetically engineered hPSC-CM lines comprehensively recapitulated the most prominent clinical features of HCM, rendering them a patho-physiologically relevant platform to investigate disease mechanisms, provide new diagnostic tools and ultimately enhance drug discovery.

P227

Lentiviral-mediated SCN5A delivery rescues electrophysiological defects in iPSC-based cardiac models of laminopathy

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LMNA mutations lead to a heterogeneous group of diseases called laminopathies that, at the heart level, manifests with dilated cardiomyopathy typically anticipated by various conduction defects. LMNA gene encodes two nuclear lamina proteins, Lamin A and C, that are involved in the regulation of diverse biological processes, from maintenance of nuclear structure to mechano-sensing, transcription and chromatin organization. Despite the advancements in the field, studies addressing the pathophysiological mechanisms underlying defective Lamin A/C in cardiomyocytes (CMs) and myocardial diseases are still at their infancy. To this aim, in the last years we have generated and deeply characterized patient-specific models of the disease by induced pluripotent stem cell (iPSC) technology. In details, through comprehensive electrophysiological and morphological analyses, we found that CMs differentiated from iPSCs carrying either K219T or R190W mutations associate dilatation to sarcomeric abnormalities, defects in the main action potential properties, reduction of the peak sodium currents and diminished conduction velocity. Molecular studies indicated that a reduction SCN5A gene transcription, mediated by a Lamin A/C, was at the basis of the observed phenotype. We therefore generated a lentiviral construct encoding the GFP-tagged SCN5A gene, encoding the main sodium channel protein Nav1.5, and found that its administration to LMNA mutant CMs ws sufficient to restore their functional phenotype *in vitro*. In conclusion, we demonstrated the efficacy of SCN5A gene replacement therapy for electrophysiological defects of LMNA-CMP in human cardiac-specific model systems, indicating SCN5A as a potential target for gene-therapy approaches to treat conduction defects associated to LMNA mutations.

P228

Immunotoxin-based conditioning facilitates autologous haematopoietic stem cell engraftment and multi-lineage development in a Fanconi anemia mouse model

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Hematopoietic stem cell (HSC) gene therapy is a promising treatment approach for inherited marrow failure disorders such as Fanconi Anemia (FA). While most transplantation protocols utilize conditioning chemotherapy to deplete host HSCs in the marrow and facilitate engraftment of donor cells, this is associated with genotoxicity in FA patients and increases the risk of leukemogenesis. Antibody-drug conjugates targeting HSCs is an emerging non-genotoxic method for promoting engraftment of transplanted cells. This platform would be ideal in diseases such as FA which are associated with sensitivity to DNA damage and cancer predisposition. We used immunotoxins as an alternative conditioning regimen in an FA mouse model of autologous transplantation. Antibodies targeting either CD45 or CD117 epitopes were conjugated to saporin (SAP), a ribosomal toxin. FANCA knockout mice were conditioned with either CD45-SAP or CD117-SAP prior to receiving marrow from a heterozygous donor. Mice conditioned with either immunotoxin exhibited equivalent HSC depletion in the marrow and lack of CFU potential, similar to cyclophosphamide (Cy). Furthermore, both immunotoxin-treated groups displayed similar levels of engraftment in a cell dose dependent manner as Cy-treated controls. Bone marrow chimerisms also showed equivalent engrafted HSC populations between immunotoxin- and Cy-treated groups. Our findings demonstrate CD45-SAP and CD117-SAP are effective at both depleting diseased stem cells and promoting engraftment of donor cells for gene therapy-based protocols and HSC transplantation. These studies highlight the feasibility and benefit of this approach for gene therapy and transplantation and should provide the groundwork for the next-generation conditioning in diseases like FA.

P229

Human iPSC-based models reveal defective neuronal and glial differentiation from neural progenitor cells (NPCs) in globoid cell leukodystrophy

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Globoid Cell Leukodystrophy (GLD) is a rare neurodegenerative lysosomal storage disease caused by the genetic deficiency of β-galactocerebrosidase (GALC). The rapid neurological deterioration consequent to glycosphingolipid accumulation in glia and neurons represents a major challenge for the development of effective gene therapy (GT) approaches. Therapeutic efficacy relies on sustained GALC availability, optimal cross-correction and clearance of storage in affected cells and tissues. Still, the pathological cascade downstream the primary storage and the traffic of GALC in neural cells are poorly elucidated. Also, whether the observations made in murine systems recapitulate critical aspects of the human disease is still to be defined. We have generated a panel of induced pluripotent stem cell (iPSC) lines from GLD patients and normal donors (ND). All GLD iPSC lines show psychosine storage that is cleared upon LV.hGALC-mediated rescue of GALC activity (GLD-GALC iPSCs). Compelling evidence in two GLD lines shows that GALC deficiency severely hampers the differentiation of iPSC-NPCs into neurons, astrocytes, and oligodendrocytes, also highlighting a mutation-specific phenotype. The pathological hallmarks are only partially rescued in GLD-GALC iPSC-derived neuronal/glial cell cultures and are recapitulated in ND-GALC counterparts. Collectively, our results emphasize that a stringent time- and cell type-dependent regulation of GALC expression is needed for efficient neural commitment and differentiation. Preliminary data suggest that an unbalance of bioactive sphingolipids, an altered trafficking of the mutated GALC proteins or the excessive functional GALC protein (in GT settings) might contribute to driving the early dysfunction of GLD neural cells, with important therapeutic implications.

P230

Interleukin-25 restores vessel-forming capacity of dysfunctional endothelial progenitor cells under high glucose condition

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In diabetes, the impairment of vascular repair processes and angiogenesis are partially caused by endothelial progenitor cell (EPC) dysfunction. A previous study demonstrated that interleukin 25 (IL-25), a member of IL-17 family, increases neovascularization in the bronchial airway of asthma patients. In this study, we investigate the ability of IL-25 to enhance the vessel-forming capacity of dysfunctional EPCs under high glucose condition using an *in vitro* model. The EPCs were cultured in 100 and 295.5 mg/dl of D-glucose representing normal control and diabetes with poor glycemic control, respectively. We found that the vessel forming capacity of EPCs cultured in high glucose was significantly decrease in comparison to their normal glucose counterparts. The addition of IL-25 increased both number and vessel-forming capacity of EPCs cultured in high glucose. Moreover, IL-25 also augmented the positive effect of vascular endothelial growth factor (VEGF) on the number and vessel-forming capacity of dysfunctional EPCs under high glucose condition. The gene expression study shows that IL-25 exerted its effect, at least in part, by upregulating the expression level of several pro-angiogenic cytokines including ANGPT1, VECAD, VEGF and VEGF receptor KDR gene. IL-25 also up-regulated the expression level of its own receptor IL25R gene, thus creating a positive-feedback response. Furthermore, the positive effects of IL-25 were restricted to the dysfunctional EPCs cultured in high glucose without affecting normal EPCs. We conclude that IL-25 can restore the impaired functions of EPCs caused by high glucose and might be used as an additional therapeutic agent for treating diabetic vasculopathies.

P231

Keratin 14 labelling with a fluorescence molecule facilitates efficiency analysis of RNA therapeutics in epidermolysis bullosa simplex

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Epidermolysis bullosa simplex is a blistering skin disease caused by dominant mutations within the KRT14 gene leading to impaired keratin 14 (K14) functionality and consequently to an instable intermediate filament network. We developed an RNA trans-splicing-based therapy approach to correct the genetic cause of the disease. During RNA trans-splicing, the spliceosomal machinery may be used to recombine two RNA molecules into a new, chimeric gene product, and thereby replace a mutated gene region by its wild-type copy. The splicing reaction is induced by an RNA trans-splicing molecule (RTM) via binding to a defined pre-mRNA target region. In this study, we generated a keratinocyte cell line expressing fluorescently labelled K14 to enable direct visualization of RNA repair at protein level. We applied the CRISPR/Cas9 system to permanently integrate GFP in-between the KRT14 promoter and the KRT14 gene in a wild-type keratinocyte cell line, leading to the expression and integration of a functional GFP-K14 fusion protein into the intermediate filament cytoskeleton. The treatment of the cell line with a KRT14-specific RTM, carrying dsRed fused N-terminally to KRT14 exons 1-7, led to the exchange of the fluorescence molecules (from GFP to dsRED) and the respective KRT14 coding region via RNA trans-splicing. The integration of resulting dsRED-K14 fusion proteins into the intermediate filament network of the cells facilitated the RNA repair efficiency analysis as green filaments turn red upon RTM treatment. Thus this fluorescence-based RTM screening system is well suitable for analysis and comparison of RTMs specific for any KRT14 gene region of interest.

P232

Human iPSC derived retinal organoids display synaptic contacts and follow native retina layer patterning

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Retinal organoids derived from human pluripotent stem cells that mimic human retinal cellular composition, architecture, and functional features have revolutionized the field of retinal research and opened new avenues to study regulatory events that govern unique human retinal development and disease and facilitate the development of therapeutic approaches for retinal dystrophies. It has been demonstrated that extended retinal organoid culture allows the formation of mature photoreceptor traits and retinal cell organization patterns which have not been possible to generate in 2D cultures. Further characterization of human retinal organoids and their relationship to human retina is necessary in order to fully exploit the possibilities of this promising tool. We describe here the synaptic contacts, advanced retinal layer formation and map the generation of retinal cell types along the differentiation culture using a human iPSC line obtained by mRNA reprogramming.

P233

A new mouse model of HDV infection based on AAV vector mediated delivery of the HDV genome reproduces important characteristics of human HDV infection

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Hepatitis delta virus (HDV) is a defective RNA virus that depends on hepatitis B virus (HBV) for the formation of new virions. HDV infection is associated with the more severe form of viral hepatitis and no treatments are currently available. Studying HDV and developing new treatments is hampered by the limited availability of small animal models. HDV and HBV used the same human receptor, hNTCP, but not the murine counterpart. Herein, to generate an HDV-mouse model, HDV and hepatitis B virus (HBV) replication competent genomes were delivered to the mouse liver using adeno-associated viruses (AAV-HDV and AAV-HBV). AAV-HDV infection initiated HDV replication in mouse hepatocytes that was detected for at least 45 days. The presence of both large and short HDV antigens in the liver indicates that the HDV genome was correctly edited in mouse liver. Furthermore, in the presence of HBV, HDV infectious particles are generated. As observed in patients, co-infection was associated with the reduction of HBV antigen expression and the onset of liver damage. Using this model we found that HDV replication induced a sustained type I interferon response, which was mainly dependent on the activation of the mitochondrial antiviral signalling protein (MAVS). Interestingly, we found that HDV replication was associated with a significant reduction of AAV genomes that cannot be by hepatocytes death or liver regeneration. Thus, a mouse model of HDV infection is described, which mimics several important characteristics of the human disease.

P234

Towards high throughput drug screening for human retina organoids

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Inherited retinal dystrophies resulting in blindness are the consequence of a large diversity of mutated genes. By studying the degenerative mechanism, we observed some common death pathways, suggesting that single or combined drug administrations may interfere with this process and delay vision loss. In order to screen drugs, we developed a standardized approach to reliably generate mouse retina organoids (RO) that can be individually followed and analyzed. Mouse ES cells expressing GFP, under the activation of the photoreceptor-specific gene Crx, were cultivated in an array of microwells composed of poly(ethylene glycol)-based hydrogels to form single aggregates (one per well) and induced to differentiate into RO. More than 95% of the aggregates gave rise to an eyecup and the retinal tissue was comprised of around 80% of photoreceptors. In addition, a large percentage of the sensory cells are cones (21.5%), necessary for day and color vision. With the aim of developing a similar approach for human photoreceptors, we used gene editing to insert a comete-GFP at the UTR site of the CRX gene. gRNAs were screened for their efficacy and specificity in 293T cells. In iPSCs, three out of 26 clones showed correct integration of the transgene. Different protocols were tested for RO induction and the appearance of fluorescence helped to determine the period of photoreceptor genesis. Immunohistological examination of RO confirmed the production of a large number of photoreceptors. We will couple both systems to attempt to standardize the production of human RO for drug screening and human photoreceptor studies.

P235

Restoration of normoglycemia in diabetic models via insulin gene therapy

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Steady glycaemic control is crucial for Type 1 Diabetes patients, however it remains difficult to achieve with exogenous insulin therapy. An interesting and unexplored therapeutic option is the use of genetically modified adeno-associated virus (AAV) vectors that have proved successful in the cure of monogenic disorders and have shown negligible toxicity and immunogenicity. In the present study, we have investigated ways of restoring a base level of insulin production in various diabetic mouse models via administration of liver tropic AAV2/8 vector containing a codon-optimised human insulin gene. We achieved restoration of euglycemia in chemically induced diabetic C57BL/6 mice that showed no immunological barriers to efficacy of insulin gene therapy and enjoyed long-lasting correction of hyperglycemia. Euglycemia was also restored in spontaneously diabetic Non Obese Diabetic (NOD) mice, although these mice required a 7-10 fold higher dose of vector to achieve similar efficacy as the C57BL/6 mice and the immunodeficient NOD SCID mice. We detected CD8+ T cell reactivity to insulin and mild inflammatory infiltration in the livers of gene therapy recipient NOD mice. Efficacy of the gene therapy in NOD mice was partially improved with a non depleting anti-CD4 antibody, while passive immunisation of gene therapy-treated NOD SCID recipients with AAV2/8-reactive serum prevented successful restoration of euglycemia. Our data indicate that both immune cells and antibodies form a barrier to successful restoration of euglycemia in autoimmune diabetic mice with insulin gene therapy, but that this barrier can be overcome by increasing the dose of vector and by suppressing immune responses.

P236

Linking non-coding RNAs to osteogenic differentiation

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Bone remodeling and regeneration can be impaired by several diseases such as osteoporosis or tumors. Those processes encompass distinct biological mechanisms regulated by non-coding RNAs (ncRNAs). Although some ncRNA are currently being used in clinical trials to treat human diseases, the vast majority remains unexplored. In this study, we aim to identify long ncRNAs (lncRNAs) involved in the osteogenic differentiation. In the long term, our goal is to use lncRNA molecules as novel tools to promote bone regeneration. LncRNA profiling upon induction of osteogenic differentiation was evaluated using Mouse LncRNA MicroArray v3.0 from ArrayStar, which comprises intergenic lncRNAs (lincRNAs), UCR and other lncRNAs from public transcriptome databases. Bioinformatics tools were used to evaluate the degree of conservation along evolution. Microarrays results show that several lncRNAs are involved in osteogenic differentiation. Importantly, a large number of the differently expressed lncRNA identified is conserved between mouse and human. Moreover, we identified uc.64+, an intron sense transcript overlapping with EHBP1 gene, as consistently overexpressed during osteogenic differentiation by RT-qPCR. Furthermore, UCR can potentially act as competing endogenous RNAs since bioinformatic tools show predicted binding sites for multiple microRNAs. In particular, uc.64+ potentially binds to miR-125a-3p, which is a known inhibitor of osteogenic differentiation. In conclusion, lncRNAs are involved in osteogenic differentiation and might be used as potential novel therapeutics tools to promote cell differentiation into osteoblasts. Work funded by FCT in the framework of the project POCI-01-0145-FEDER-031402 R2Bone; and by NORTE-01-0145-FEDER-000012, under the PORTUGAL 2020, through ERDF. MIA is supported by FCT fellowship.

P237

Generation of a 3D human neural system for Parkinson's disease modeling

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Early triggering mechanisms in Parkinson's disease (PD) are still poorly understood. Animal models have been widely used to model PD dynamics but they cannot recapitulate the complexity and sensitivity of the human brain. Therefore we strongly need to generate *in vitro* models of human brain tissues that are mainly affected in PD such as the nigrostriatal pathway. To this aim we combined cell reprogramming, bio-engineering and biofabrication technologies to design a 3D *in vitro* model of human nigrostriatal pathway. We therefore optimized protocols to generate human dopaminergic neurons, medium spiny GABAergic neurons, astrocytes and microglia by forcing specific cell-lineage transcription factors. In order to connect different neuronal populations we employed axon guidance molecules in a 3D culture system and combined them with the microfluidics-based system OrganoPlate®. The final aim of the project is to compare healthy and PD patient-derived *in vitro* models to identify early neurodegeneration markers as diagnostic biomarkers and potentially new early therapeutic targets.

P238

Hematopoietic differentiation of induced pluripotent stem cells derived from patients with Griscelli syndrome type 2

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Griscelli Syndrome Type 2 (GS-2) is a rare genetic disease caused by mutations in the RAB27A gene. GS-2 affects immune system and causes functional impairments of cytotoxic T cells and Natural Killer cells. Currently, the only available treatment is hematopoietic stem cell (HSC) transplantation. Here, we developed and characterized induced pluripotent stem (iPS) cells from three GS-2 patients, and differentiated them into HSCs for disease modelling. Bone marrow Mesenchymal Stem cells (MSCs) from three GS-2 patients were cultured and transduced with bicystronic or polycystronic lentiviral vectors carrying Oct3/4, Sox2, KLF4 and cMyc. IPS cells were maintained in iPS Brew medium (Miltenyi) supplemented with bFGF. Expression of pluripotency markers TRA-1-60, TRA-1-81, SSEA1 and SSEA4 (Miltenyi) was confirmed by FACS and/or immunofluorescent staining. RT-PCR was performed for SOX2, NANOG and OCT4. Teratoma assays were performed to confirm capacity to differentiate cells from all three germ cell layers. GS-2 IPS clones were co-cultured on Op9 stromal cells layers for 5 days in HSC expansion medium (Miltenyi) supplemented with SCF, TPO, Flt 3 ligand and BMP4. Cells before co-culture showed typical iPS morphology and expressed all pluripotency markers, although in varying levels. During co-culture, isolated islands of hematopoietic cells were observed. Up to 15% of iPS cells co-cultured on Op9 stromal cells expressed the CD34 antigen, and formed typical CFU-GM and BFU-E colonies in Methocult (Stem Cell Technologies), that showed positivity for CD14, CD16, CD33 and CD45. In conclusion, iPS cells derived from BM-MSCs from GS-2 patients were able to differentiate into CD34+ HSCs.

P239

***in vitro* evaluation of AAV capsid variant transduction efficiency**

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Recombinant adeno-associated virus (AAV) vectors for gene therapy have demonstrated safety and long-term transgene expression in various tissues including the retina. Nevertheless, existing AAV serotypes remain limited in their ability for panretinal transduction and thus unable to treat retinal dystrophies efficiently. As such, novel AAV vector variants are being engineered, using capsid diversification strategies, with the aim of optimal uptake in the retina and critical subpopulations like cone photoreceptors. Evaluation of this AAV plethora is key in identifying meaningful candidates for use in a therapeutic context. While *in vivo* assessment is imperative, *in vitro* models can serve as a swift and inexpensive

intermediate step for candidate selection. To this end, we performed *in vitro* characterization of the transduction efficiency for two novel AAV capsid variants, termed herein AAV2-GL and AAV2-NN, using the murine cone photoreceptor-like 661W cell line. AAV2-GL and -NN vectors expressing sc-CMV-eGFP were administered at various multiplicities of infection (MOI) to 661W cells and compared against the parental AAV2-WT and the previously published AAV2-7m8. The vectors were applied to 661W cells at optimal MOI with or without prior challenge (e.g. different handing conditions) to determine their stability and efficiency under these conditions. Cell transduction was assessed 24-48 hours post-infection by EVOS epifluorescence microscopy and FACS analysis of eGFP signal. The overarching outcome of this study was a significantly higher transduction efficiency of the novel capsid variants and served as a proof of principle for subsequent *in vivo* evaluation.

P240

MyoD-directed reprogramming of fibroblasts with DYSF gene mutation for human dysferlinopathy modeling

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Mutations in DYSF gene lead to dysferlinopathy which is one of muscular dystrophies. This disease results in gradual skeletal muscle degeneration primarily in calf muscles in the lower limbs. There is no effective treatment for these diseases yet. Thus production of skeletal myocytes from mutant fibroblasts isolated from patients with dysferlinopathy can be useful to investigate the effectiveness of new therapy approaches. Fibroblasts were isolated from gingival connective tissue of the patient with dysferlinopathy (mutation in DYSF gene exon 26). The patient fibroblasts were immortalized using lentivirus encoding p53 shRNAs. MyoD-directed genetic reprogramming of patient gingival fibroblasts was mediated by lentivirus encoding transcriptional activation domain VP64 genetically fused to the N-terminus of human wild-type MyoD. Recombinant lentiviruses were produced by co-transfection of the HEK293FT packing cell line with packaging plasmid (Addgene, #22036), envelope plasmid (Addgene, #8454) and vector plasmid (Addgene, #60629). Patient immortalized gingival fibroblasts were transduced with the lentivirus carrying VP64MyoD gene with tetracycline-inducible (Tet-ON) promoter. The cells were selected in 1 μg/mL puromycin for 10 days to obtain a pure population of transduced cells. Selected cells were grown to 50% confluence and MyoD transgene expression was induced by supplementing the medium with 3 μg/mL of doxycycline. Cell type differentiation resulted in cell fusion and myofiber formation. The resulting MyoD-induced skeletal myocyte model cell line can be used for new dysferlinopathy therapy approaches screening.

P241

AAV8-mediated gene expression in pigs with neutralizing antibody against vector capsid

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Adeno-associated virus (AAV) vectors can transduce hepatocytes efficiently *in vivo* in various animal species including humans. However, few reports exist concerning pig liver. Pigs are potentially useful in preclinical study showing high similarity to humans in both anatomical and physiological aspects. In this study, we evaluated the utility of micro mini pigs by testing liver-mediated gene expression. AAV8 vector encoding luciferase was administered into the 1st animal intravenously at higher dose and gene expression in the liver was assessed seven days later using IVIS system. Robust transgene expression was observed almost exclusively in the liver. This animal showed low titer (x1) neutralizing antibody (NAb) against AAV8 capsid. Next, we assessed the action of NAb against AAV, which is known to interfere with AAV vector-mediated gene transfer by intravascular delivery. Relationship between the titer of neutralizing antibody and transgene expression was tested using four additional animals. When a standard dose (2.0×1012 vg / kg) of vector was administered intravenously, transgene expression in the liver was observed in both NAb-negative and low titer (x1)-positive subjects, whereas gene expression was not observed in animals with higher titer (x4), implying that the threshold value for the transgene expression lies around low NAb titer (x1), in this experimental settings. These results are compatible with our previous observations using non-human primates, indicating that pigs are useful in gene therapy experiments, and the role of low titer NAb upon gene expression by intravenous administration of AAV vector shows similarity across species.

P242

Human skeletal muscle xenograft as a tool to assess transduction efficiency of AAV serotypes

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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 muscular dystrophies. These disorders represent an heterogeneous group of diseases affecting muscles and typically characterized by a progressive skeletal muscle wasting and weakness and the development of fibrosis. The tropism of each AAV serotype has been largely studied using systemic delivery routes, but very few studies have looked at their efficiency via direct intramuscular injection. Yet, in some diseases, where 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 the different AAV serotypes available is then needed. Here, we investigated the transduction efficiency of AAV serotypes by local injection in mouse skeletal muscle. We used a CMV-nls-LacZ reporter cassette to precisely localize transduced nuclei/cell type. 10 different serotypes of AAV-CMV-nls-LacZ were injected in control C57BL/6 mice and dystrophic fibrotic sgca-null/scid/bg mice, allowing us to compare muscle transduction in normal and fibrotic environment. The transduction efficiency was evaluated by detecting beta-galactosidase activity on TA muscle cryosections. Our preliminary results show that AAV 7, 8, 9 and 10 are the most efficient in both mouse strains analysed, with equivalent transduction efficiency in both strains, indicating that extracellular matrix accumulation did not interfere with the diffusion of the AAVs throughout the TA. Using

human muscle xenografts in immunodeficient mice, we further tested the transduction efficiency of these same AAVs to evaluate their efficiency in a human context.

P243

Analysis of ectopic dysferlin expression effect on the proliferative activity of HEK293A cells after electroporation

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Mutations in human dysferlin gene (DYSF) lead to the development of dysferlinopathies. Accumulation of dysferlin protein in muscle cells-sarcolemma's sites of damage has been described. Therefore, participation of this protein in sarcolemma repair processes can be assumed. Synthesis of a non-functional protein or its insufficiency due to mutations in DYSF gene leads to sarcolemma recovery processes and vesicular transport disruption. To achieve ectopic dysferlin expression HEK293A cells were transfected with previously obtained plasmid vector pCMV-DYSF encoding human dysferlin gene. Transfection was performed using TurboFect kit according to manufacturer's recommendations. To evaluate the efficiency of transfection and comparative analysis, HEK293A cells were transfected with the plasmid vector pEGFP-N2. Damage to the cell membrane by cell electroporation was performed 48h after transfection at 200 V (25 ms pulse time, 0.4 m cuvette). MTS proliferation assay was performed 48h after electroporation. Proliferative activity of HEK293A cells transfected with plasmid pCMV-DYSF (HEK293A-DYSF) decreased by 20% after electroporation compared to HEK293A-DYSF cells without electroporation. Interestingly, electroporation decreased the proliferative activity of HEK293A cells transfected with plasmid pEGFP-N2 (HEK293A-EGFP) by 40% compared to HEK293A-EGFP cells without electroporation. Thus, after electroporation, the proliferation activity of HEK293A-DYSF cells was 20% higher than in control HEK293A-EGFP cells. These results may indicate positive effect of wild-type dysferlin expression on membrane repair processes in human cells. However, further studies using other cell types and membrane damaging effects are needed to confirm this theory.

P244

Persistence of CRISPR/Cas9-edited hematopoietic stem and progenitor cells and reactivation of fetal hemoglobin in nonhuman primates

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A promising treatment strategy for hemoglobinopathies consists in the genome engineering of patients' hematopoietic stem and progenitor cells (HSPCs) to reactivate fetal hemoglobin (HbF) as substitute for defective or absent adult hemoglobin. Here, we evaluated CRISPR/Cas9-induced small deletions in HSPCs that are associated with hereditary persistence of fetal hemoglobin (HPFH) in a nonhuman primate (NHP) transplantation model. A first cohort of three rhesus macaques received 70-75% HPFH-edited bone marrow (BM)-derived $CD34^+$ HSPCs. All animals showed rapid hematopoietic recovery and peripheral blood (PB) editing levels stabilized at 12-30% for at least a year post transplantation. HbF production, determined by circulating F-cells, persisted at frequencies of 8-22% and correlated with PB editing. To circumvent challenges associated with the manipulation and engineering of the large number of CD34+ HSPCs, we transplanted a second cohort of three animals by solely editing the hematopoietic stem cell (HSC)-enriched $CD34^+CD45RA^-CD90^+$ population, shown to be required for short- and long-term multilineage reconstitution. This approach reduced the number of target cells by over 10-fold without impacting hematopoietic recovery, *in vivo* editing, and HbF reactivation. Robust engraftment of gene-edited HSPCs in the BM compartment was confirmed in all animals, and safety of our approach was confirmed with the absence of off-target activity and clonal expansion. These results demonstrate persistence of CRISPR/Cas9-edited HPSCs with therapeutically relevant HbF reactivation in a NHP transplantation model. Targeting of the HSC-enriched population should facilitate clinical translation of this approach, bypassing the need for scaled up parameters without compromising editing or engraftment efficiencies.

P245

Using hiPSC-derived retinal organoids to model Ush2a pathophysiology

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Human communication and perception of the environment are mostly conveyed by information perceived through the ear and the eye. A number of different inherited conditions impair both sight and hearing. The most common inherited deaf-blinding disease is USHER syndrome (USH), with USH2 patients accounting for almost half of all the USH cases. Here we use induced pluripotent stem (iPS) cells to model disease *in vitro*. We have generated iPS cell lines from USH2A patients and utilized our recently described 2D/3D protocol to differentiate these into retinal organoids. Immunohistochemistry and real time PCR analysis were used to assess the expression of cilial and USH genes in controls and USH2A retinal organoids. Electron microscopy was used to evaluate photoreceptor ultrastructure. Photoreceptor degeneration was evaluated by Tunel staining. Retinal organoid cultures generated photoreceptors containing synapses, connecting cilia, inner segments and outer segments. USH proteins were present in iPSC-derived USH2a photoreceptor cells and electron microscopy did not demonstrate morphological abnormalities in cilia and outer segment formation. However, increased photoreceptor cell death was observed in USH2a retinal organoids when compared to control organoids. We have now started to evaluate cellular metabolism pathways such ER stress, autophagy and oxidative stress to further understand the cause of degeneration *in vitro*. These data suggest that USHER2a iPSC-derived retinal organoids represent a potential tool to model disease and therefore enhance our

understanding USH pathophysiology and the mechanism of degeneration. Most importantly, diseased retinal organoids will aid the development of new treatments such as drug screening and gene therapy.

P246

Human induced pluripotent stem cell-derived astrocytes are differentially activated by multiple sclerosis-associated cytokines

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Astrocytes occupy a central place in the homeostasis of the central nervous system (CNS). Recent studies highlighted the importance of astrocytes in neuroinflammatory diseases, especially in multiple sclerosis (MS), interacting closely with other CNS cells but also with the immune system. However, due to the difficulty in obtaining human astrocytes, their role in these pathologies is still poorly characterized. Here, we describe a new protocol to differentiate astrocytes from hiPSCs derived from the blood of MS patients and healthy donors and we studied their response to MS-associated cytokines. So far, we generated iPSC clones from PBMC of eight donors (three MS patients, three healthy donors and one NIND) and subsequently differentiated them in astrocytes. We developed a new serum-free protocol to differentiate human iPSCs into astrocytes. Gene expression and functional assays showed that our protocol consistently yields a highly enriched population of resting mature astrocytes across the thirteen hiPSC lines differentiated (five hiPSC lines from healthy donors and eight hiPSC lines from MS patients). Using this new model, we first highlighted the importance of serum-free media for astrocyte culture to generate resting astrocytes. Second, we assessed the astrocytic response to IL-1β, TNFα and IL-6, all cytokines important in neuroinflammation, such as multiple sclerosis. Our study reveals very specific profiles of reactive astrocytes depending on the triggering stimulus. This new model provides ideal conditions for in-depth and unbiased characterization of astrocyte reactivity in neuroinflammatory conditions.

P247

High-fidelity disease modelling of skeletal muscle laminopathies using LMNA-mutant human iPS cells and bioengineered muscles

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Laminopathies are heterogeneous genetic diseases caused by mutations in A-type lamins, which are encoded by the LMNA gene. These proteins together with Lamin B1 and B2 form the nuclear lamina: a mesh-like structure located underneath the nuclear membrane which helps maintaining nuclear shape and regulating gene expression. Laminopathies affect multiple cell types and can be tissue-specific or systemic, with some subtypes affecting striated muscle, peripheral nerve and adipose tissue, while others cause multisystem disease with accelerated aging. Although several mechanisms have been proposed, the exact pathophysiology of laminopathies remains unknown; additionally, the rarity of the disorder and lack of easily accessible cell types for *ex vivo* studies negatively impact on therapy development. To overcome these hurdles, here we used induced pluripotent stem (iPS) cells from patients with skeletal muscle laminopathies such as LMNA-related congenital muscular dystrophy and limb-girdle muscular dystrophy 1B, to model disease phenotypes *in vitro*. iPSC lines from three skeletal muscle laminopathy patients were differentiated into skeletal myogenic cells and myotubes. Disease-associated phenotypes were observed in all genotypes, including abnormal nuclear shape and mislocalisation of nuclear lamina proteins. Notably, complex modelling in three-dimensional artificial muscle constructs resulted in recapitulation of nuclear abnormalities with higher fidelity than standard bi-dimensional cultures and identified nuclear length as a robust and objective outcome measure. These results demonstrate that patient-specific iPS cells can model cellular hallmarks of skeletal muscle laminopathies with high fidelity upon differentiation *in vitro*, laying the foundation for future drug screening platforms and gene therapy programmes for skeletal muscle laminopathies.

P250

CRISPR/Cas9- and TALEN-mediated disruption of aberrant regulatory elements restores normal splicing and gene function

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Gene therapy using designer nucleases for precise genome editing suffer from low efficiencies, as most of the correction approaches are based on homologous recombination, impeding their progress to clinical trials. In this study, we established a novel mutation-specific approach, where correction of the common HBBIVSI-110(G>A) β-thalassemia mutation, creating an exon-proximal aberrant splice acceptor site, can be achieved through the more efficient non-homologous end joining-mediated disruption of the aberrant regulatory elements (DARE) caused by the mutation. Proof of principle of DARE was initially established in a transgenic HBBIVSI-110(G>A) cell model, where treatment with a CRISPR/Cas9 RNA-guided nuclease (RGN) or TAL effector nucleases (TALEN) restored correct splicing. Clonal analyses showed that disruption of the upstream region of HBBIVSI-110(G>A) was sufficient to correct splicing. In patient-derived CD34+ cells, DARE mediated by nucleofection of ribonucleoprotein for the RGN and *in vitro* synthesized mRNA for TALENs, was characterized by targeted deep sequencing, which demonstrated up to

95% on-target disruption, with comparable performance by both platforms. Based on conditions and designs that minimized off-targeting at the HBB paralog HBD, DARE achieved significant correction of two key parameters defining β-thalassemia pathology, globin-chain synthesis and erythroid differentiation, in nuclease-treated CD34+ bulk populations. DARE would be applicable to a plethora of human diseases caused by aberrant regulatory elements outside open reading frames. The present study validates DARE as a novel virus-free and DNA-free mutation-specific therapy at efficiencies in primary patient-derived cells suitable for direct clinical translation without enrichment of modified cells.

P251

Preclinical assessment of *in vivo* gene editing efficiency, specificity, and tolerability of EDIT-101, an investigational CRISPR treatment for Leber congenital amaurosis 10 (LCA10)

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1: Editas Medicine

Leber Congenital Amaurosis 10 (LCA10) is an early-onset retinal degenerative disease caused by mutations in the CEP290 gene. Because of its large size ($\sim$7.5kb cDNA), CEP290 is not amenable to AAV-mediated gene augmentation therapy. EDIT-101 is an AAV5 vector expressing SaCas9 driven by the photoreceptor-specific GRK1 promoter and gRNAs selectively targeting the common CEP290 IVS26 c.2991+1655 A>G mutation. To support the clinical development of EDIT-101, we evaluated the PK/PD profile of EDIT-101 in human CEP290 IVS26 knock-in mice and CEP290 gene editing efficiency of surrogate NHP vector in cynomolgus macaques following subretinal delivery. Targeted editing in human photoreceptors was also assessed in human retinal explants. In NHP, tolerability was assessed using indirect ophthalmoscopy, slit-lamp biomicroscopy, and intraocular pressure measurements at 6 and 13 weeks post-injection. Immunogenicity to AAV5 capsid and SaCas9 was assessed by periodic ADA and ELISPOT assays. Guide RNA specificity was evaluated in two stages: a discovery phase utilizing in-silico prediction, GUIDE-Seq and Digenome, followed by a verification phase utilizing targeted NGS in human retinal explants and cell lines. EDIT-101 and the surrogate NHP vector achieved therapeutically relevant editing of the CEP290 gene in photoreceptors in mice and NHP. Both vectors were well tolerated based on clinical examinations despite an ADA response to AAV5 in NHP. Targeted NGS verified the high specificity of EDIT-101 and supports the development of EDIT-101 towards the clinic. The *in vivo* CRISPR approach may have broad application to other inherited retinal diseases with significant unmet medical need.

P252

In vivo genomic deletion of expanded CTG repeats reduces pathological signs of myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1), the most common inherited myopathy in adults, is caused by a CTG repeat expansion located in the 3′ untranslated region (3′UTR) of the myotonic dystrophy protein kinase gene (DMPK). Mutated DMPK transcripts are trapped into the nucleus, where they aggregate in foci with RNA-binding proteins, leading to defects in the alternative splicing of numerous pre-mRNAs and cellular dysfunction. To date, there is no curative treatment for DM1. Here, we investigated a gene editing strategy using the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system to delete the CTG repeat in the human DMPK locus. Co-expression of two single guide RNAs (sgRNAs) complementary to genomic regions situated on each side of the trinucleotide expansion and Staphylococcus aureus Cas9 (SaCas9) in cultured DM1 myoblasts carrying 2600 CTG repeats resulted in targeted DNA deletion, nuclear foci disappearance and correction of splicing abnormalities in various transcripts. Based on these *in vitro* results, we generated serotype 9 recombinant adeno-associated virus (rAAV9) vectors expressing SaCas9 under a muscle-specific promoter and a selected pair of sgRNAs flanking the trinucleotide repeat. Intramuscular dual vector delivery in DMSXL mice, which harbor a human pathogenic DMPK allele, resulted in the excision of the genomic CTG repeat region, a reduction of foci in myonuclei and reversion of aberrant splicing. These results establish the proof of concept that expanded CTG repeat deletion is feasible *in vivo* and can ameliorate pathological signs of the disease, which may represent a promising approach for the treatment of myotonic dystrophy.

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CRISPR/Cas9-mediated therapeutic editing of Rpe65 ameliorates the disease phenotypes in a mouse model of Leber congenital amaurosis

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Background: Leber congenital amaurosis (LCA), one of the leading causes of childhood-onset blindness without a cure, is caused by autosomal recessive mutations in several genes including RPE65. In this study, we performed CRISPR/Cas9-mediated therapeutic correction of a disease-associated nonsense mutation in Rpe65 in rd12 mice, a model of human LCA. Results: Subretinal injection of adeno-associated virus carrying CRISPR/Cas9 and donor DNA resulted in >1% homology-directed repair (HDR) and $\sim$1.6% deletion of the pathogenic stop codon in Rpe65 in retinal pigment epithelial tissues of rd12 mice. Our approach recovered scotopic a- and b-waves to levels up to 21.2$\pm$4.1% and 39.8$\pm$3.2% of their normal counterparts in rd12 mice at 7 months after injection. There was no definite evidence of histologic perturbation or tumorigenesis during 7 months of observation. Conclusions: Collectively, we present the first therapeutic correction of an Rpe65

nonsense mutation by HDR using CRISPR/Cas9, providing new insight for developing therapeutics for LCA.

P254

Optimization of a CRISPR/Cas9-based strategy for the correction of CD40LG gene in human haematopoietic stem cells and T cells

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X-linked hyper-IgM syndrome (HIGM1) is caused by mutations in CD40LG. CD40L protein loss in $CD4^+$ T-cells impairs their ability to signal for B-cell activation and immunoglobulin class-switching. Since CD40L unregulated expression caused lymphoproliferation/lymphomas in mice, we aimed to correct CD40LG while preserving its physiologic regulation. Corrected autologous T-cells could provide immediate therapeutic benefit to patients by resolving pre-existing infections and bridge them towards a possible cure by hematopoietic-stem/progenitor-cell (HSPC) transplant. To confirm this, we infused different doses of wild-type T-cells into HIGM1 mice, with or without preconditioning, and reached long-term, stable T-cell engraftment and partial rescue of antigen-specific IgG response upon vaccination. Additionally, we optimized a CRISPR/Cas9-based protocol to insert a corrective cDNA into the first intron of CD40LG in human T-cells, obtaining $\sim$30% correction while preserving the long-term repopulating T-stem-memory-cells. CD40L expression and physiologic regulation was restored on edited $CD4^+$ T-cells from HIGM1 patients, which provide normal contact-dependent helper function to B-cells by measuring their in-vitro proliferation, class switching, and IgG secretion assays. To increase the purity of edited T-cells before transplant, we coupled the corrective cDNA with a clinically-compatible selector gene and confirmed that enriched T-cells preserve their engraftment capability in NSG mice. To define a threshold of HSPC correction for a life-long therapy of HIGM1, we performed competitive transplants in the mouse model and found that as low as 10% of wild-type HSPCs restored serologic immunity. Utilizing an optimized RNP/AAV6 editing protocol, we reached this correction threshold in human HSPCs ($\sim$30% editing in the primitive subpopulation).

P255

Homology-independent targeted integration for gene correction in photoreceptors

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Retinitis pigmentosa (RP) is a heterogeneous group of inherited ocular diseases affecting 1/3.000-5.000 people worldwide. Mutations in the rhodopsin gene (RHO) are responsible for about 20% of cases of dominant RP. We have developed an allele-independent genome editing strategy for correcting the RP mouse retinal phenotype by editing murine rhodopsin (mRho) after its cleavage using Cas9 and the homology-independent targeted integration (HITI) system. We have designed a gRNA specific for mRho Exon 1, and used dsRED as an insert for proof of concept studies both *in vitro* and in the retina. In HEK293 cells co-transfected with a template CMV-mRho plasmid, Cas9 cleaves both the target locus and the donor DNA, and integration occurs in 77,6% of cells, with surprising precision. We used subretinal injection to deliver 2,5*109 genome copies of each of two AAV8 vectors, encoding Cas9 and the donor DNA respectively, in the retina of 4-week old C57BL/6 mice, and 30 days post-injection we observed up to 10% efficiency of dsRED integration in rod photoreceptors, only when the donor DNA is delivered together with mRho specific gRNA. We are currently using this same approach to integrate a correct copy of human RHO into the mRho locus in order to achieve rescue of dominant RP in a mouse model.

P256

Silencing Huntingtin in the hypothalamus of a transgenic Huntington disease rat model using AAV-mediated microRNA strategy

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Huntington Disease (HD) is an autosomal dominant neurodegenerative disorder with a prevalence of 4-10 individuals per 100000. It is an adult-onset, chronic disease with progressive motor, cognitive, psychiatric and metabolic impairments. The sole cause of HD is the abnormal expansion (>40 Q) of the polyglutamine (polyQ) tract in exon 1 of the huntingtin (HTT) gene encoding the huntingtin protein (htt). Despite recent advances, neither disease-modifying nor preventive treatments are available at present. Existing medications are limited and only provide temporary symptomatic relief to the patients. Due to the monogenic nature of HD, strategies that delete or suppress mutant htt (mhtt) could become an effective disease-modifying treatment for HD. In this study, we examined the therapeutic efficacy of mhtt downregulation in a transgenic HD rat model, the BACHD rat, which carries the full-length human mHTT with 97 polyQ repeats and displays HD-like phenotypes. A recombinant adeno-associated virus (AAV)-mediated microRNA that could suppress mHTT was delivered to the lateral hypothalamus of the BACHD rats at 1 and 6 months of age representing the early disease and symptomatic stages respectively. Behavioural and post-mortem analyses showed that improvements were not limited to metabolic but also motor phenotypes at both therapeutic time points when mhtt was downregulated. Our study demonstrates that the

beneficial effects of this treatment strategy are not confined and defined by the vector delivery site in the central nervous system.

P257

Highly efficient single and multi-gene knockout with CRISPR/Cpf1 in T cells for the development of improved cell therapies

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Gene editing using RNA-guided nuclease technology has gained widespread attention for its potential to improve current cell therapies. The CRISPR-Cpf1 (also known as Cas12a) system is complementary to Cas9 with several distinct differences. Cpf1 uses a single ~40 nucleotide crRNA and can target T- and C- rich PAMs with the WT and engineered PAM variants. The expanded targeting space, when compared to the purine rich PAMs of Cas9, makes it an attractive addition to enable broader targeting opportunities. Unlike SpCas9, Cpf1 makes a staggered cut in the DNA leaving behind a 4-5 nucleotide 5′-overhang, which could result in different editing outcomes. We screened multiple loci of therapeutic interest in T cells with AsCpf1 and its engineered RR and RVR PAM variants and optimised multiple components of the Cpf1 RNP assembly and nucleofection process to improve editing efficacy without compromising cell viability. Robust single (>95% KO) and multiplexed (80-90% double KO) gene disruption was observed with Cpf1 enzymes when delivered as an RNP. Multiple published studies have shown that Cpf1 is highly intolerant to DNA:RNA mismatches in biochemical and cellular assays. We conducted specificity studies using GUIDE-Seq, Digenome-Seq, and in silico modeling followed by targeted NGS sequencing. Our results are consistent with Cpf1 being a highly specific enzyme. Taken together, these data suggest that Cpf1 is both a robust and specific technology for developing T cell-based medicines.

P258

***In utero* AAV-based genome-editing to cure a mouse model of human hereditary tyrosinemia type 1**

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In utero gene therapy is an advanced approach that offers significant advantages over postnatal therapy. *In utero* gene therapy for liver disease can be curative while also addressing liver disease before any clinical consequences occur. In addition, the liver is an ideal organ for gene therapy *in utero* due to its easy access and rapid growth. We show here for the first time that *in utero* gene editing can cure a mouse model of liver disease—in this case, hereditary tyrosinemia type I (HT1). Two AAV vectors expressing S. aureus Cas9 with gRNAs and a 1.2kb homology repair template were developed to correct the point mutation in fumarylacetoacetate hydrolase (FAH)-deficient mice. We performed fetal intrahepatic injections of both vectors in Fah-/- mice at E15 ± 1. Pups were withdrawn from 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) at the time of weaning (21 days) and were weighed daily for NTBC-independent growth. Dosed mice demonstrated the presence of FAH positive cells in the liver at birth (3.99%) and showed healthy NTBC-independent growth after weaning, with many animals requiring no NTBC therapy. By 70 days of life all animals were phenotypically cured, with complete liver repopulation by FAH-positive hepatocytes and normal plasma tyrosine levels. The cure was durable when followed to 186 days of life. This report is the first ever to use *in utero* AAV-based genome editing to cure a mouse model of a human disease and acts as a proof-of-concept approach that would prevent liver damage, neurological crises, and noncompliance issues in HT1 patients.

P259

Efficient *in vivo* delivery of CRISPR ribonucleoprotein using polymer nanoparticles

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CRISPR associated proteins have shown their potential to revolutionize the world of gene editing therapeutics, but an unmet need for their translation is achieving effective and safe delivery. In our studies, we have demonstrated that it is possible to improve the gene editing capabilities of CRISPR using both engineering and novel delivery mechanisms. We have achieved successful delivery of the CRISPR using polymer nanoparticles, which can facilitate the simultaneous delivery of CRISPR ribonucleoproteins (RNPs). In the brain, we have demonstrated that polymer nanoparticle delivery of the CRISPR system can be used to treat Fragile X Syndrome in mice. Specifically, delivery of the CRISPR-polymer nanoparticle into the brain via intracranial injection was shown to knock out the target gene resulting in edited mice exhibited fewer exaggerated repetitive behavior phenotypes in FMR1 knock-out mouse. Additionally, polymer nanoparticles are effective at delivering CRISPR into muscle tissue. We have demonstrated that it is possible to induce homology-directed repair in muscle tissues to correct a mutation in dystrophin gene causing Duchenne Muscular Dystrophy in mouse. Lastly, to expand the applicability of CRISPR, we have explored the editing potential of Cpf1. With our polymer nanoparticle system, we successfully delivered Cpf1 into cells for the first time using a non-viral delivery mechanism. Engineered the Cpf1 enhanced its gene editing efficiency and polymer encapsulation capability. Overall, our discoveries have shown the promise for its therapeutic applications. We will present the essence of works published in two Nature BME articles and one accepted article in Nature Comms.

P260

NHEJ-mediated gene editing phenotypically corrects Fanconi anemia A patients' haematopoietic stem and progenitor cells

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Gene editing is considered a promising alternative to correct patients' hematopoietic stem and progenitor cells (HSPCs). Since non-homologous end-joining (NHEJ) is the preferential DNA-repair mechanism in HSCs –particularly in the case of Fanconi anemia (FA) cells– we aimed at exploiting this pathway to correct FANCA gene mutations, mimicking spontaneous reversions observed in FA mosaic patients. To demonstrate the feasibility to target FANCA in repopulating HSCs, healthy donor (HD) hCD34+ cells were electroporated with designed CRISPR/Cas9 nucleases and transplanted into immunodeficient mice. Edited cells demonstrated unaltered long-term engraftment and differentiation capacities, both in primary and secondary recipients. Interestingly, Next-generation sequencing (NGS) evidenced that the percentage of insertions/deletions (indels) in the pool of hCD34+ cells was similar to the one obtained in primary and secondary recipients, demonstrating the possibility to target true HSCs. Moreover, an *in vivo* clonal succession of edited HSCs was evident over time. As a next step, mobilized peripheral blood hCD34+ cells from FA-A patients harboring the c.295C>T mutation in FANCA (the most frequent in Spain) were targeted as described with HD hCD34+ cells. NGS analyses showed between 0.45-8.0% of therapeutic indels. As a consequence, a marked *in vitro* and *in vivo* proliferative advantage was observed, as well as a significant increase in the mitomycin C resistance of hematopoietic progenitor cells. Finally, NGS confirmed no unspecific editing in the top-five in silico predicted off-target. These results demonstrate for the first time the possibility to correct HSPCs from FA patients by a safe and simple NHEJ-based gene editing strategy.

P261

Single cell transcriptomic analysis of gene edited HSPC uncovers molecular targets to improve long-term cell repopulation ability

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Gene editing in Hematopoietic Stem/Progenitor cells (HSPC) holds the promise to provide a safe and effective therapeutic option for many blood-related disorders. Yet, little is known about the cellular responses triggered by programmable nucleases and viral vectors in ex-vivo edited HSPC, which could adversely affect the long-term stability of the hematopoietic graft after transplantation. Here, we induced site-specific breaks by optimized CRISPR/Cas9 and new-generation Zinc-Finger nucleases and used AAV6 as vehicles for the donor DNA template, to study their impact on HSPC biology and functionality. By unbiased single-cell transcriptomic analysis of primitive cell subpopulations (>15.000 cells), we uncovered several extents of DNA Damage Response (DDR) activation that were consistent across different HSPC types/states. P53 pathway activation and downregulation of cell-cycle progression resulted the predominant/almost-only detectable responses to the DNA DSB and were virtually harmless to HSPC when using highly specific nucleases transiently acting on the single/few intended target sites. Instead, the extent of such responses substantially increases with lower specificity reagents or AAV6 transduction, up to the activation of pro-inflammatory programs, partially affecting the in-vitro clonogenic and in-vivo repopulating activities of edited HSPC. Short transitory dampening of DDR during the editing procedure, achieved by delivering an mRNA for a dominant-negative p53-peptide, increased the efficiency of homology-driven repair (>40% in primitive CD90+ cells) and the in-vivo repopulating capacity of edited HSPC (2-fold over control) in serial NSG transplantations. These findings provide molecular evidence of the feasibility of seamless targeted gene editing in HSPC, giving confidence to its prospective translation in humans.

P262

Novel vector systems for transient delivery of CRISPR-nucleases

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The method of delivery of CRISPR-nucleases into target cells is a strong determinant of efficacy and specificity in genome editing. Even though high efficiency of RNA-guided nuclease delivery is necessary for optimal editing, its long-term expression correlates with increased off-target activity and risk of immune responses against targeted cells. To address these issues we developed two novel vector systems for genome editing resulting in the transient delivery of CRISPR/Cas9. 1) lentiSLiCES: a lentiviral-based delivery system encoding a Self-Limiting Cas9 circuit for Enhanced Specificity, which consists of a Cas9 expression unit, a self-targeting sgRNA and a second sgRNA targeting a chosen locus. After circuit inhibition to achieve viral particle production, the lentiSLiCES self-limiting circuit switches on into target cells, editing the intended genomic locus while simultaneously inactivating Cas9. By controlling levels and timing of nuclease expression, lentiSLiCES results in increased genome editing specificity. 2) VEsiCas: a delivery platform based on VSV-G vesicles carrying CRISPR/Cas9 ribonucleoprotein complexes (RNPs). A crucial step for VEsiCas production is the synthesis of sgRNAs by the T7-RNA-polymerase in the cytoplasm of producing cells as opposed to canonical U6-driven Pol-III nuclear transcription. VEsiCas are free of DNA encoding Cas9/sgRNA, which allows rapid clearance of Cas9-RNPs from target cells, minimizing genome-wide off-target cleavages. VEsiCas delivered with high efficiency and low toxicity Cas9-RNPs in several transformed cells, iPSCs and

cardiomyocytes *in vivo*. Being a traceless and efficient CRISPR-nuclease delivery tool, VEsiCas represents an advancement toward the therapeutic use of the CRISPR/Cas9 technology.

P263

A minicircle-based screening system facilitates the selection of promising CRISPR/Cas9 double nicking pairs for homology-directed repair of genetic mutations

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Double nicking using Cas9 nickase pairs represents a promising option to induce double strand breaks (DSB) at a target gene locus of interest, which is the basis for the subsequent correction of pathogenic mutations via homology-directed repair (HDR). Compared to the commonly used spCas9 from Streptococcus pyogenes the use of a guide-RNA pair together with the D10A/Cas9 nickase reduces the likelihood of unwanted off-target events but at the same time increases HDR efficiency. Whereas specificity, cutting activity and repair efficiency are mainly determined by the selected gRNAs, the presence of homologous DNA sequences for HDR is a prerequisite. We designed and established a minicircle-based gRNA screening and HDR detection system and applied it to the COL7A1 gene, in which mutations are the main cause of the dystrophic form of epidermolysis bullosa (DEB), to pre-select most promising double-nicking gRNA pairs for gene repair. With this antibiotic selection-based system, we were able to detect HDR already 4 days after transfection into patient keratinocytes and reached targeting efficiencies between 50% and 98% for the most functional gRNA pairs 2 weeks after treatment. We analyzed and compared HDR efficiencies in various settings, including single nicking, double nicking or spCas9-mediated DSB induction via next generation sequencing (NGS), thereby confirming double nicking as the most efficient technique for HDR induction. In conclusion, this plasmid-based screening system represents a robust tool for the selection and optimization of HDR-mediated gene therapy approaches.

P264

All-in-one delivery of gene-editing system into primary cells and *in vivo* using LentiFlash®, a MS2-chimeric viral RNA delivery tool designed for clinical applications

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Gene editing shows great promises for a wide range of therapeutic areas such as genetic disorder treatment, cancer immunotherapy and antiviral strategies. Nevertheless, the use of gene editing systems must now face a number of challenges in *ex vivo* and *in vivo* clinical programs. The transfer of gene-editing systems, mediated by DNA delivery tools like viral vectors or non-viral transfection protocols have been widely used in research. These tools show major drawbacks such as toxicity, phenotype modifications, chromosomal integrations and long-term nuclease expression (i.e. maximization of off-target events) and are thus incompatible with clinical applications. As a game-changing RNA carrier, LentiFlash overcomes DNA delivery issues. It allows the encapsulated RNAs to be directly delivered and transiently expressed into the cytoplasm. LentiFlash technology is a bacteriophage-lentivirus chimera that efficiently transfers RNA *in vivo* and *ex vivo* without any risk of integration. LentiFlash particles are able to deliver non-viral RNA in any cell types such as T cells, HSC and muscle cells. Here, we demonstrate that all-in-one LentiFlash particles carrying the CRISPR/Cas9 technology can be efficiently used for knock-out, knock-in and exon skipping applications. The combination of genome-editing and immunotherapy approaches that aims to design one-time therapies will be presented. LentiFlash benefits from the same production process as lentiviral vectors and uses the whole cell manufacturing platform validated in clinical settings. LentiFlash-mediated transduction preserves the viability and original cell phenotype. These properties offer an important safety consideration for clinical development and therapy in Human.

P265

Specific knock-down of C-terminal dominant mutation in Rhodopsin gene by CRISPR/Cas9 system

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A common cause of blindness is represented by mutations in Rhodopsin (RHO) gene, accounting for 25% of autosomal dominant Retinitis Pigmentosa (adRP). Therapeutic benefit for adRP may be achieved by using CRISPR/Cas9 system to precisely and permanently inactivate the mutated allele. In this study, we employed CRISPR/Cas9 nucleases as genome-editing tools to knock out a RHO allele carrying a C-terminal mutation (P347S-RHO, c.1040C>T), while preserving the wild-type (wt) allele. We compared mutation-specific targeting of wild-type SpCas9 guided by a gRNA (gRNA1) carrying the C-to-T transition on the nucleotide next to NGG PAM, and high-fidelity VQR variant (SpCas9-VQR-HF1) guided by a gRNA (gRNA5) with NGAG PAM generated by the point mutation. We tested the designed gRNAs *in vitro* on HeLa clones stably expressing P347S or wt RHO. Frequency and type of insertions/deletions (Indels) were analysed by TIDE analysis, single amplicon sequencing and NGS. Genomic analyses clearly showed that the SpCas9 is more efficient in editing the P347S target locus (SpCas9 vs SpCas9-VQR-HF1: 72% vs 40%), with almost undetectable editing of the wt allele. RTqPCR on edited P347S HeLa clones demonstrated a strong reduction (up to 50%) of the mutant RHO mRNA level compared to un-transfected cells. Western Blot analysis confirmed specific knockdown of Rhodopsin in edited P347S clones. Ongoing analyses are evaluating expression, localization and potential cytotoxic effects of the most frequent mutants resulting from CRISPR treatment. Our results will provide clear evidences about the employment of CRISPR/Cas9 system to selectively target C-terminal dominant mutations responsible of adRP. (Funding: Fondazione Roma)

P266

Demonstrating therapeutic applicability of homology-independent targeted integration (HITI) in a fluorescent reporter cell line

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Homology directed repair (HDR) is often selected for correction of genomic mutations for therapeutic benefit due to its favourable repair fidelity. The activity of the main repair pathway, non-homologous end joining (NHEJ) however, is several-fold higher than HDR in most systems. Homology-Independent Targeted Integration (HITI) allows for precise integration of a chosen sequence by cutting both the genome and donor sequence. We developed a HITI (mNeonGreen and mCherry) reporter cell line to assess the different configurations of homology-independent DNA integration and demonstrated the feasibility of all four possible permutations. mNeonGreen can be expressed efficiently with or without mCherry expression based on the inclusion of an IRES or transcription blocker and the location of mNeonGreen integration relative to mCherry. Whilst less efficient, it is also possible to target both gRNA sites flanking mCherry to replace it with mNeonGreen, emulating the targeted replacement of a mutated gene or exon. Usually, the HITI integration is seamless ($\sim$90%, 18 of 20 clones average, n = 3) with most mutations comprising 1 or 2bp indels, implying that NHEJ is not as error-prone as expected. We have utilised one of the HITI permutations to express mNeonGreen from the endogenous alpha-1 antitrypsin (AAT) promoter whilst simultaneously preventing endogenous AAT expression in Huh-7 liver cells. This provides an opportunity for treatment of the PI*Z mutation in AAT deficiency that can be toxic in hepatocytes. This HITI reporter cell line provides proof of concept for the investigation of in-vivo NHEJ-mediated DNA integration as an approach for correction of genetic diseases.

P267

Enhancing ZFN expression and nuclease activity in a genome editing construct leads to improvement of an *in vivo* genome editing platform

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Sangamo Therapeutics is evaluating a Zinc Finger Nuclease (ZFN)-mediated genome editing strategy to permanently modify patient liver cells through insertion of a corrective transgene at the Albumin locus, following systemic AAV2/6 delivery. We are currently evaluating this approach using donor constructs encoding the genes that are defective in Hemophilia B and mucopolysaccharidosis types I and II. Therapeutic transgene insertion into the Albumin locus, co-opting its high transcriptional activity, could potentially provide stable, long-term expression of the corrective transgene in stably modified hepatocytes. This *in vivo* genome editing approach depends upon effective ZFN expression and nuclease activity in patient hepatocytes. The work described here highlights the potential for next-generation *in vivo* genome editing constructs, demonstrated by improvements in both ZFN expression and nuclease activity through the rational enhancement of a) the AAV-ZFN expression construct backbone and b) the coding ZFN sequences by modulating both the DNA-binding and nuclease domains of the ZFNs. Importantly, selective substitution of ZFN amino acid residues at the protein-DNA interface allows for increased ZFN activity, the ability to tolerate a SNP in the ZFN Albumin target site, and greatly increased specificity. These improvements were achieved while preserving the original ZFN target site, which allows use of the original transgene donor construct. These enhancements further highlight the advantages of using ZFNs as a tool for the correction of monogenic disease via *in vivo* genome editing.

P268

Haematopoietic stem cell gene editing for the treatment of Wiskott-Aldrich syndrome

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Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency caused by mutations in the WAS gene and characterized by severe platelet abnormalities, defective immunity and development of autoimmune diseases and cancer. Although gene therapy approaches using integrating lentiviral vectors showed encouraging results, full immune and platelet reconstitution is not always achieved. Moreover, lentiviral vectors bear the potential risk of genotoxicity and non-physiological transgene expression in target cells. It is therefore desirable to develop new strategies for targeted gene correction. The goal of our study is to use CRISPR/Cas9-based genome editing to knock-in a wild-type WAS cDNA in its first coding exon, allowing transcriptional regulation from WAS endogenous promoter and functional correction of the mutations in primary human hematopoietic stem and progenitor cells (HSPCs). By co-delivery of Cas9-gRNA ribonucleoprotein complexes together with an AAV donor vector for targeted integration of a promoterless WAS cDNA, we achieved high rates of homology directed repair in HSPCs from multiple WAS patients, without impairing cell viability and differentiation potential. Optimization of culture conditions and editing protocols allowed targeted gene insertion in the most primitive stem cell population. Delivery of the editing reagents to WAS HSPCs led to full rescue of WAS expression and correction of functional defects in T cells, macrophages and platelets. We are currently assessing the long-term engraftment and differentiation potential of corrected HSPCs in animal models, to determine the best editing strategy and establish a viable therapeutic approach to treat WAS deficiency.

P269

Cultivation and characterisation of human airway epithelial cells with potential for CFTR editing for the development of cystic fibrosis therapies

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Cystic fibrosis (CF) is the most common heritable disease among people with Northern European ancestry, but, for most patients, there is no cure. It is caused by mutations in the CF gene, CFTR. Gene therapy by gene editing with CRISPR/Cas9 could provide precise and permanent correction of the CFTR gene. We aim to correct and expand epithelial progenitor cells *in vitro* and subsequently deliver them to the airways as cell therapy. We have optimised the culture conditions for expansion of human airway epithelial cells by culturing them in the presence of an irradiated mouse fibroblast feeder layer. This allows us to expand adult primary cells for over 10 passages (and more than 40 population doublings) while maintaining their ability to differentiate *in vitro* in Air Liquid Interface (ALI) cultures. The differentiated ALI cultures were shown to be positive for airway epithelial cell markers and differentiation markers. The cells also demonstrated electrical responses and the expected chloride channel activity, according to their phenotype (CF and Normal cells), in Ussing Chambers. We have optimised GFP transfection of primary CF epithelial cells with an efficiency of approximately 60%. CRISPR/Cas9 mediated double strand breaks were created in CFTR with optimal guide RNAs in 45% of cells. We have also developed a donor repair plasmid with a selection cassette, which will facilitate the correction of nasal epithelial cells with the most common CF mutation, ΔF508, exploiting the cells homology directed repair pathway. Corrected cells will be investigated for their potential to repair the CF epithelium.

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A new generation self-inactivating editing system with improved delivery

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Huntington's disease (HD) is a fatal neurodegenerative disorder caused by CAG expansion in the huntingtin (HTT) gene. Genome editing with the recently discovered CRISPR/Cas9 system represents an exciting alternative to tackle dominantly inherited genetic disorders such as HD. In a previous study, we developed a KamiCas9 self-inactivating editing system to achieve transient expression of the Cas9 protein and high editing efficiency using a double viral vector approach. Mutant huntingtin (HTT) was efficiently inactivated in mouse models of HD, leading to an improvement in key markers of the disease. Sequencing of potential off-targets with the constitutive Cas9 system in differentiated human iPSC revealed a very low incidence with only one site above background level. This off-target frequency was significantly reduced with the KamiCas9 system. In the present study, we improve this system in term of editing and delivery. At first, we combine the KamiCas9 in a single vector to avoid editing of the Cas9 gene during viral vector production in HEK-293T cells using the inhibitor proteins encoded by Listeria monocytogenes prophages (acrIIA4). Furthermore, to maximize viral-mediated gene transfer and retrograde transport, we took advantage of the neuronal circuitry using an improved version of HiRet lentiviral vector. This FuG/B2 envelope efficiently transduces neuronal and glial cells around the injection site and lead to high retrograde transport. This new generation of the KamiCas9 self-inactivating system will facilitate pre-clinical validation of gene editing in the CNS.

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Generation of memory stem T cells specific for tumour antigens and resistant to inhibitory signals by genome editing

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We recently showed that a large fraction of CD8+ cells, including long-living memory stem T cells (TSCM), infiltrating the bone marrow of patients with acute myeloid leukemia relapsing after allogeneic transplantation co-express multiple IRs, thus suggesting the need to overcome IR expression in adoptive T cell therapy approaches. Here we aim to simultaneously redirect T cell specificity and permanently disrupt IRs by CRISPR/Cas9 in TSCM for adoptive cell therapy. Primary T cells activated with CD3/CD28-conjugated beads and cultured with IL7+IL15 were electroporated with Cas9/gRNA ribonucleoproteins (RNPs) targeting Tim3, LAG3 and the TCR a and b chain constant region (TRAC and TRBC1/2) genes. The frequency of NHEJ was assessed with FACS analysis, surveyor assay and ddPCR. T cell specificity was redirected using a lentiviral vector (LV) encoding for an NY-ESO1-specific TCR (LV-NYESO1-TCR). The simultaneous TRAC and TRBC1/2 gene disruption resulted in 98% CD3neg cells, that were then efficiently transduced (59-68%) with LV-NYESO1-TCR. We then combined one IR disruption with TCR gene editing in a single protocol. We obtained an average of 65% and 66% of Tim3neg and LAG3neg NY-ESO1-TCR redirected T cells respectively. IR-disrupted TCR edited cells showed a TSCM functional phenotype and proved effective in specifically killing NY-ESO1+ tumor targets. More interestingly, the frequency of IFNg+ or TNFa+ cells was higher in Tim3neg and Lag3neg NY-ESO1-TCR redirected T cells compared to wild type TCR edited T cells. By exploiting the plasticity and multiplexity of CRISPR/Cas9 we generated innovative tumor-specific cellular products resistant to inhibitory signals.

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Cas9/gRNA selective targeting of the Beethoven tmc-1 mutant allele for treating progressive hearing loss by AAV-based delivery

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Hearing Loss (HL) is the most prevalent sensorineural disorder in humans affecting approximately 1/500 newborns and 4% of people at 45 years of age and younger, reaching 50% by 80 years of age. Among alternative therapeutic strategies, gene therapy holds promise for 50% of pre-lingual deafness cases

with a genetic cause. Transmembrane channel-like Protein 1 (Tmc-1) was identified as the gene underlying both dominant and recessive forms of non-syndromic sensorineural HL at the DFNA36 and DFNB7/11 loci, respectively. Families with DFNA36 present a Tmc-1 point mutation c.1253T>A (p.M418K) which is orthologous to the murine Beethoven (Bth) mutation (Tmc-1 c.1235T>A [p.M412K]). The natural progression of hearing loss in humans is closely replicated in the Bth-heterozygous mouse (Tmc-1Bth/+), therefore considered a useful model to test therapeutic approaches to prevent progressive HL in this genetic form of deafness. We are developing an AAV-based gene editing strategy using the CRISPR/Cas9 technology to selectively disrupt and knock-out the Bth-Tmc1 allele, and evaluate this strategy as a potential gene therapy to prevent hearing loss in humans. Efficacy of various gRNAs to accurately target the Bth-Tmc1 allele was evaluated in mouse and human cells in-vitro. To deliver the system into mouse cochlea, two AAV vectors carrying the CRISPR system were co-injected in the inner ear of Tmc-1Bth/+ mice and functional assays were performed to assess treatment efficacy. Following vector administration, Tmc-1Bth/+ mice presented functional hearing recovery suggesting that disruption of the mutated allele is sufficient to prevent hearing loss in a DFNA36 HL model.

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Precise *in vivo* genome editing using two gRNAs targeting nearby genomic regions

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The liver is affected by many genetic disorders associated with the accumulation of toxic metabolites. The inhibition of enzymes that generate these products may lead to metabolic correction and amelioration of disease phenotype. CRISPR/Cas9 is a precise gene editing tool that can be applied for this purpose. Recently, we have demonstrated that this strategy can be applied to treat primary hyperoxaluria, a disease associated with a malfunction of the glyoxylate detoxification pathway and accumulation of oxalate (OR30 ESGCT 2017). The elimination of glycolate oxidase (GO) using CRISPR/Cas9 resulted in amelioration of the disease. In this work, we have characterized by NGS the indels produced in the liver after NHEJ in CRISPR/Cas9-induced DSBs using two different gRNAs targeting Hao1 gene (coding for GO) and its effect over RNA and protein expression when administered individually or in combination using AAV8 vectors. The administration of a unique gRNA or the co-expression of 2 gRNAs targeting nearby regions resulted in a highly significant reduction GO expression. Indel profile was the same in all the animals treated with each gRNA, showing a sequence-dependent repair pattern, which allowed to predict the effect over the reading frame. Moreover, when the 2 gRNAs were co-expressed indel profile changed, generating an exact deletion of the region between both DSBs in a high percentage of sequences. In addition, the combination of the 2 gRNAs and D10A nickase Cas9 is being analyzed. Overall, our findings demonstrated that the ''non-specific'' NHEJ is predictable due to the sequence specificity of the repair.

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Reversal of spliceopathy in cardiomyocytes derived from myotonic dystrophy patient-specific iPS cells by gene editing with CRISPR/Cas9 ribonuclear protein complexes

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Myotonic dystrophy type-1 (DM1) is a dominant genetic muscle disorder due to a CTG-repeat expansion in the 3′-untranslated-region (UTR) of the human myotonic dystrophy protein kinase (DMPK) gene (Klein et al, Curr. Gene Ther., 2015). It is characterized by abnormal splicing in several genes involved in muscle and cardiac function. In the current proof-of-concept study, we explored the use of non-viral transfection with Cas9-sgRNA ribonucleoprotein (RNP) complexes for gene editing in DM1 patient derived induced pluripotent stem cells (DM1-iPSCs). To eliminate the pathogenic gain-of-function mutant DMPK transcript, we therefore designed a dual guide RNA-based strategy that excises the CTG-repeat expansion with high efficiency, as confirmed by Southern blot, triplet-repeat primed PCR and target region sequencing. CRISPR/Cas9 RNP transfection resulted in correction efficiencies upto 90% in DM1-iPSCs as confirmed in individual clones without the need for selective enrichment, (Dastidar et al, Nucl. Acids Res.,2018). Expanded CTG repeat excision resulted in the disappearance of ribonuclear foci in DM1-iPSCs and DM1-iPSC-derived cardiomyocytes. The CTG repeat excision restored the normal intracellular localization of the muscleblind-like splicing regulator 1 (MBNL1) and resulted in the correction of the characteristic spliceopathy upon cardiac differentiation. This study validates the use of transient transfection with Cas9-sgRNA ribonucleoprotein

(RNP) complexes as an efficient ''hit-and-go'' strategy for correction of DM1 iPSCs resulting in normalization of cardiac splicing patterns.

P275

AAV-mediated gene therapy for fALS

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The non-invasive administration of viral vectors derived from Adeno Associated Virus (AAV) to transduce cells in the central nervous system (CNS) opened new perspectives for the treatment of motor neuron disorders (MND). In particular, our group demonstrated the valuable therapeutic potential of self-complementary AAV9 vectors to transduce the CNS following systemic delivery (Barkats, PCT/EP2008/063297). A gene therapy approach, based on this method, was tested for Spinal Muscular Atrophy (SMA), in the severe mouse model of the disease and has been translated to SMA type I patients with encouraging results. Our team also exploited AAV vectors for the treatment of the most common MND in adults, Amyotrophic Lateral Sclerosis (ALS), a fatal condition with limited therapeutic options. While the majority of ALS cases are sporadic (90%), a small percentage of ALS cases have a familial history (fALS, 10%). We recently tested an AAV-mediated gene therapy for SOD1-linked ALS (representing about 20% of fALS cases). Specifically, we used the small nuclear RNA U7 carrying antisense oligonucleotides to silence the expression of toxic SOD1 through exon skipping. With this method we reported the best therapeutic effect obtained so far in SOD1G93A mice, the commonest ALS model for preclinical tests. We are now assessing the therapeutic potential of a similar approach for the most common form of fALS, linked to the hexanucleotide repeat expansions in intron 1 of the uncharacterized gene C9ORF72. Overall, these studies will contribute to the development of new treatments for MNDs.

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CRISPR/Cas9-AAV mediated editing for disorders of the central nervous system

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Allele specific genome editing rescues cystic fibrosis splicing mutations in patients organoids

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Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the CFTR gene. A significant number of mutations ($\sim$13%) alter the correct splicing of the CFTR gene, causing the production of aberrant mRNA transcripts and non-functional protein channels. One of these mutations, 3272-26A>G, involves an A to G substitution in position 3272-26 of intron 19 generating an alternative 3′ acceptor splice site followed by the inclusion of 25 nucleotides which determines frame shift and lack of CFTR expression. To permanently correct this splicing defect, we designed a genome editing approach, using a minigene model which reproduces the genetic defect. Full recovery of properly spliced CFTR mRNA was obtained using Acidaminococcus sp. Cpf1, AsCpf1, with a single crRNA which efficiently abrogated the mutated splice acceptor site. The AsCpf1 mediated repair is highly precise since no off-target was detected by genome-wide GUIDE-seq analysis and no modifications observed in the wild-type allele, analysed by deep sequencing. Finally, the efficacy of this gene correction was proven in organoids derived from a CF patient with the heterozygous 3272-26 A>G mutation, showing nearly complete

repair of the affected allele and full organoids functional recovery. A similar approach was applied to another CF splicing mutation, 3849 + 10Kb C>T, showing an almost complete recovery of CFTR function in patient organoids. These results demonstrate that allele specific genome editing with AsCpf1 can correct aberrant CFTR splicing paving the way for permanent splicing correction in genetic diseases.

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A high-throughput deep sequencing approach for CRISPR off-target assessment in therapeutic genome editing applications

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Presented is a high-throughput next-generation sequencing approach for investigating off-target effects of CRISPR genome-editing experiments. This approach involves the design of TruSeq Custom Amplicon (TSCA) primers for multiplexed target amplification and deep sequencing of predicted off-target sites. Specificities of sgRNAs were assessed in K562 myelogenous leukaemia cells and CD34+ human pluripotent stem cells. 3000 amplicons across 24 samples were pooled for parallel sequencing on a single Mi-Seq run. Results show limited cross-hybridization across TSCA primers indicating high specificity to the target loci. Over 80% of amplicons achieve uniform coverage, negating target region bias across the 125 predicted sites. CRISPR editing events were detected at the predicted on-target site as well as several predicted off-target sites. Mutations in the population were detected to a sensitivity of 0.1% minor allele frequency. This approach presents a scalable option for discovering, validating, and assessing the off-target profile of genome-editing modifications.

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Reversal of fetal globin silencing in primary cells through isoform-specific knockout of the BCL11A transcription factor

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Sickle-cell disease and β-thalassaemia are common and potentially life-threatening monogenic disorders. Both are caused by defects in β-globin and are suitable targets for gene-therapy approaches. Pathology for both disorders is significantly alleviated by elevated levels of the fetal β-like globin, γ-globin, whose expression is curtailed in most adults by the BCL11A transcription factor. BCL11A is essential for the survival of lymphoid cells, and its extra-long (XL) isoform has recently been demonstrated to be sufficient and required for γ-globin suppression in erythroid cell lines. Utilising the CRISPR/Cas9 system, we identified highly efficient sgRNAs for the disruption of BCL11A and its XL isoform in HUDEP-2 cells and patient-derived CD34+ cells. In HUDEP-2 cells, BCL11A-XL-specific knockouts gave high-level γ-globin expression, albeit with intermittent delay in erythroid differentiation. Likewise, isoform-specific knockout of BCL11A-XL resulted in γ-globin induction in CD34+ cells, and in patient-derived cells with different β-thalassaemia genotypes achieved partial correction of defective erythroid differentiation and β-like globin expression.

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Extension of the crRNA enhances Cpf1 gene editing and *in vivo* delivery with polymer nanoparticle

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Engineering of the Cpf1 crRNA has the potential to enhance its gene editing efficiency and non-viral delivery to cells. Here, we demonstrate that extending the length of its crRNA at the 5′-end can enhance the gene editing efficiency of Acidaminococcus sp. BV3L6 Cpf1 (AsCpf1) both in cells and *in vivo*. Extending the 5′-end of the crRNA improves the activity of the Cpf1 ribonucleoprotein (RNP) in three ways. First, extending the 5′-end of the crRNA enhances the gene editing efficiency of the Cpf1 RNP to induce non-homologous end joining and homology directed repair using electroporation in cells. Second, chemical modifications on the extended 5′-end of the crRNA result in enhanced serum stability. Third, extending the 5′-end of the crRNA by 59 nucleotides increases the delivery efficiency of Cpf1 RNP using in cells and *in vivo* cationic delivery vehicles including polymer nanoparticle. The efficient gene editing with Cpf1 RNP and polymer nanoparticle was demonstrated in gastrocnemius muscle of ai9 mouse. Thus, 5′-extension and chemical modification of the Cpf1 crRNA is an effective method for enhancing the gene editing efficiency of Cpf1 and its delivery *in vivo*.

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Guide RNA selection and homology arms design influence the efficiency of CRISPR-mediated integration of donor DNA

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Targeted insertion of therapeutic transgenes into predetermined sites in the genome, such as ''safe harbor'' loci, makes gene therapy safer and more efficient. For this purpose HDR-mediated genome editing using programmable endonucleases and DNA repair template can be used. We studied the effect of different guide RNAs and various structure of homology arms in DNA template on the efficiency of knock-in of marker gene to CCR5 locus. Seven sgRNAs for SpCas9 (including 4 previously described) and three sgRNAs for Cpf1 targeting CCR5 gene were generated. The RNA-guided cleavage of CCR5 was assessed in indicator HT1080 CCR5-EGFP cells and three most potent sgRNAs for SpCas9 and one for Cpf1 were selected for the following HDR experiments. For these four sgRNAs we constructed corresponding donor DNAs containing

EGFP marker gene flanked by homology arms and packaged them in AAV vectors. The efficiency of donor DNAs insertion for different sgRNAs was compared in HT1080 cells. The percentage of cells with integrated marker varied between 8% and 40%. We have shown that the efficiency of knock-in depends on donor DNA design as well as on the selection of guide RNA. The advantage of the arms overlapping DSB site has been demonstrated for SpCas9 and Cpf1 nucleases.

P282

In vivo multiple gene targeting for pancreatic cancer modeling with adeno associate virus and CRISPR/Cas9

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Pancreatic ductal adenocarcinoma is one of the most lethal cancer type in human, but animal model is limited due to its need of multiplex gene mutation. Thus, the aim of this study is to develop pancreatic cancer in mice with *in vivo* multiple gene editing. First, Streptococcus pyogens Cas9 (SpCas9-RFP) overexpression mice were generated by piggyback system, and Cas9 expression in pancreas was confirmed by RFP signal detection. Second, sgRNAs on SpCas9 and Campylobacter jejuni Cas9 (CjCas9) for KrasG12D, Trp53, Ink4a, Brca2 and Smad4 were designed and they exhibited $12.5 \sim 87.5\%$ indel efficiency. Third, AAV2-eGFP was injected to pancreas via common bile duct. *In vivo* transduction and tropism of AAV2 in pancreas was confirmed with high eGFP expression. Taken together, 3 experiment groups were prepared as; C57BL/6 (B6) mice with 3 AAVs (AAV2-SpCas9 and 2 different AAV2-3sgRNAs) in group I, B6.PB-SpCas9 with 2 AAVs (2 different AAV2-3sgRNAs) in group II, and FVB with 2 AAVs (2 different AAV2-CjCas9-3sgRNAs) in group III. Five month after 1.29 X 1010 AAVs transduction in each group, pancreas tissues were collected and indel formation and neoplasm development was analyzed. Genotyping with PCR, heteroduplex formation and PAGE gel running, exhibited indels in every group, but only mice from group II (B6.PB-SpCas9 and AAV-sgRNAs) and group III (FVB and CjCas9-sgRNAs) developed abnormal lesion, and mice from group III showed overall severe neoplasm formation. In conclusion, our study demonstrates that *in vivo* gene editing with CRISPR/Cas9 in applicable for cancer modeling with multiplex genes.

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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 of the third most common form of SCID, and the majority of T-B+NK+ cases. Gene therapy has proved to be a powerful tool to treat rare genetic diseases affecting the hematopoietic system avoiding these complications. However, pre-clinical gene therapy studies using viral vectors to introduce a correct copy of IL7R in HSPCs showed that constitutive and unregulated expression of the gene predisposes to leukemia. CRISPR-mediated genome editing enables the correction of the mutated IL7R locus with a regulated and controlled transgene expression. To achieve this purpose, we have targeted several guide RNAs (gRNAs) in combination with Cas9 protein to the IL7R locus, detecting up to 70% of indels in hematopoietic stem and progenitor cells (HSPCs). CRISPR-system delivered with an Adeno-associated virus (AAV) donor template containing a GFP cDNA resulted in the knock-in of the cassette in up to 35% of HSPCs. As a proof of concept for our strategy, we showed that the delivery of an AAV encoding a codon optimized IL7R cDNA cassette in an IL7R-deficient model cell line restores IL7R expression. Our final goal is to correct IL7R-deficient HSPCs derived from SCID patients restoring T-cell production. For this purpose, successful lymphopoiesis will be assessed through ongoing optimizations of an *in vitro* T-cell differentiation protocols and *in vivo* xeno-transplantation assays.

P284

Gene editing of PKLR gene in hematopoietic cells for the efficient correction of pyruvate kinase deficiency

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Pyruvate Kinase Deficiency (PKD) is a low prevalent erythroid metabolic disease caused by mutations in the PKLR gene which encodes the erythroid specific Pyruvate Kinase (RPK) enzyme. The defective enzyme fails to produce normal ATP levels and consequently, erythrocytes from PKD patients show an energetic imbalance and hemolysis. Site-specific hematopoietic stem cell gene therapy using gene editing would be a safe strategy to treat PKD patients. We have developed a gene editing approach that addresses the treatment of most PKD patients through a knock-in strategy using PKLR specific engineered nucleases (TALEN), which has been shown effective to reverse PKD phenotype in PKDiPSC lines. However, its direct application in hematopoietic progenitor and stem cells (HPSCs) is still challenging due to the high sensitivity of these cells to *in vitro* manipulation and the toxicity associated with the delivery of the gene editing tools. We have implemented a new and optimized gene editing approach based in the delivery of CRISPR/Cas9 system as ribonucleoprotein (RNP). We were able to generate up to 60% indels in PKLR locus of CD34+ without toxicity was associated with RNP delivery. For matrix delivery, viral and non-viral platforms have been used. Up to 35% of homologous recombination has been achieved in hematopoietic progenitors grown on semisolid culture. Additionally, NSG mice engraftment of gene edited cells has been obtained demonstrating the feasibility of these strategies to gene edit HPSCs in the PKLR locus. Collectively, these results lay the foundation to address PKD treatment by means of gene editing.

P285

Development of a nanoparticle formulation for delivery of a CRISPR/Cas9 gene therapy for cystic fibrosis

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Cystic Fibrosis (CF) is the most common, recessively-inherited genetic disorder, affecting around 1 in 2,500 babies born in Europe. CRISPR/Cas9 could be an attractive therapeutic option to target the underlying cause of the disease, however a major hurdle to overcome is how to deliver the technology with sufficient efficacy to the lung. Our approach is to deliver CRISPR with a non-viral nanoparticle, previously described for *in vivo* DNA and siRNA delivery. These nanoparticles comprise peptide and lipid components, which package nucleic acids and target their delivery to epithelial cells. Gene editing of airway epithelial cells is permanent, therefore repeated delivery with these non-immunogenic nanoparticles could be performed to reach a sufficient level of genetic correction. We first engineered primary, human bronchial epithelial cells to stably express GFP by lentiviral transduction and used this model to compare nanoparticle formulations for knockout of GFP by CRISPR/Cas9. Nanoparticles were formulated to package GFP-targeting gRNAs and Cas9 mRNA or Cas9 protein. Using Cas9 mRNA, we were able to achieve levels of around 50% GFP knockdown by flow cytometry with optimal formulations. Nanoparticles packaging the Cas9 protein, pre-assembled with GFP-targeting gRNA in a ribonucleoprotein complex, elicited 65% GFP knockdown. Both of these transfection levels are higher than that achieved by commercially available reagents. The nanocomplexes have been biophysically characterized, and found to have desirable size, charge and polydispersity indexes appropriate for delivery to the lung. This work provides the foundation for the development and delivery of a CF gene editing strategy *in vivo*.

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CRISPR/Cas9-mediated genome edited human embryonic stem cells as *in vitro* models of Batten disease

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Batten disease (BD) is a rare, progressive neurodegenerative disorder. It is one of the most common forms of a group of disorders known as neuronal ceroid lipofuscinoses; it is caused by a number of mutations in CLN genes across the genome. BD biology has proven complex to study, as the function of the causative genes remain elusive and there are few tools available to elucidate the molecular mechanisms of the proteins. Although transgenic animal models of the disease are currently employed in BD research, they have shown limitations in the efficacy of recapitulating Batten disease pathology. Hence, there is a requirement for *in vitro* human models to expedite the development of new therapeutics. We have utilised plasmid-based CRISPR/Cas9 technology to generate homozygous, clonal CLN-knockout human embryonic stem cell lines. Beyond the homozygous CLN null mutation, the parental control hESC line is genetically identical. This makes the mutant and parental lines ideal for comparative proteomic, metabolomics evaluations of drug library screens after differentiation to multiple neural cell types. Independently, these cell lines produced using state-of-the-art technologies are invaluable tools to enable a better understanding of Batten disease biology and potentially the discovery of novel therapeutic interventions.

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Truncated gRNAs result in efficient *in vivo* liver-directed gene inactivation using CRISPR/Cas9

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In vivo tissue-directed gene editing at a particular locus remains a challenge and in this project we look into the prospects of liver-directed gene editing using CRISPR/Cas9. We report the use of a dual AAV9 mediated delivery of Cas9 and truncated or full-length guide-RNAs (gRNAs) under the control of a computationally designed hepatocyte-specific promoter in C57BL/6 mice. The use of truncated gRNAs has been proposed as a means to augment targeting specificity based on *in vitro* studies in cell lines, though concerns remained about potential loss of efficacy. We therefore tested the potential of using truncated gRNAs for gene targeting *in vivo*. This resulted in a sequence-specific targeting in the mouse factor IX (FIX) gene. It was particularly encouraging that *in vivo* targeting efficacy of truncated gRNA (17nt) was as high as that of full-length gRNA (20nt). The targeted mutation resulted in a substantial loss of FIX activity and the development of a bleeding phenotype, consistent with hemophilia B condition. T7E1 assays, site-specific Sanger sequencing and deep sequencing of on-target and putative off-target sites assessed the efficiency of *in vivo* targeting. Cas9 expression was restricted to liver and was transient in neonates, due to hepatocyte turnover, signifying an attractive 'hit-and-run' paradigm. Consequently, the indel frequency was robust in the liver (up to 50%) in the desired target loci of the FIX gene, with no evidence of significant off targeting. Our results have possible extensive implications for somatic gene targeting in the liver using the CRISPR/Cas9 platform (Singh et al., Mol. Ther.;2018).

P288

Comparing methods of targeted integration to correct Bruton's tyrosine kinase defects

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Homology Directed Repair (HDR) is commonly employed to achieve targeted integration. However, studies using HDR to

treat hematopoietic disorders have often found editing rates drop between *in vitro* and *in vivo* measurements, likely due to HDR's restriction to the S/G2 phases of the cell cycle, whereas hematopoietic stem cells are quiescent and predominantly in G0. Two alternative pathways for targeted integration are Homology Independent Targeted Integration (HITI) and Perfect Integration into Target Chromosome (PITCh) which utilize Non-Homologous End Joining (NHEJ) and Microhomology Mediated End Joining (MMEJ), respectively. NHEJ occurs throughout the cell cycle, while MMEJ is mostly during S phase, making HITI the method most likely to function in quiescent cells. We compared these three pathways for integration of a Bruton's Tyrosine Kinase (BTK) cDNA into intron 1 of the BTK locus in K562 cells. From plasmid donors, HDR integrated in 14.7% of cells, HITI in 6.9%, and PITCh in 4.2% (n = 2-4). In cells reversibly pre-synchronized into G1 with hydroxyurea, HDR and PITCh integration efficiencies dropped to 1.4% and 0.1% while HITI increased to 13.1% (n = 2-4). 100% (n = 39) of HDR 5′ integration junctions were base perfect, while HITI was error prone and only 10% (n = 30) of junctions had the predicted sequence. In 80% of PITCh junctions (n = 45), an 8 bp microhomology-guided deletion was found. This comparison suggests that HITI is optimal for integrating into quiescent cells at sites where aberrant integration and indels can be tolerated to some degree, while HDR is the best option when integration must be seamless.

P289

Characterisation of novel putative human safe harbour loci for controlled transgene expression

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Controlled expression of transgenes in cells is essential for both therapeutic and research purposes. Traditionally such transgene expression has been accomplished via viral targeting and integration in the host genome in a generally random fashion. The advent of gene-engineering technologies, such as zinc finger nucleases and CRISPR/Cas9, has allowed for directed integration of transgenes in the cell of interest. The ideal integration sites for the foreign genetic material are 'safe harbour' sites, which allow for controlled expression of a transgene, without perturbing endogenous gene expression patterns. To date only a limited number of safe harbour loci have been reported and characterised in the human genome, these include for example the AAVS1 site, CCR5 locus and the human homolog of ROSA26. We sought to increase the number of putative human safe harbour loci, by first identifying loci in the human genome that lie outside DNAse clusters, gene transcription units and ultra-conserved regions, as well as being >300kb away from any known oncogenes, miRNAs and lncRNAs. We further refined our list candidate safe harbour loci by cross referencing against a set of stable housekeeping genes, defined from the GTEx dataset, and highly active chromatin compartments, inferred from published chromatin conformation data from 21 human primary tissues and cell types. Our final list has approximately 1000 of candidate human safe harbour loci and we are currently characterising selected loci *in vitro* using hES cells and CRISPR/Cas9 targeting.

P290

New generation of the minimised UCOEs for direct transgene expression from the innate HNRPA2B1 promoter

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The ubiquitous chromatin opening element (UCOE) derived from the human HNRPA2B1-CBX3 housekeeping gene locus (A2UCOE) is able to provide highly reproducible and stable expression even from transgenes integrated within extreme heterochromatic regions. The A2UCOE has been shown to provide highly reproducible and stable transgene expression from within lentiviral vectors (LVs) both *in vitro* and more importantly *in vivo* following *ex vivo* gene transfer to mouse bone marrow haematopoietic stem cells (HSCs). 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 1.2-1.7A2UCOEs 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 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.

P291

Efficient genome editing system using short-term and strong expression of Cas9

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The genome editing offers a new development for gene therapy. This system is applicable not only to genetic disease, but also to intractable viral infections. However, possible adverse effects on cells with persistent high expression of Cas9 have still been discussed. In order to solve this problem, we have developed ''excisional''-expression system of Cas9 using adenovirus vector. In this system, Cas9-expression unit is excised out as a circular DNA when Cre is supplied, and consequently, strong expression occurs because of connection of the strong promoter to Cas9 gene. This expression unit is present on an excised circular DNA, which is much more unstable than viral genome. Therefore, the ''excisional''-expression vector can achieve the short-term and strong expression. In this study, we demonstrated that the ''excisional''-expression system of Cas9 showed more than 90% reduction of the HBV covalently closed circular DNA (cccDNA) in human hepatocyte cells. And also, to enhance the safety of gene therapy, we constructed the cell-

specific genome editing system by cell-specific promoter applying ''excisional''-expression system. We succeeded in eliminating cccDNA efficiently only in liver cells while retaining high cell specificity. This system may be useful as an effective and safety genome editing system in gene therapy.

P292

TALEN-mediated inactivation of dominant-negative keratin alleles for general phenotypic alleviation of epidermolytic ichthyosis

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Epidermolytic ichthyosis (EI) is a skin fragility disorder caused by dominant-negative mutations in KRT1 or KRT10. As EI is difficult to treat and currently lacks a cure, there is an acute need for novel therapies. Keratin 1 and keratin 10 specifically polymerise to form the intermediate filament cytoskeleton of epithelial cells. Integration of dominant-negative mutant keratins into this results in fragility, collapse upon mild stress and skin blistering. Mutant keratin elimination should therefore result in curation of the disease. Transcription activator-like effector nucleases (TALENs) are designer nucleases. They can be used to introduce double-strand breaks at specific target sites, leading to inactivation of target genes. Few design parameters are required and off-target effects are rare. We are developing an *ex vivo* gene therapy for EI, using TALENs to knockout mutant KRT10 alleles in patient keratinocytes. TALENs targeting upstream of a stop codon known to induce KRT10 knockout were constructed. These cleave efficiently at the target site, modifying 52% of single cell clones without selection. A correctly modified clone was isolated. Mutant KRT10 knockout was subsequently confirmed at RNA and protein level. Phenotypic restoration was demonstrated following immunofluorescent analysis of differentiated monolayers, concurrent with murine xenograft examination. Off-target activity was not observed at 22 predicted sites via next-generation sequencing. We aim to take a skin biopsy from an EI patient, isolate, grow and treat KSCs with TALENs to phenotypically correct cells prior to grafting these onto the patient's skin as an effective *ex vivo* therapy for 95.5% of dominant-negative EI cases.

P293

ssODN design is essential to ensure an accurate gene correction in fibroblasts derived from primary hyperoxaluria type 1 patients

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Gene corrected autologous hepatocyte-like cells generated by means of cellular reprogramming is proposed as an alternative cellular source in patients with Primary Hiperoxaluria Type 1 (PH1). The development of optimized tools for the specific gene correction of the described mutations is essential to address this strategy. Primary fibroblasts from a set of PH1 patients carrying the same mutation in exon 7 of AGXT gene (c.853T>C) have been used to design single-stranded oligodeoxynucleotides (ssODN) and guideRNAs (gRNA) driven Cas9 cleavage to correct this specific mutation. Three different gRNAs, targeting exon 7 of AGXT, and three different ssODN with lengths ranging 100-150 bp and carrying the wild-type nucleotide and synonymous changes to identify recombinants by RFLP, have been designed. Efficient cleavage in exon 7 of AGXT gene has been performed with the different gRNAs after nucleofection of ribonucleoprotein complexes. Proper recombination with ssODN as repair templates resulted challenging, with a high proportion of partial insertions of these DNA templates. Specific correction of the mutation was achieved in PH1 derived-fibroblasts when using some of the tested combinations being ssODN design and length crucial for minimizing undesirable partial insertions of the DNA template. Once genetically corrected, direct reprogramming of gene edited PH1-derived fibroblasts will be conducted, following the hepatic direct reprogramming we have previously optimized in healthy donor-derived fibroblasts, offering a new cell source alternative for PH1 treatment.

P294

An optimized platform for efficient CRISPR editing of iPSCs using synthetic sgRNA

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1: Synthego

CRISPR/Cas9 technology has enabled efficient genome editing in a variety of cell types. However, efficient editing of human iPSCs - relevant for generating disease models or *ex vivo* therapies - remains a challenge. Here we demonstrate an optimized method that utilizes synthetic modified sgRNAs in an RNP delivery format to successfully generate gene knockouts and knock-ins in a human iPSC line that can be used to develop models for disease. This method makes it possible to achieve CRISPR/Cas9 indel frequencies up to 75% and allows for knock-in efficiency of up to 15% using single stranded DNA repair templates. We further demonstrate the use of this method in generating a clonal disease model line.

P295

Generation of insulin deficient pancreatic beta cells for insulin gene replacement therapy

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Insulin is a crucial polypeptide for glucose metabolism, lack of which causes metabolic diseases like diabetes. Likewise, genetic mutations interfering with insulin gene synthesis like seen in MODY10 also leads to a similar phenotype. Insulin gene

replacement therapy is required to compensate insulin deficiency under these circumstances.1 A novel lentiviral vector expressing insulin gene under the control of insulin promoter (LentiINS) was constructed using Gateway technology to test its therapeutic potential in insulin knockout (KO) pancreatic beta cell line. To accomplish this, a mouse pancreatic beta cell line (Min6) was transfected with a dual gene knockout plasmid system involving CRISPR / Cas9. In this scenario, knockout plasmids carry insulin gene targeted sgRNA and Cas9 coding sequence along with GFP expression cassette, while donor plasmids possess red fluorescent gene (RFP) and homology arms for insulin gene. Following transfection of dual plasmids, GFP expression revealed successful transfection while RFP expressing cells indicated homology directed gene insertion. TUBITAK(215S820) 1- Insulin Gene Therapy From Design to Beta Cell Generation. Sanlioglu AD, Altunbas HA, Balci MK, Griffith TS and Sanlioglu S. Expert Rev Mol Med. 2012 Oct 15;14:e18. doi: 10.1017/erm.2012.12.PMID: 23062285

P296

Cellular delivery of CRISPR/Cas9 ribonucleoproteins via biomimic lipid nanoparticles

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Clustered Regularly Interspaced Palindromic Repeats and CRISPR-associated protein-9 (CRISPR/Cas9) is part of the natural defense system of bacteria against invading viruses. The endonuclease can introduce double strand cuts in DNA in a sequence-specific manner. It has been engineered for gene editing purposes in mammalian cells and has great potential to revolutionize modern medicine including gene therapy. One central question is how to deliver the editing system to the cells with minimal off-target effects and cellular immune response. Non-viral delivery systems, such as biomimic lipid nanoparticles, are promising tools to efficiently deliver CRISPR/Cas9 in a ribonucleoprotein (RNP) complex with single guide RNA (sgRNA) along with a template DNA to allow homology-directed repair (HDR) genome editing. This study shows the first steps in producing a Cas protein variant, SpCas9 (Streptococcus pyogenes), activity of RNP complex and efficient delivery to cells in culture.

P297

Adenine base editing in a mouse model of Duchenne muscular dystrophy

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Taeyoung Koo, Seuk-Min Ryu, Kyungmi Kim, Kayeong Lim, and Jin-Soo Kim Adenine base editors (ABEs) composed of an engineered adenine deaminase and the Streptococcus pyogenes Cas9 nickase enable adenine-to-guanine (A-to-G) single-nucleotide substitutions in a guide RNA (gRNA)- dependent manner. Here we demonstrate application of this technology in a mouse model of Duchenne muscular dystrophy. We delivered the split ABE gene, using trans-splicing adeno associated viral vectors, to muscle cells in a mouse model of Duchenne muscular dystrophy to correct a nonsense mutation in the Dmd gene, demonstrating the therapeutic potential of base editing in Duchenne muscular dystrophy.

P298

Coupling AAV-mediated promoterless gene targeting to SaCas9 nuclease to efficiently correct liver metabolic diseases

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AAV-mediated gene replacement therapy in the liver has demonstrated efficacy in adult patients, but faces potential limitations associated with episomal DNA loss during hepatocyte proliferation, a major concern when considering paediatric disorders. Gene targeting is a promising approach, overcoming these limitations by means of the permanent modification of the genome. We successfully performed a gene targeting strategy without nucleases in Crigler-Najjar mice, rescuing neonatal lethality by inserting a promoterless UGT1A1 cDNA just upstream of the albumin stop codon, such that both proteins are produced from a chimeric mRNA. To further increase recombination rate and therapeutic efficacy, we combined GeneRide with the CRISPR/SaCas9 platform. Neonatal WT mice were i.v. injected with two AAVs: one expressing the SaCas9 and sgRNA, and one containing the albumin homology regions plus a promoterless cDNA (eGFP, or human coagulation factor IX, hFIX). Targeting efficiency increased up to ~100-fold compared to the group without nuclease, reaching ~25% of eGFP-positive hepatocytes and ~200% of normal hFIX levels, respectively. Next, we applied the strategy to neonatal Crigler-Najjar mice. All treated mice were rescued from neonatal lethality, with normal plasma bilirubin levels at 10 months after administration. Protein levels were ~6-fold higher than in WT liver, and immunofluorescence analysis showed ~5% of hUGT1A1-positive hepatocytes. Liver histology was normal and no off-targets were detected in predicted sites. In conclusion, we demonstrated enhanced homologous recombination when the GeneRide strategy was coupled to the CRISPR/SaCas9 platform. The improved efficacy and safety supports the potential for this type of approach when considering clinical application.

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evoCas9, a highly specific SpCas9 variant from a yeast *in vivo* screening

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CRISPR–Cas9 nucleases represent the method of choice to perform genome editing, however, potential off-target cleavages raise concerns over their application, especially for therapeutic purposes. By engineering a yeast strain for simultaneous

detection of on- and off-target activity of Streptococcus pyogenes Cas9 (SpCas9) we have recently set up an *in vivo* screening platform to identify optimized variants. The first screening of a library of SpCas9 variants randomly mutated in the REC3 domain produced evoCas9, carrying four beneficial substitutions that increased editing accuracy and preserved catalytic activity. Side-by-side comparisons on eight endogenous genomic loci performed by genome-wide approach (GUIDE-seq) between evoCas9, the high-fidelity variants eSpCas9(1.1) and SpCas9-HF1 demonstrated that our mutant has a substantially improved specificity while maintaining near wild-type on-target editing efficiency (90% median residual activity). Moreover, the higher precision profile is preserved on difficult-to-discriminate off-target sites during long-term expression (40 d). Given the widespread use of SpCas9 ribonucleoprotein (RNP) delivery in genome editing protocols, we further evaluated the possibility to directly deliver our high-specificity variant as RNPs into cells. This required further optimization of the combination of the mutations used to generate evoCas9 in order to maximize on-target cleavage while reducing off-targeting to a minimum. Compared to previous structure-guided mutagenesis, our semi-rational *in vivo* screening proved successful in identifying the best-fit combination of amino acid substitutions for error-free SpCas9 variants optimized for different delivery methods. Altogether, this study proves that our approach represents a valid strategy to enhance the specificity of SpCas9 as well as other RNA-guided nucleases.

P300

Efficient genome editing in primary human T, B and HSCs using Baboon envelope gp pseudotyped virus derived "Nanoblades" loaded with Cas9/sgRNA ribonucleoproteins

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Programmable nucleases have enabled rapid and accessible genome engineering in cells and living organisms. However, their delivery into target cells can be challenging, specially into primary cells. Here, we have designed ''Nanoblades'', a new technology to deliver a genomic cleaving agent into cells. These are MLV- or HIV-derived virus like particles (VLPs), in which the viral structural protein Gag has been fused to the Cas9, which are thus loaded with Cas9 protein together with guide RNAs. Nanoblades are extremely efficient in delivery of their Cas9/sgRNA cargo for entry into human T, B and HSCs thanks to their surface co-pseudotyping with baboon retroviral and VSV-G envelopes. We were able to induce efficient and rapid genome-editing in human IPS cells reaching up to 70% in the empty spiracles homeobox 1 gene. A brief nanoblade incubation of human T and B cells resulted in 40% and 20% editing of the Wiskott-Aldrich syndrome (WAS) gene locus, while HSCs treated for 18 h with nanoblades allowed 30-40% gene editing in the WAS gene locus and 80% for the Myd88 genomic target. No cell toxicity was detected. Additionally, nanoblades can be complexed with donor-DNA for ''all-in-one'' homology-directed-repair' in order to correct a gene or programmed with modified Cas9 variants to mediate transcriptional up-regulation of target genes. Summarizing, this technology is simple to implement in any laboratory, shows high flexibility for different targets including immune cells of murine and human origin, is relatively inexpensive and has important prospects for basic and clinical translation for gene therapy.

P301

CRISPR-mediated gene editing using a cGMP-compliant non-viral cell engineering: from iPSC disease modelling to clinically-meaningful correction of monogenic disease mutations in patient cells

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More robust and scalable cell cultivation methods of patient cells, stem cells, and human induced pluripotent stem cells (hiPSCs) has revolutionized the fields of regenerative medicine and cell and gene therapy. These cell populations often require engineering for their generation, reprogramming, differentiation and/or for targeted gene editing prior to their therapeutic use. Although cell and gene therapies have been approved by the FDA, it remains imperative that researchers consider early in development whether their cell engineering method meets the stringent demands of clinical use - - namely the ability to safely and reproducibly modify human primary or stem cells with high efficiency, low cytotoxicity, and at the scale required to treat patients. Flow Electroporation® Technology, a non-viral cell engineering technology designed to fulfill these demands, has an established record of rapidly advancing cell- and gene-based therapies to the clinic. In this poster we will demonstrate the high efficiency, high viability, fully scalable engineering of a variety of patient cells, stem cells and hiPSCs as well as their real-world application in pre-clinical disease modeling of Amyotrophic Lateral Sclerosis (ALS) & cardiac arrhythmia and IND-enabling studies for the therapeutic correction of monogenic disease mutations such as X-linked chronic granulomatous disease (X-CGD) and sickle cell disease (SCD).

P302

Clinically-relevant correction of recessive dystrophic epidermolysis bullosa by dual sgRNA CRISPR/Cas9-mediated gene editing

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Gene editing constitutes a novel approach for precisely correcting disease-causing gene mutations. Frame shift mutations in COL7A1 causing Recessive Dystrophic Epidermolysis Bullosa are amenable to open reading frame restoration by non-homologous end joining repair-based approaches as previously shown by us using TALENs. Efficient targeted deletion of faulty (mutation carrying) COL7A1 dispensable exons in polyclonal patient keratinocytes would enable straightforward translation of this

therapeutic strategy to the clinic. In this study, using a dual sgRNA guided-Cas9 nuclease delivered as a ribonucleoprotein complex through electroporation, we have achieved very efficient targeted deletion of COL7A1 exon 80 in RDEB patient keratinocytes carrying a highly prevalent frameshift mutation. This non-viral approach rendered a large proportion of corrected cells including the stem cell population producing a functional collagen VII variant enabling long-term regeneration of a properly adhesive skin upon grafting onto immunodeficient mice. The strategy overcomes the constraint of isolating edited stem cell clones. In addition, characterization of potential off-target sites by NGS analysis did not reveal any off-target activity. Our strategy could potentially be extended to a large number of COL7A1 mutation-bearing exons within the long collagenous domain of this gene, opening the way to precision medicine for RDEB.

P305

OCT4 and SOX2 are required at the M-G1 transition to re-establish chromatin accessibility at enhancers in pluripotent stem cells

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Fast-cycling pluripotent stem cells maintain cell identity through indefinite cell divisions. During mitosis, enhancers are inactivated, transcription stops, and most proteins are evicted from chromatin. This raises the question of how transcriptional programs are reactivated following division. A subset of transcription factors remains bound to the mitotic genome, including the pluripotency regulators OCT4 and SOX2. Degradation of OCT4 or SOX2 at the M-G1 transition hampers pluripotency maintenance, suggesting that mitotic binding is required for their action on cell fate. Because OCT4 has been shown to recruit chromatin remodellers, we reasoned that OCT4 and SOX2 might be involved in reopening and reactivating enhancers after mitosis. We transiently degraded OCT4 or SOX2 at the M-G1 transition and measured chromatin accessibility throughout the cell cycle and compared to cells without M-G1 degradation. A large proportion of OCT4/SOX2-bound sites exhibited reduced accessibility upon M-G1 removal of either OCT4 or SOX2. Strikingly, some loci did not recover their accessibility even at the end of the cell cycle, when protein levels had been restored for several hours. These sites were enriched for mitotic binding and enhancers that regulate the expression of pluripotency genes. We posit that OCT4 and SOX2 act to reopen closed cell fate enhancers after mitosis, and that their failure to do so has prolonged effects that result in pluripotency loss.

P306

Role of OCT4 in establishing and maintaining chromatin architecture during stem cell self-renewal

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Chromatin architectural features are key in governing cell type-specific gene expression programs. During mitosis (M), chromatin accessibility and promoter enhancer contacts are disrupted and gene transcription strongly decreases. After mitosis, these chromatin architectural features are re-established to initiate and sustain cell type specific gene expression. While pioneer transcription factors are known to play a crucial role in mediating cell type specific chromatin architecture, their role in re-establishing promoter-enhancer contacts at the M-G1 is unclear. In ES cells, the pioneer transcription factor OCT4 is indispensable in mediating promoter-enhancer contacts and accessibility of a set of pluripotency-associated regions. We thus hypothesized that OCT4 could also be essential for re-establishing these chromatin architectural features after mitosis. Supporting our hypothesis, we have recent evidence that the absence of OCT4 during M-G1 transition affects the re-establishment of chromatin accessibility of a set of regions throughout the cell cycle. These regions are typically enriched with OCT4 cognate binding sites, suggesting that OCT4 occupancy at the M-G1 transition is crucial for re-establishing accessibility of these regions. We are now aiming to determine whether OCT4 occupancy also plays a role in maintaining chromatin accessibility in later cell cycle stages. To do so, we are now developing an inducible degradation approach to acutely degrade OCT4 for 2 hours at different cell cycle phases. We will also use this method to determine whether OCT4 occupancy is crucial for re-establishment and maintenance of OCT4-associated promoter-enhancer contacts known to regulate expression of pluripotency associated genes.

P307

Empowering CAR-T-cell immunotherapies by precise genome editing

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The last years have seen the adoptive transfer of engineered autologous and more recently allogenic off-the-shelf T-cells making great strides in the development of new treatments against cancer. The clinical outcome of CAR T-cell therapies is intimately linked to the ability of effector cells to engraft and proliferate in order to eradicate tumor cells within patients. Although the transient activity of off-the-shelf T-cells represents an important safety feature, the possibility to extend their therapeutic window may be desirable. Thus, we are developing novel approaches to render these cells less visible to both host T- and NK-cells. The single genetic disruption of the beta-2 microglobulin (B2M) leads to the resistance to host CD8+ T-cells but may trigger NK-cell activation and cell rejection. We therefore further engineered these B2M negative T-cells to express elements allowing to escape NK-cell attack. Additional challenges arise when considering parameters such as accessibility to the tumor, immunosuppressive microenvironment, and recruitment of host immune cells able to improve antitumor response, for example in solid tumor patients. The delivery of therapeutic molecules in a spacio-temporal controlled manner directly by CAR T-cells will greatly improve their efficiency and safety. Using gene editing, we have repurposed endogenous immune pathways to not only express a CAR, but also to secrete key cytokines such as IL-12 and IL-15 upon tumor engagement. In summary, we are integrating state-of-the-art gene editing into the development of next generation CAR T-cells, opening a new avenue for synthetic biology approaches within the field of adoptive T-cell immunotherapy.

P308

Antibody gene transfer for prophylaxis of respiratory syncytial virus (RSV) infection

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Respiratory syncytial virus (RSV) infection is the single most common cause of hospitalisation in infants under 1 year of age, with no currently licensed vaccine available. Prophylaxis with monoclonal antibody palivizumab is effective but costly due to the complexity of large-scale manufacture and is only offered to select vulnerable infant populations. We are developing gene delivery approaches to directly express palivizumab in patients as an alternative to parenteral administration. Recombinant adeno-associated virus (rAAV2/8) was used for intramuscular delivery in mice and recombinant simian immunodeficiency virus (SIV) pseudotyped with Sendai virus envelope proteins F and HN (rSIV.F/HN) was delivered to the murine lung via insufflation. When BALB/c mice were dosed with either vector configuration containing the secreted Gaussia luciferase (GLux) or palivizumab cDNA, dose-dependent, sustained, transgene expression was observed in the lung lumen and circulation for at least 12 months. When rAAV expressing palivizumab was delivered to mice, therapeutically relevant serum levels of the antibody (89.3 μg/mL; $p < 0.001$) were observed for a minimum of 6 months post-delivery. Delivery of either vector to BALB/c mice 28 days prior to challenge with 7.5e5 pfu of RSV significantly reduced weight loss ($p < 0.05$ and $P < 0.0001$ for 1e8 TU rSIV.F/HN and 1e11 VG rAAV2/8, respectively). Translation of this approach could reduce the overall 'per dose treatment cost' allowing for wider use of prophylaxis against RSV infection in at risk human populations despite the continued absence of an effective vaccine.

P309

Alteration of CD28 signaling motifs increases anti-tumour activity and persistence of chimeric antigen receptor-modified T cells

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The limited expansion and persistence of CAR-T cells in solid tumours is a challenge to the field. We previously demonstrated that CAR-T cells containing the ICOS intracellular domain display improved persistence when compared to CD28 in animal models of cancer. Here, we hypothesised that fine-tuning of the CD28 signalling motifs to resemble ICOS signalling could improve the persistence and anti-tumor effects of CD28-based CAR-T cells. We report the generation and preclinical characterisation of an ICOS-tuned (I-tuned) CD28-based CAR. We show that signalling through the I-tuned CD28 intracellular domain results in enhanced Akt phosphorylation with reduced PLCg and Vav phosphorylation when compared to conventional CD28-based CARs. Treatment of animals with pancreatic tumours with conventional CD28-based CAR-T cells induced an initial decrease in tumor burden, but T cells did not persist, and tumours eventually progressed. Animals treated with ICOS-based CAR-T cells showed a slower anti-tumor effect with enhanced persistence, that was not sufficient to induce complete responses. Interestingly, mutation of the CD28 motif enhanced T cell persistence, which resulted in complete responses in all the animals treated. Transcriptional profile of CAR-T cells isolated from tumours two weeks after injection revealed that the I-tuned CD28-CART cells exhibited a distinct gene expression profile when compared to the conventional CD28-CART cells, with a reduced expression of key signature markers of T cell differentiation an exhaustion. All together, these results indicate that the poor persistence and anti-tumor activity of conventional CD28 co-stimulated CAR-T cells can be enhanced through mutation of the CD28 tail.

P310

Integration of immune stimuli at the single-cell level expands the spectrum of macrophage activation states and identifies targets for functional reprogramming

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Macrophages are specialized cells capable of translating complex environmental signals into specific and partially reversible activation states. Yet, while the genomic features of responses to individual immune stimuli are well characterized, it remains unclear how complex inputs, entailing multiple signals with distinct biological properties, are computed and translated into transcriptional outputs. We have recently showed that co-exposure of bone marrow-derived macrophages (BMDMs) to the paradigmatic cytokines IFN-gamma and IL-4 leads to broad transcriptional cross-antagonisms driven by specific genomic features of co-targeted gene regulatory elements (Piccolo et al., Nature Immunology 2017). Here, we expand these studies using bulk and single-cell genomics to show that macrophages are intrinsically able to integrate relevant combinations of immune stimulatory and suppressive signals into coherent gene expression programs. This type of response was globally homogeneous, generalizable across multiple combinations of stimuli, and resulted in the acquisition of unique and emerging transcriptional features distinct from those of cells exposed to individual stimuli. Mechanistic analyses revealed common principles underlying selectivity and specificity of transcriptional cross-antagonisms, which we incorporated into gene therapy approaches for functional macrophage reprogramming in cancer. Our analyses expand the spectrum of macrophage activation states by investigating a novel dimension, namely integration of antagonistic stimuli into emerging and unique transcriptional programs. Since macrophages, *in vivo*, are invariably exposed to multiple stimuli at the same time, we argue that these data will help to better interpret macrophage polarization in homeostasis and disease.

P311

Hunting novel WT1-specific T-cell receptors for immune gene therapy of acute myeloid leukaemia

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T-cell receptor (TCR) gene therapy is a potent immunotherapeutic approach designed to unleash the immune system against cancer. By TCR gene editing, we showed that it is possible to completely redirect T-cell specificity against a tumour antigen. Unfortunately, the paucity of tumour-specific TCRs has limited its wide-range exploitation. Here, we aim at the generation of a library of TCRs encompassing different human leukocyte antigen (HLA) restrictions with specificity for Wilms Tumour 1 (WT1), a tumour-associated antigen overexpressed in acute myeloid leukaemia, an aggressive and still largely incurable haematological malignancy. We successfully expanded tumour-specific T-cells from 10 healthy donors by repetitive stimulation with autologous antigen-presenting cells loaded with a WT1 peptide pool. The ability of WT1-specific T-cells to recognize naturally processed epitopes and their on-target specificity was demonstrated upon co-culture with antigen-expressing cell lines and primary leukaemic blasts. Over time, tracking of the clonal contribution led to the identification of 15 tumour-specific $\alpha\beta$ TCR clonotypes that recognized several tumour-associated peptides and are restricted to different HLA alleles. Simultaneous editing of endogenous TCR α and β chain using CRISPR/Cas9 technology (knock-out efficiency >90%), followed by transduction of T cells with a WT1-specific TCR, led to the expression of the inserted TCR in >95% of CD8+ T lymphocytes. Functional validation of the edited T cells is currently ongoing. In conclusion, we consistently and efficiently identified tumour-specific TCRs that can be easily and rapidly used to redirect T-cell specificity against cancer cells by TCR gene editing.

P312

Lung-targeted lentiviral vector mediates passive immunisation against influenza

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Lentiviral vectors are being investigated for passive immunisation against respiratory pathogens such as Influenza A, which causes >500,000 deaths annually. Current vaccines and prior infection offer no lasting protection against emerging strains and a new global influenza pandemic is anticipated by the World Health Organisation. Broadly neutralising antibodies (bnAbs) isolated from vaccinated volunteers can be used for passive immunisation, but have a relatively short half-life in the circulation and are expensive to manufacture. Delivery of anti-influenza bnAb genes to the lung could provide long-lasting passive immunity to widely divergent strains of influenza. We have utilised lentiviral vectors pseudotyped with the Sendai virus F and HN proteins (rSIV.F/HN) to target lung epithelial cells and the hCEF promoter for long-term lung gene expression. In mice, intranasal delivery of the vector (5e7 TU/mouse) expressing T1-3B (which cross-reacts with multiple Group 1 influenza A strains) resulted in persistent antibody secretion into the serum (340 ng/mL; $p < 0.001$ on day 28) and the lung epithelial lining fluid (ELF) (2,300 ng/mL, $p < 0.001$). Similar doses also protected mice against a lethal dose of influenza ($\sim$15 LD50 H1N1 strain A/PR/8/1934) with 50% survival ($p < 0.001$). A matched dose of rSIV.F/HN expressing T1-3B from the hCEF promoter was significantly more potent compared with expression from the CMV promoter (0% survival) and the mock treated control group (0% survival). We speculate that expression of broadly neutralising antibodies could be both time-responsive and cost-effective when deployed as a first line of defence against the next human influenza pandemic.

P313

Development of analytical strategy to ensure production efficiency, and consistency of a WT1-TCR immunotherapy

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1: Cell Therapy Catapult 2: Cell Medica

TCR/CAR-T immunotherapies have shown remarkable clinical successes with two products having obtained marketing authorisation and more being assessed. Manufacturing of these therapies typically involves use of viral vectors for delivery of a CAR/TCR construct to patient T-cells. However, the use of patient specific starting material can lead to variability in cell behaviour during manufacture and can affect transduction efficiency and product potency. Characterisation is therefore critical both during and post-manufacture to ensure consistency and sufficient function. Here we used an exemplar retrovirally transduced WT1-T-cell-product to develop an advanced panel of in-process and product release assays. γ-retroviral vectors only transduce dividing cells with reagents added to induce T-cell activation and division. This process is conventionally monitored qualitatively by visualization of cell size. To improve this, we use a quantitative measurement of mitochondrial metabolism to determine the timepoint at which the T-cell become activated and prepare to divide. By combining this with a panel of 96-gene markers analysed using principal component analysis we further determine when the cells are in pre-, early- and late-activation and track this in a donor dependant manner. To demonstrate how the donor material impacts product potency we developed a novel cytotoxicity assay using the electrical impedance-based xCelligence system. This approach allowed real-time and label-free assessment of T-cell-mediated killing of target cells. In conclusion, we have developed a suite of assays that could be used to monitor and control the manufacture of TCR-immunotherapies that accounts for donor variability in starting material and minimises the risk of batch failure.

P314

CD44v6 CAR-T cells display antitumor activity against CD44v6+ human solid tumors

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Identification of suitable target antigens is one the greatest challenge in developing CAR T cell therapy for solid tumors.

The hyaluronate receptor CD44 is implicated in a several physiological and pathophysiological processes. In particular the isoform variant 6 (CD44v6) is homogenously expressed in squamous cell carcinomas and adenocarcinomas. A CD44v6-specific chimeric antigen receptor (CAR) containing as spacer the extracellular domain of the low-affinity nerve-growth-factor receptor (NL), has been shown to mediate a antitumor effects against human acute myeloid leukemia (AML) and multiple myeloma (MM) cells in immunocompromised mice. Aim of this study is to investigate the antitumor activity of CD44v6NL CAR T cells in human solid tumor models. T cells were transduced with a retroviral vector carrying the HSV-TK suicide gene and the CD44v6NL CAR. Antitumor activity of CAR T cells was investigated in tumor-bearing NSG mouse models. Tumor growth was monitored both by *in vivo* luciferase imaging and by measuring tumor size and weight. Several independent experiments with CAR T cells from different donors showed that a single infusion of CD44v6NL T cells mediates significant anti-tumor effects and enhances survival of treated mice. At sacrifice, tumor infiltrating cells were characterized for activation and differentiation markers. A high amount of CAR T cells was detected only in tumors of CD44v6NL CAR T cells treated mice, but not in those treated with control CAR T cells. Phenotype of the infiltrating cells suggested local differentiation of CAR T cells into effector cells responsible for tumor cell killing.

P315

Enhancing activity of established anti-myeloma regimens by gene therapy-driven Interferon-a delivery

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Multiple Myeloma (MM) is an incurable malignancy of plasma cells accounting for 2.1% of cancer deaths. Immunotherapy is showing encouraging responses in relapsed/refractory patients, but the immunosuppressive myeloma microenvironment represents a challenge for long-term disease control. We employ a gene therapy (GT) approach based on transplantation of hematopoietic stem and progenitor cells (HSPC) transduced with a lentiviral vector driving myeloid-specific expression of Interferon-alpha (IFNa-LV) to reprogram the tumor microenvironment towards activation of innate and adaptive immunity against the tumor (Escobar et al, Nat Commun in press). NSG mice were transplanted with IFNa-LV or control-LV-transduced human HSPC and challenged intravenously with the MM.1S-LUC myeloma line. GT significantly reduced bone disease as assessed by whole-body bioluminescence and bone marrow analysis. Next, we combined GT with pharmacological consolidation treatment with either Lenalidomide (R) or Daratumumab (D). R had little anti-myeloma activity in mice transplanted with control HSPC, leading instead to a reduction in the human HSPC graft. Strikingly, R enhanced the anti-tumor activity of IFNa GT, even when the IFNa-LV graft made up less than 10% of hematopoiesis, without increasing R-hematotoxicity. D showed activity against the human CD38+ cells in NSG mice, as evidenced by a depletion of human B cells while sparing myeloid cells. When combined with GT, D markedly reduced myeloma growth suggesting at least additive effects of the two treatments. In summary, in the context of a human myeloma xenograft model we provide a rationale for clinical translation of IFNa GT as a platform to enhance of state-of-the-art myeloma treatments.

P316

Generation of universal "off-the-shelf" chimeric antigen receptor (CAR)-engineered T cells

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Having rapid access to ''off-the-shelf'' T cell products, which can be applied across histocompatibility limitations, would greatly benefit the broader applicability of adoptive T cell therapy. To this end, we use induced pluripotent stem cells (iPSC) as an ideal platform for an unlimited source of cells that can be readily engineered to produce ''off-the-shelf'' tumor-reactive T cells. We have previously shown the feasibility and effectivity of successful engineering of T cell-derived iPSC (TiPSC) with a tumor specific chimeric antigen receptor (CAR). Constitutive CAR expression, however, arrested proper T cell development and resulted in an innate-like phenotype and *in vivo* anti-tumor function. We therefore hypothesized that a controllable CAR expression, induced after T cell development, could allow the generation of mature CD8+ CAR-T cells. To test our hypothesis, we generated TiPSC-derived T cells with a doxycycline inducible CAR (indCAR), which was introduced into the TCR-alpha locus via CRISPR/Cas9 gene editing, to eliminate the expression of the endogenous TCR. This strategy resulted in successful development of mature, expandable, TCR negative, CD4+, CD8alpha/beta double positive T cells, which were further differentiated into CD8 single positive T cells expressing the CAR (CD38 or CD19 indCARs) upon doxycycline treatment. The generated effector cells elicited significant, cell dose-dependent, CAR-specific tumor cell lysis. In conclusion our results provide the basis for further genetic manipulations and future translational use of synthetic TiPSC-derived CAR-engineered T cells for immunotherapy.

P317

Engineering CAR-T cells with an integrated on/off switch

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Adoptive cell therapy with reprogrammed T cells has shown great promise against various tumor types. However, adverse events including on-target/off-tumor toxicity, neurological toxicity and cytokine release syndrome have necessitated interventions including the incorporation of suicide genes, inhibitory antigen receptors and dual-antigen recognition systems to prevent or manage these toxicities. However, these interventions are either unable to inhibit ongoing toxicities or will completely ablate the therapy. Because of this, a more controlled approach in which the adoptive T cell therapy can be manipulated in a non-lethal fashion is needed. Such an approach will mitigate

systemic toxicities and improve tumor targeting and therapeutic efficacy by controlling the time and place of adoptive T-cell activity. Here, we describe a single component system to control chimeric antigen receptor (CAR) T-cell cytolytic properties with a small molecule drug in a switch OFF fashion. To do so, we combined a protease with a degron to control CAR stability in primary T-cells via the protease inhibitor Asunaprevir (ASN). We show that the addition of ASN leads to a selective and dose-dependent decrease of CAR expression and cytolytic properties. These results describe a simple and robust methodology for fine tuning CAR T-cell activity with a small molecule to improve the safety and efficacy of adoptive T-cell therapies.

P318

Characterization of tisagenlecleucel, a CAR-T cell product manufactured from patients with pediatric ALL

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Tisagenlecleucel consists of T cells expressing a CAR that recognizes CD19, a protein found exclusively on B cells. We characterized 51 batches of tisagenlecleucel that were manufactured for the ELIANA, a phase 2 trial of tisagenlecleucel in pediatric and young adult patients with r/r ALL. Tisagenlecleucel characteristics were evaluated by flow cytometry for identification of cell phenotypes and T-cell subsets and for their functional response to CD19 expressing target cells by means of cytokine release (ELISA) and cytotoxicity. Tisagenlecleucel contained mostly T cells despite very highly variable cell composition of the leukapheresis material. No B cells/B lymphoblasts/other cells originating from the leukapheresis starting material and only occasional residual NK cells were detected. The relative amount of CAR-positive cells was covering a wide range resulting in varying mixes of non-transduced (CAR-negative) and CAR-positive CD4+ and CD8+ cells. The ratio of transduced CAR-positive CD4+:CD8+ cells was also varying and no relation to clinical outcome in pediatric ALL was observed. The T cells in tisagenlecleucel were predominantly less mature transduced T cells which were highly activated. Immunophenotyping showed high cellular fitness with minimal immunosenescent or exhausted phenotype. Tisagenlecleucel demonstrated functional activity upon CD19-specific stimulation resulting in IFN-gamma release and cytotoxic capacity, however with a wide range among different batches. Despite the vast heterogeneity in regard to key product attributes such as T cell composition and functional response, tisagenlecleucel resulted in high rates of tumor remissions with 83% CR/CRi.

P319

Lentivirus integration site analysis characterization of CTL019

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Tisagenlecleucel-T (CTL019) is an autologous immunocellular cancer therapy of T cells encoding a chimeric antigen receptor that eliminate CD19 expressing B cells in an antigen-dependent but MHC-independent manner. Cells are transduced *ex vivo* with a replication-deficient human lentiviral vector with EF1α promoter, CD8α leader sequence, murine anti-CD19scFv, CD8 hinge and transmembrane region and 4-1BB/CD137 and CD3ζ/TCRζ signalling domain. We determined genomic insertion sites (IS) from 12 GMP-produced cancer patients (6 ALL and 6 DLBCL) and 2 healthy controls after measuring vector copy number (qPCR: 0.04 – 0.71) and transgene expression (flow cytometry: 3.7 – 42.3%). IS were recovered using shearing extension primer tag selection ligation-mediated PCR (S-EPTS/LM-PCR) followed by deep sequencing (MiSeq; Illumina) in triplicates and analyzed using adapted Gene-IS pipeline. We retrieved 33,324 IS within the 14 samples, unique IS number per sample ranged from 267 to 4,678, in line with the transduction percentage. For all but one sample, the ten strongest clones represented less than 10 % of the read count indicating a polyclonal, not-clustered integration profile. Consequently, all samples showed polyclonal scores on traditional metrics (richness, Shannon, Simpson and Eveness) or the recently developed pmdIndex. We looked at IS targeting LMO2, CCND2, MECOM, IKZF1 and HMGA2. Only CCND2, MECOM and IKZF1 gene loci harbored 4, 1 and 5 IS, whose relative abundance was far below 1%. No substantial hotspots of vector IS were identified. The 12 clinical samples from the CTL019 project showed conventional lentiviral IS in T-cells, without evidence for preferential integration near genes of concern.

P320

Highly efficient multiplex genome editing and lentiviral transduction in cord blood derived CD8+ T cells; towards a novel cellular treatment for acute myeloid leukemia relapse in pediatric patients

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Hematopoietic stem cell transplantation (HSCT) is the potential cure for pediatric patients with acute myeloid leukemia (AML). However, a large portion of transplanted patients still die mainly due to relapse. To improve overall survival, we developed a strategy based on the use of cord blood (CB) derived gene modified CD8+ T cells by introducing a recombinant T cell receptor (TCR) against Wilms tumor 1 (WT1) and at the same time eliminating endogenous TCR expression. We optimized CRISPR/Cas9 gene-editing of CD8+ T cells, as well as transduction by lentiviral vectors. A CRISPR/Cas9 multiplexing approach was used to gene-edit the TCR alpha or beta chain, to eliminate endogenous TCR expression and avoid mispairing between the introduced recombinant TCR and the endogenous TCR. High efficiency of up to 98% knock down was achieved, which was confirmed at a genomic level. High transduction efficiencies (up to 80%) and expression levels were obtained with lentiviral constructs containing viral and housekeeping gene promoters. Finally, WT1 TCR expression in a single lentiviral vector was optimized. Altogether, we developed a tumor antigen-specific T cell product with excellent expression of a WT1-specific TCR and very efficient elimination of endogenous TCR expression. These cells could be used to eliminate AML blasts and increase survival chances in pediatric relapse patients.

P321

Anti-CD19 CAR-T cells are efficient against CD19-positive 3D bioprinted solid tumor models

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Application of CAR-T cells demonstrated remarkable success in treatment of hematologic malignancies (HM), however this novel cell therapy is yetto prove its efficiency against solid tumors (ST). Poor clinical performance of CAR-T therapy in ST is primarily accounted for biological differences between ST and HM. Therefore it is important to develop models simulating *in vivo* conditions for testing effectiveness of CAR-T therapy against ST. In current study we evaluated anti-CD19 CAR-T cells against several 3D bioprinted solid tumor models. We constructed plasmid with 2nd-generation anti-CD19 CAR and also recombinant vector containing CD19 gene under control of internal ubiquitin C promoter and puromycin resistance gene. T-cells obtained from healthy donor were activated and transduced with lentivirus. CD19-positive cells were generated by transduction of MDA231, MDA468, A431, H522 solid tumor cell lines with CD19_p2a_PuroR recombinant lentiviral vector and further incubation with puromycin for selection. After that anti-CD19 CAR-T cells were applied onto CD19-positive tumor cell 3D constructs bioprinted using hydrogel composition. Efficacy of anti-CD19 CAR-T cells was assessed using viability assay and confocal microscopy. We propose that reported approach might be useful for screening and evaluating CAR-T against 3D solid tumor models.

P322

Consequences of maternal microchimerism upon CAR-T cell treatment

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With the use of efficient gene transfer technologies, T cells can be genetically modified to stably express antibody receptor (chimeric antigen receptors = CAR) on their surface, conferring new antigen specificity. The consequences of maternal microchimerism (MM) in newborns of CART-treated women and the risk for newborns to suffer from B cell depletion are unknown. MM is acquired by an infant during pregnancy. Currently, two CART19 constructs are used in clinics. To stay close to the clinical setting, we cloned two 2-cistronic-lentiviral constructs containing CAR19-CD28 or CAR19-4-1BB and mCherry connected with T2A site using a lentiviral construct and tested them *in vitro* and *in vivo*. To achieve adequate transduction efficiency (TE), lentiviral constructs were concentrated and TE efficacy was confirmed using several methods. As immuno-competent female bl/6 mice were used, preconditioning with cyclophosphamide was necessary to ensure engraftment of transferred CAR-T cells. Two cyclophosphamide concentrations were tested to determine a safe (effect on reproduction and on offspring rate) but effective cyclophosphamide concentration. Our observation showed decrease in lymphocyte population, but neither malformations nor effects on offspring rates were seen. Subsequently, mice were pre-treated with cyclophosphamide and dosed with 1×106 CAR-T cells (CAR19-CD28-mCherry, CAR19-4-1BB-mCherry or CAR19-mCherryctrl). Localization and effects of CAR19 T cells were analyzed in both treated female mice and offspring. Furthermore, to ensure all facets on MM, we further improved the CAR-19 construct by cloning 3-cistronic-retroviral constructs consisting of Pmscv-P2A-mCherry-T2A-CAR19CD28 or 4-1BB-IL-12. Functionality is assessed by IncuCyte and Amnis technologies and ongoing *in vivo* mouse studies.

P323

Automated end-to-end manufacturing solutions for CAR-T immunotherapies

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1: Lonza

Autologous cell therapies, such as CAR-T cells for cancer therapy, pose major cost and manufacturing challenges. The ideal solution to many of these challenges is to have the entire manufacturing process performed via a closed and automated system specifically designed to meet the needs. Our work using the novel CocoonTM system highlights the successful translation of a typical manual CAR-T process into the closed and automated CocoonTM system to reduce cost and maximize process efficiency and quality. The CAR-T process was performed using critical parameters such as starting inoculation of 100 million PBMCs, CD3/CD28 activation, IL-2 and IL-7 supplemented into T-cell growth media for culture expansion with an optimized and defined feeding strategy. The system was programed to run the entire process after inoculation automatically, without manual intervention. The in-process samples were drawn for cell counts and viability. At the end of the harvesting process, FACS analysis and killing assay were performed, the CAR-T cells reached approximately 2 Billion cells. Automated runs and associated manual controls were able to maintain both CD4+ and CD8+ T cell subsets. There was a higher detection of NGFR in the CD4 fraction than in the CD8 fraction in all samples. In summary, automated CAR-T process in the Cocoon system yields a healthy populations of T cell subsets. This system is a viable solution to translate labor-intensive CAR-T process into a fully automated system, thus allowing scalability, high yield, reduction of manufacturing cost, and better process control to yield high quality CAR-T cells.

P324

Analysis of lentivirus integration site distributions in CTL019 immunotherapy

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Tisagenlecleucel (CTL019) is an cancer immunocellular therapy reprogramming autologous T cells with a transgene encoding a chimeric antigen receptor (CAR), to target and destroy CD19 positive malignant cells. This report investigates

lentiviral integration site distributions and implications for safety. Specifically, we investigated whether the CTL019 lentiviral vector might integrate into sites of concern preferentially. Adverse events have taken place in human gene therapy in which gammaretroviral vectors integrated near the 5′ ends of cancer-associated genes such as LMO2, CCND2, and MECOM. Cells proliferated, accumulated more genetic lesions, and eventually evolved to frank leukemia. Clonal expansions without clinical consequences have been observed for lentiviral vectors in HMGA2; a recently reported integration event in TET2 might have assisted CART19 therapy for CLL. Here fourteen DNA samples were studied from experimental infections of T-cells with the CTL019 lentiviral vector from 12 cancer patients (6 ALL and 6 DLBCL) or 2 healthy controls. Integration site sequences were determined and analyzed using the INSPIIRED pipeline (integration site pipeline for paired end reads). All samples were highly polyclonal, either by traditional measures (S. chao, Gini or Shannon indexes) or the recently developed UC50. Integration site distributions were as expected for lentiviral integration in human T-cells, showing integration in gene rich regions, within transcription units, and near epigenetic marks associated with active transcription. There was no indication of preferential integration near genes of concern, or preferential outgrowth of cells harboring integration events near genes of concern in the pre-infusion cell expansion.

P325

Automated determination of the purity and packaging of gene therapy delivery platforms using transmission electron microscopy

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1: Vironova

The morphological characterization of gene therapy delivery platforms can support the drug development process by identifying typical product-related impurities and analyzing of the capsids packaging supports. Gene delivery empty viral capsids may be the result of failure in packaging or occur as a result of structural changes of the capsid along the manufacturing process – the latter being not only a risk for lowered potency but also representing a safety issue for the patient, as particles with a damaged capsid can provoke immunogenicity issues. Transmission electron microscopy (TEM) can provide unique insights in the characterization of viral gene delivery platforms such as adeno-associated viruses (AAVs), adenoviruses and retroviruses. TEM analysis of negatively stained samples (nsTEM) can provide insights on the morphology of the viral vectors present in a specimen, where a qualitative assessment can be made on the intactness of the particles, and on the presence and characterization of impurities. MiniTEM, a compact low voltage electron microscope with automation features specifically designed for the characterization of nanoparticles can be used to automatically analyze specimen and give indications on the integrity of the particles and on the presence and nature of process related impurities. TEM analysis of Cryo-preserved samples (cryoTEM) is used to assess the encapsulation of vectors such as AAV and is, in combination with the Vironova image analysis software (VAS), a GMP validated method suitable for QC testing of the ratio of full AAV particles. In this presentation results obtained from using both these techniques will be presented.

P326

The comparative analysis of Treg-cells and transcription factor Foxp3 in lung diseases

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It is necessary to study chronic obstructive pulmonary disease (COPD) and atopic bronchial asthma (ABA) at the level of immune cell interaction due to the presence of a common target tissue and the data of the association with chronic inflammation. Treg-cells (Tregs) and transcription factor Foxp3 play a key role in maintaining of immune homeostasis. The function of the Tregs is aimed at suppressing the effector action of immunocompetent cells in normal. It is known that post-translational modifications of Foxp3 occur due to the action of histone-acetyltransferase p300 and can influence the stability Foxp3. The aim of this study was to analyze and compare the immune status of Treg-cells in ABA and COPD. It was found that the number of Treg cells (5.17%) is higher in COPD patients with and is lower in ABA patients (2.76 %) than in healthy individuals (9.29%). The level of protein Foxp3 expression is higher in Tregs in the group of the patients with COPD and is lower in the group of the patients with ABA than in healthy ones. Besides, it was found that the protein p300 was expressed in the Tregs. There was a tendency to increase levels of protein p300 expression in Treg-cells in ABA and COPD patients. Decrease of the level of Foxp3 protein expression when the Tregs were cultivated with the inhibitor of the p300 (Garcinol) indicates on a regulatory effect of p300 on Foxp3. This study was supported by Program of Competitive Growth of Kazan Federal University.

P327

Alternatives to viral vectors for nucleic acid-mediated therapies

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1: Polyplus Transfection

Nucleic acids have considerable potential as therapeutic agents in the treatment of pathologies including genetic diseases, viral infections, and cancer therapies. The major challenge for the use of nucleic acids in therapy lies in safely delivering these anionic macromolecules to their intended sites of action. At Polyplus-transfection, we develop powerful non-viral vectors to safely deliver nucleic acids *in vivo* to target a wide range of tissues, through various routes of administrations. Of these reagents, *in vivo*-jetPEI® is widely acknowledged to deliver nucleic acids in animals; and coherently is selected as the delivery vector of choice in several drug development programs, notably for immunotherapies. To fulfill all the quality requirements associated to its use in Human, Polyplus-transfection® supplies preclinical grade and cGMP grade *in vivo*-jetPEI® reagents for a growing number of plasmid and oligonucleotide based-preclinical studies and clinical trials.

P328

CRISPR/Cas9 unites with Sleeping Beauty to generate CAR-T cells with enhanced therapeutic index for fighting against immunosuppressive tumour microenvironment

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Adoptive immunotherapy with CD19-specific chimeric antigen receptor (CAR) is effective for treating B-cell malignancies. However, extrapolating this paradigm to other hematologic and prevalent solid tumors requires more advanced bioengineering of CAR T cells and likely, the ability for concomitant and multiplex genome editing to augment their therapeutic potency. Therefore, we developed a platform that enables simultaneous and virus-free Sleeping Beauty (SB) gene transfer and multiplex CRISPR/Cas9 genome editing in T cells to create CAR T cells with improved antitumor function. We generated CAR T cells against ROR1 antigen (breast cancer) being edited at TGFβ-RII, PD-1 or both loci to abrogate the signaling mediated by these receptors which leads to impaired T-cell function within tumor microenvironment. A high rate of CAR gene-transfer ($\approx$40%, n=6) and knockout (TGFβ-RII $\approx$50%, PD-1 $\approx$ 80%, double KO $\approx$50%, n=6) was achieved. High-throughput sequencing revealed a high rate of SB CAR transposition in genomic safe harbor regions, and no guide RNA off-target activity. Compared with non-edited CAR T cells, genome-edited CAR T cells conferred more potent antitumor functions *in vitro*. Experiments that demonstrate enhanced therapeutic efficacy of the CAR T cells *in vivo* are currently completing follow-up. Collectively, we developed a novel T-cell engineering platform to accomplish concomitant CAR gene transfer and multiplex genome editing in a single-step and virus-free manner. Genome-edited CAR T cells possess enhanced therapeutic potential and a safe genomic integration and genome editing profile. Manufacturing is currently adopted under GMP-conditions in preparation for clinical trials that evaluate efficacy of these advanced CAR T-cell products.

P330

Ex vivo gene therapy for Fabry disease

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1: AVROBIO

Ex vivo gene therapy involves transplantation of genetically modified autologous stem cells to compensate for an underlying genetic defect. Lysosomal storage disorders (LSDs) are inherited diseases that result from defective lysosomal function, leading to intra-lysosomal accumulation of non-metabolized macromolecules and subsequent cellular and organ dysfunction. LSDs are attractive candidates for *ex vivo* gene therapy based on the potential to transform a patient's own cells into a drug product to deliver functional protein after a single treatment. Fabry disease (FD) is an LSD caused by mutations in the GLA gene that result in a functional deficiency of the enzyme, alpha-galactosidase A (AGA), which leads to pathological accumulation of glycosphingolipids throughout the body. Significant morbidity and early mortality result from damage to kidneys, heart, and brain. AVR-RD-01 is an *ex vivo*, lentiviral vector-mediated gene therapy being developed for the treatment of patients with FD. In an ongoing Phase 1 clinical trial, two male patients with classic FD have been treated and the therapy was well tolerated. Both had nearly undetectable levels of plasma AGA activity before receiving AVR-RD-01, which increased to well above the range for males with classic FD within days after treatment and have remained above the classic FD range for 12 and 3 months, respectively. Additionally, results have demonstrated LV-vector gene marking in the patients' blood and bone marrow, indicating survival of the transduced cells. Based on the initial results from the Phase 1 trial, a Phase 2 trial has been initiated and the first patient treated.

P331

Liver directed gene therapy using AAV9 to treat a new murine model of propionic acidemia caused by Pccb deficiency

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Propionic acidemia (PA) is a rare inborn error of metabolism caused by deficient propionyl-CoA carboxylase (PCC) activity, and equally attributed to mutations in either the PCC alpha (*PCCA*) or beta (*PCCB*) genes, which encode the two subunits of PCC. Individuals with PA display chronic morbidity and early mortality indicating that new treatment options are needed. Previous studies have shown that AAV gene delivery can rescue murine models of Pcca and lower disease related metabolites. However, gene therapy for PCCB deficiency has not been described. Here, we report new murine models of Pccb deficiency that recapitulate the biochemical and clinical features of the human conditon and demonstrate the potential of AAV gene delivery as a new therapy for PA caused by PCCB deficiency. First, we created new *Pccb* alleles using genome editing and characterized four unique *Pccb* mutations, all of which resulted in elevated levels of 2-methylcitrate and neonatal lethality in the homozygous state. Next, an AAV configured with a shortened human alpha-1-antitrypsin (hAAT) promoter driving the expression of the human *PCCB* gene (AAV9-hAAT-PCCB) was generated, packaged as a serotype 9 vector, and delivered at a dose of 1x10^11 GC to $Pccb^{-/-}$ pups at birth. A single treatment with AAV9-hAAT-PCCB efficiently rescued the $Pccb^{-/-}$ pups from neonatal lethality and reduced the plasma levels of the methylcitrate. The new models of PA, and vectors used to demonstrate proof of concept efficacy, acheive critical first steps toward the development of AAV gene therapy for humans with propionic acidemia caused by *PCCB* mutations.

P332

Characterization of hematopoietic system reconstitution *in vivo* in metachromatic leukodystrophy gene therapy patients

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Here we report the molecular analysis of hematopoietic reconstitution in 20 patients enrolled in a self-inactivating lentiviral vector-based hematopoietic stem cell (HSC) gene therapy clinical trial for metachromatic leukodystrophy conducted at SR-Tiget (up to 7 years' follow-up) and in 7 additional patients treated via early access programs. We retrieved integration site

(IS) from CD34+, myeloid and lymphoid cells purified at different time points after therapy from bone marrow and/or peripheral blood using PCR protocols. From each patient, we retrieved from 6,000 to 65,000 IS, many of which persisted long term with multi-lineage potential. In line with the clinical data, we did not observe clonal dominance events, no bias to integrate near cancer genes and no common insertion sites generated by genetic selection in any patient. The clonal dynamics of hematopoietic reconstitution of the different lineages showed that circulating lymphoid cells were oligoclonal at early time-points and progressively switched to polyclonal after 6 months, whereas myeloid cells were polyclonal from the first time points. Estimations of the HSC activity, obtained by mark-and-recapture statistics of IS observed over time in short-lived cells, showed that at earlier time points the population size was >26,000 cells that then progressively stabilized to ~10.000 from 9 months post-transplantation, suggesting that the initial waves of reconstitution are sustained by short-lived progenitors. Our data indicate that the treatment results in a highly diversified polyclonal and multilineage reconstitution of hematopoiesis without signs of genotoxicity.

P333

Gene therapy for Crigler-Najjar syndrome with AT342, a liver-targeted AAV8-UGT1A1 vector: preliminary results from a Phase 1/2 study (VALENS)

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Crigler-Najjar (CN) syndrome, a severe, autosomal-recessive, monogenic disease, is characterised clinically by jaundice due to high serum levels of unconjugated bilirubin that can lead to irreversible bilirubin-induced brain injury (kernicterus) or death. Mutations in the uridine diphosphate glucosyltransferase gene (UGT1A1) can lead to the absence or diminished expression of the hepatic enzyme involved in normal bilirubin conjugation and excretion. Standard-of-care phototherapy (≥10-12h/day) reduces but does not normalise bilirubin levels and most patients eventually require liver transplantation. We report preliminary results from the ongoing VALENS trial, a randomized, open-label, ascending-dose, Phase 1/2 study of AT342 in CN. Eligible patients are ≥1 year of age, have bi-allelic mutations of UGT1A1, and require ≥6h/day phototherapy. VALENS was designed to enroll three patients and one-delayed treatment control patient per evaluated dose level. Treated patients receive a single intravenous infusion of AT342, and oral prednisolone daily for 16 weeks as prophylaxis against immunological reactions. Phototherapy (PT) is continued at prescribed levels until criteria are met to allow weaning off PT from Weeks 13 to 17. Primary endpoints include safety and change in bilirubin levels at Weeks 12 and 18. The first treated patient received an AT342 dose of 1.5x1012 vector genomes/kg, which was well tolerated. An initial rapid reduction in bilirubin by 2 weeks post-dose was followed by a slow return to baseline levels by Week 12, similar to low-dose results in animals. Subsequent patients will be enrolled at a higher dose-level. The latest clinical data will be presented.

P334

AAV9-GCDH gene therapy for glutaric aciduria type I

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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, 3-hydroxyglutarate and glutarylcarnitine in tissues and body fluids. Clinically, GA-I patients display macrocephaly, progressive dystonia and dyskinesia. Most affected individuals experience acute encephalopathic crises during the first 6 years of life. 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 striatal degeneration with irreversible brain damage. We have explored the feasibility of *in vivo* gene therapy in the preclinical model of the disease, the Gcdh -/- mice. These animals show a biochemical profile and neuropathological alterations that resemble the human disease. Moreover, exposure of young animals to a lysine overload enhances the severity of the disease leading to loss of weight and motor impairment. Intravenous administration of an AAV9-GCDH in Gcdh -/- mice restores GCDH expression and partially rescues the accumulation of glutarylcarnitine and glutaric acid in liver and serum. In summary, these data suggest the potential of an AAV-mediated gene therapy approach for glutaric aciduria type I.

P335

Intravenous AAV9-mediated gene therapy ameliorates neuronal disease of mucopolysaccharidosis type ll (MPSll)

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Mucopolysaccharidosis type ll(MPSll) is a lysosomal storage disease caused by deficiency of iduronate-2-sulfatase (IDS) which leads the accumulation of glycosaminoglycans (GAGs) in brain. Intra brain AAV gene therapy for brain disease of MPS II was reported, however this approach required surgical procedure and was invasive. Thus, we evaluated the effectiveness of the intravenous administration of AAV9/IDS to brain disease of MPS II mice. In this study, we administered AAV9 vector expressing IDS to murine model of MPS II and evaluated brain related parameters. IDS activities in peripheral blood at 4weeks after treatment achieved 130-fold higher ($n=10$ 1317.4 ± 534.4 nmol/4h/ml) than wild-type levels ($n=91.11 \pm 0.40$nmol/4h/ml) and these values maintained until 16 weeks after treatment with a marginal decrease. GAG contents in brain cortex decreased significantly from 176.7 ± 141.9(Non-treated MPS ll group) to 25.43 ± 22.9 pmol/mg protein(AAV-treated group), $p=0.007$, corresponding to 14% of MPS II mice. IDS activities in brain had also increasing tendency from 0.104 ± 0.082 to 0.168 ± 0.137 nmol/4h/mg protein, $p=0.138$. The histological finding showed a reduction of Subunit C Mitochondrial ATP Synthase positive cells at cortex, which is the

one of surrogate maker of brain disease of MPS II. The fear conditioning test had also improving tendency. In conclusion, a small amount of IDS decreased GAG in brain and intravenous AAV9-mediated intravenous gene therapy might be feasible for brain disease of MPS II mice.

P336

Targeted integration of *MUT* into Albumin using a promoterless AAV vector (GeneRide^{TM}) confers a hepatocellular growth advantage in mice with methylmalonic acidemia (MMA)

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MMA is genetic disorder most typically caused by a deficient of methylmalonyl-CoA mutase (MUT) activity. Patients with MMA suffer from frequent, and potential lethal, episodes of metabolic instability, which accounts for the severe morbidity and early mortality observed in the patients. To minimize the potential of vector related insertional mutagenesis and to preserve MUT expression after gene therapy, we have designed a promoterless AAV vector that utilizes homologous recombination to achieve site-specific gene addition of human *MUT* into the mouse albumin (*Alb*) locus. This new vector, AAV-Alb-2A-MUT, contains arms of homology flanking a 2A-peptide coding sequence proximal to the *MUT* gene, and generates *MUT* expression from the endogenous *Alb* promoter after integration. We have previously reported that AAV-Alb-2A-MUT, delivered at a dose of 8.6E11-2.5E12 vg/pup at birth, reduced disease related metabolites, and increased growth and survival in murine models of MMA. RNAscope of older AAV-Alb-2A-MUT treated MMA mice revealed robust *MUT* expression, and *MUT* positive hepatocytes appeared as distinct and widely dispersed clusters, consistent with a pattern of clonal expansion. In addition, the RNAscope studies revealed that after treatment the *MUT* expression was present in $\sim$5-40% of the hepatocytes in MMA mice versus 1% as noted in wild-type controls. Finally, hepatic MUT protein expression and the number of *Alb*-integrations were also observed to increase overtime in treated MMA mice. The aggregate findings suggest that a selective advantage for corrected hepatocytes can be achieved in the murine models of MMA after treatment using MUT GeneRide^{TM}, an observation with immediate clinical relevance.

P337

Anc80 and AAV8 vectors mediate equivalent long-term hepatic correction of methylmalonyl-CoA mutase deficiency in a murine model of isolated methylmalonic acidemia (MMA)

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Other than dietary and cofactor therapy, no alternative to organ transplantation exists for patients with isolated methylmalonic acidemia (MMA), a common and severe organic acidemia most frequently caused by mutations in the enzyme methylmalonyl-CoA mutase (MUT). In these studies, we used a model of severe MMA, *Mut* $^{-/-;TgINS\text{-}MCK\text{-}Mut}$ mice, to test the effects of either an Anc80 (Anc80-hAAT-MUT) or AAV8 vector (AAV8-hAAT-MUT) to mediate long-term hepatic expression of MUT. MMA mice treated with $5x10^{12}$ GC/kg of either AAV experienced life-long maintenance of weight and metabolic stability, as evidenced by stable, but reduced, levels of circulating metabolites, and preserved 1-^{13}C propionate oxidative capacity. More than one year after gene therapy, MUT protein was detected in hepatic extracts, near the level of controls, in the treated MMA mice. Of note, all groups of mice that received AAV were without evidence of hepatocellular carcinoma or dysplasia, and had LFTs equal to age- and diet- matched littermates. RNA in situ hybridization experiments, performed on the livers from treated *Mut* $^{-/-;TgINS\text{-}MCK\text{-}Mut}$ mice, revealed transduced cells that were scattered throughout the parenchyma and stained positive for *MUT*. These studies demonstrate the functional equivalency of AAV8 and Anc80 vectors to mediate robust hepatic correction of Mut deficiency in MMA mice, with durability and lack of toxicity, and have immediate relevance for patient translation.

P338

Liver-based expression of the human alpha-galactosidase A gene in a murine Fabry model results in continuous therapeutic levels of enzyme activity and effective substrate reduction

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Fabry disease, caused by mutations in the GLA gene encoding alpha galactosidase A (a-GalA), leads to systemic accumulation of globotriaosylceramide (Gb3) and lyso-Gb3 in plasma and tissues and renal, cardiac and cerebrovascular disease. Fabry disease is commonly treated by enzyme replacement therapy, which requires a lifetime of biweekly infusions and may not clear all substrate from secondary organs. The development of a one-time, durable treatment is, therefore, desirable. To this end, an AAV-mediated, liver-targeted gene therapy approach was developed and evaluated in a knock-out mouse model for Fabry disease (GLAKO) that lacks α-GalA activity and accumulates high levels of Gb3/lyso-Gb3 in plasma and tissues. This strategy employs an episomal AAV vector encoding human GLA cDNA (hGLA) driven by a liver-specific promoter. Administration of a single AAV dose resulted in supraphysiological expression of plasma α-GalA, reaching stable levels by day 14 up to 200 fold of wild type. α-GalA activity in heart and kidney of GLAKO mice treated with this hGLA cDNA averaged 20- and 3- fold over wild type levels, respectively, and Gb3/lyso-Gb3 in these tissues were near normal levels. Appropriate glycosylation of the α-GalA enzyme produced from liver cells was confirmed by *in vitro* experiments to ensure efficient mannose-6-phosphate mediated lysosomal uptake in target tissues. These studies provide ''proof-of-concept'' for AAV-mediated targeting of hepatocytes *in vivo* to express therapeutic levels of human α-GalA, resulting in marked reduction of the accumulated Gb3/lyso-Gb3 in key tissues.

P339

Enhanced lentiviral transduction coupled to cell homeostasis preservation in human 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 during the in-vitro manipulation (48-72 hours). In this study, we explored the usage of small molecules, individually and in combination, for enhancing human HSPCs transduction by a therapeutic lentiviral vector (LV) designed for GT of a Lysosomal Storage Disorder. Best results were obtained by exposing adult mobilized CD34+ cells to a combination of an immunomodulator and a surfactant agent, both approved for clinical use. LV transduction increased of $\approx$ three-folds without detrimental effects on cell-viability and clonogenic potential (bulk liquid culture and clonogenic-assay). The mechanism possibly linking immunomodulation and LV transduction enhancement is currently under investigation. Besides, we added a potent aryl hydrocarbon receptor antagonist to preserve stem cell homeostasis during cell transduction, which was recently shown to increase microglial cell engraftment in NSG mice (abstract 205, 2018 BMT Tandem Meetings, Magenta Therapeutics). In-vitro characterization of human HSPCs exposed to the three compounds' combination included a comprehensive phenotypic analysis for stemness-associated markers by flow-cytometry, primary and secondary clonogenic-assay, and a genome-wide transcriptional analysis by RNA-seq. Whereas, the capability of these cells of promoting sustained, high-level gene marking in-vivo, and long-term hematopoietic and microglial reconstitution, was investigated by HSPC-transplantation assay in NSG mice.

P340

Pre-clinical safety and efficacy evaluation of haematopoietic stem cell gene therapy for MPSIIIA

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Mucopolysaccharidosis IIIA (MPSIIIA) patients suffer from severe behavioural disturbances, progressive mental decline and other debilitating non-neurological symptoms starting at an early age. MPSIIIA is caused by a defective SGSH gene resulting in accumulation of damaging partially degraded heparan sulphate in lysosomes and cells. Currently no effective treatments exist. We have previously showed that hematopoietic stem cell gene therapy (HSCGT) can effectively correct the neuronal phenotype in MPSIIIA mice (Sergijenko 2013) using a lentiviral vector (LV) encoding codon optimised SGSH driven by a myeloid specific CD11b promoter (LV-CD11b-coSGSH). In preparation for clinical trial we demonstrate efficacy and safety of GMP grade LV-CD11b-coSGSH in-vitro in human CD34+ cells and in-vivo in MPSIIIA and NSG mice. We achieved high vector copies per genome following single and double transduction as well as high levels of SGSH gene expression in human CD34+ cells, without adverse toxicity or lineage skewing. We transduced human mobilised peripheral blood CD34+ cells at large scale and demonstrated comparable engraftment of mock or double transduced, cryopreserved CD34+ cells in transplanted humanised NSG mice. Transduced cells were detected in the brain as well as in the bone marrow, thymus, spleen, liver, lung, WBCs and kidneys with an average VCN comparable to the VCN in transduced cells prior to transplant. No transduced cells were detected in the gonads, with no vector shedding. Genotoxicity analysis demonstrated low transformation potential of the SGSH LV, similar to other clinically available LVs. This work establishes the preclinical safety and efficacy of HSCGT for the treatment of MPSIIIA.

P341

Functional, biochemical and transcriptional rescue of advanced Pompe disease with liver expression of secretable GAA

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Pompe disease is a neuromuscular disorder caused by mutations in the gene encoding for the lysosomal enzyme acid α-glucosidase (GAA) which converts lysosomal glycogen to glucose. GAA deficiency leads to pathologic glycogen accumulation throughout the body. Enzyme replacement therapy (ERT) is the only available treatment for subjects affected by Pompe disease, but it suffers from several shortcomings, including limited efficacy and high immunogenicity. We recently reported full rescue of the Pompe phenotype in symptomatic four-month-old Gaa-/- mice using AAV liver-directed gene therapy with a highly secretable form of GAA (Puzzo, Colella et al., Sci Trans Med, 2017). To assess whether this approach could result in therapeutic efficacy in Gaa-/- mice with advanced pathology, we treated nine-month-old animals with an AAV8 encoding for highly secretable GAA and followed the animals for 9 months thereafter. AAV-treated Gaa-/- mice showed high and sustained circulating levels of GAA activity which correlated with a complete clearance of glycogen in cardiac and skeletal muscle, and a $\sim$30% glycogen reduction in the nervous system. Moreover, we observed an amelioration of respiratory symptoms and a complete normalization of muscle strength in the AAV-treated cohort. Detailed investigation of muscle transcriptome by RNASeq showed normalized expression of genes involved in lysosomal and mitochondrial homeostasis. These were also supported by the normalization of autophagic buildup and mitophagy in skeletal muscle. These results in Gaa-/- mice with advanced disease state shed light on the reversibility of the disease phenotype in the Pompe mouse model via liver gene transfer of highly secretable GAA.

P342

AAV gene therapy LYS-SAF302 demonstrates widespread sulfatase distribution in primate brain and correction of disease pathology in MPSIIIA mice

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Lysogene completed its program of nonclinical studies for LYS-SAF302, an optimized AAVrh.10 vector carrying the human SGSH gene. LYS-SAF302 achieves 3-fold higher enzyme expression than LYS-SAF301, which was previously tested in a phase 1/2 trial. A dose ranging efficacy study was conducted with LYS-SAF302 in 5-week-old MPS IIIA mice (15/gender/group) at three different doses (8.6E+08, 4.1E+10, and 9.0E+10 vg/animal) injected into the caudate putamen/striatum and thalamus. LYS-SAF302 was able to dose-dependently correct MPS IIIA-related brain pathology at 12-weeks and 25-weeks post-surgery. To study SGSH distribution in the brain of large animals, LYS-SAF302 (7.2E+11vg/animal) was injected into white matter of nonhuman primates ($n=2$; 2 injections of 50μl per hemisphere), using an optimized injection method. Six weeks following dosing, animals were euthanized, the brain was sliced into 3-4 mm coronal slabs, and each slab was divided into 10×10 mm sections. Viral DNA and SGSH enzyme activity were determined in each of these punches. In 97% (±2) of punches analysed, we observed a >20% increase in SGSH activity relative to non-injected controls. In parallel, a GLP toxicological and biodistribution study conducted in 12 juvenile NHP did not reveal toxicity following bilateral white matter administration of LYS-SAF302 at any dose (up to 4.9E+12vg/animal), and confirmed the broad distribution of enzymatically active SGSH throughout the primate brain. Taken together, these data validate intraparenchymal AAV administration as a promising method to achieve widespread enzyme distribution and correction of disease pathology in MPSIIIA. An international Phase 2-3 clinical trial in MPS IIIA with LYS-SAF302 is planned.

P343

Development of a novel AAV-based gene therapy in combination with tolerogenic nanoparticles for sustained treatment of ornithine transcarbamylase deficiency

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Ornithine transcarbamylase deficiency (OTCd) is an X-linked monogenic urea cycle disorder that causes hyperammonemia and related irreversible brain damage. Despite dietary restriction and ammonia scavenging drugs, many patients die early in life due to ammonia crisis and neurotoxicity. Our aim is to develop a novel strategy to treat OTCd, based on adeno associated virus (AAV) vectors encoding the human OTC gene in combination with immunomodulatory biodegradable synthetic vaccine particles containing rapamycin (SVP-R). Pre-clinical studies showed that co-treatment of AAV vectors with SVP-R resulted in the prevention of humoral and cellular response against the immunogenic AAV capsid antigens, enabling repeated doses of gene therapy treatments. This tolerogenic AAV strategy would have two major benefits: 1) ability to treat patients early in life with the possibility to re-dose to maintain therapeutic levels, and 2) prevent liver damage associated with cellular immune reaction to the therapeutic vector while minimizing the use of steroids. We generated a ssAAV8 encoding a codon-optimized human OTC transgene under the transcriptional control of a liver specific promoter, which was about 5 times more efficient than the wt hOTC cDNA in correcting the OTCspf-ash mouse model. A dose finding experiment was performed in OTCspfash mice in order to identify the therapeutic dose by determining protein levels, catalytic activity and urinary orotic acid levels. To further improve safety of the therapeutic vector, we tested a version in which liver-specific enhancer elements located next to the ITR were deleted. Preliminary *in vitro* experiments showed a 50% reduction in transgene expression.

P344

Durable correction of phenylketonuria *in vivo* following a single intravenous dose of AAVHSC15 packaging a human phenylalanine hydroxylase transgene

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Novel CladeF adeno-associated viruses isolated from human $CD34^+$ hematopoietic stem cells (AAVHSCs) show high liver tropism and potential for hepatic gene therapy. Phenylketonuria (PKU) is a rare metabolic disease arising from mutations in the PAH gene. Current treatments do not address the underlying genetic defect. PAHenu2 mice, a model of severe PKU, harbor a missense mutation (F263S) in the PAH gene reducing enzyme activity by 99% that causes a 40-fold elevation in serum phenylalanine (Phe) on normal chow diet. The human PAH transgene driven by a ubiquitous promoter packaged in AAVHSC15 (AAVHSC15-PAH) was administered as a single intravenous injection in PAHenu2 mice. Serum levels of Phe and tyrosine [metabolic byproduct of PAH] were measured weekly. Livers were harvested at periodic intervals and processed to measure vector genomes, mRNA and PAH enzyme activity. One week post-dose, Phe levels decreased from 2000uM to <150uM ($p<0.0001$) and were sustained out to >28 weeks post-dosing ($p<0.0001$). Dose-dependent increases in PAH vector genomes, mRNA, and enzymatic activity were observed. Serum AST/ALT levels in treated mice did not show significant changes. To maximize potency, vector sequences in AAVHSC15-PAH were optimized by addition of a liver-specific promoter that decreased serum Phe and increased serum Tyr in PAHenu2 mice even at ten-fold lower doses from the initial research vector with durable response seen out to >38 weeks in mice on normal chow. These data demonstrate that a single dose of optimized AAVHSC15-PAH, labeled as HMI-102, resulted in long-term correction of PKU in PAHenu2 mice while on normal chow.

P345

Optimization and scale-up of a liver-directed approach for Pompe disease based on secretable GAA

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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 and results in multi-system pathology. Enzyme replacement therapy (ERT) increases survival, stabilizes disease progression, and is the current standard of care for both infantile- and late-onset Pompe disease patients. However, ERT suffers from several drawbacks such as limited efficacy, immunogenicity of the recombinant GAA, high cost and need for frequent infusions. We have shown that liver-directed 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 (Puzzo, Colella et al, 2017). To further investigate the therapeutic potential of this approach, we treated four-month old Gaa-/- mice with vector doses ranging from 1e11 to 2e12 vg/kg of AAV expressing either secretable GAA transgenes or the native version of the transgene. Secretable GAA demonstrated superior therapeutic efficacy compared to native GAA even at the lowest doses of vector tested, as shown by enhanced survival and muscle function. Based on these promising results, and in an effort toward clinical translation, we further optimized the secretable GAA therapeutic expression cassette and selected a highly hepatotropic vector capsid. Comparability studies and scale-up to non-human primates are currently ongoing. Results support the development of liver-targeted gene therapy with secretable GAA for the treatment of Pompe disease.

P346

Early dose response data with homologous recombination-based genome editing approach using a promoterless AAV vector in mice with methylmalonic acidemia

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Methylmalonic acidemia (MMA) is an autosomal recessive inborn error of metabolism associated with mutations in the methylmalonyl-CoA mutase (MUT) gene. MMA manifests itself in the neonatal period with potentially lethal metabolic decompensation events and basal ganglia strokes. Patients have a deficiency in branched chain amino acid metabolism characterized by high levels of circulating methylmalonic acid and methyl citrate. There are no approved therapeutic interventions and patients are managed with extreme dietary regimens and liver and kidney transplants. Given the early onset of disease pathology and the potential for the development of irreversible disease sequalae, it is important to treat patients as early in life as possible. To accomplish this we have developed a novel genome editing technology platform, GeneRide™, that leverages the natural process of homologous recombination to insert a healthy version of the MUT gene into the albumin locus, resulting in expression at high levels specifically in hepatocytes. This approach addresses the risks associated with random insertion of active promoters utilized in canonical AAV therapies, and the potential for offsite mutagenesis associated with nucleases used in gene editing approaches. Here we present preliminary data from a dose ranging study with our promoterless, nuclease-free MUT GeneRide™ vector in Mut-/-Mck+ mice. Data include improvements in survival, weight gain and blood metabolite levels.

P347

Intraperitoneal delivery of LentiINS vector lowered fasting plasma glucose and improved glucose tolerance of type 1 diabetic Wistar rats

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Type 1 diabetes (T1DM) is characterized by autoimmune destruction of pancreatic beta cells leading to insulin deficiency. Thus, both short- and intermediate-acting insulin analogs are under development to mimic endogenous insulin response. Normally, basal insulin is continuously secreted at low levels in response to hepatic glucose output, while post-prandial insulin is released periodically in response to high blood glucose levels following a meal. Consequently, 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.1 Compared to other cell types, pancreatic beta cells possess unique features such as controlled transcription and translation of proinsulin, presence of glucose-sensing machinery, prohormone convertase expression, and a regulated secretory pathway. Since previous insulin gene delivery to non-beta cell types did not produce successful results, a new lentiviral vector (LentiINS) with insulin promoter hooked up to proinsulin encoding gene was generated using Multisite Gateway Technology to sustain pancreatic beta cell-specific insulin gene expression. Intraperitoneal delivery of HIV-based LentiINS gene therapy vector resulted in lowering of fasting plasma glucose and improved glucose tolerance of Type 1 diabetic Wistar rats. While diabetic rats exhibited weight loss following STZ injection, LentiINS injected rats retained normal body weight. TÜBİTAK-215S820 [1]: Sanlioglu, A. D., et al. (2012). ''Insulin gene therapy from design to beta cell generation.'' Expert Rev Mol Med 14: e18.

P348

FoxO3a overexpression prevents both glycogen overload and autophagic buildup in Pompe disease

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Pompe disease is a lysosomal storage disorder caused by the mutation of acid α-glucosidase (GAA), the enzyme degrading glycogen in glucose into lysosomes. A massive glycogen overload is described in Pompe patient, mainly in skeletal and cardiac muscles. Furthermore, severe impairment of autophagic flux highlights by autophagic build-up. Currently, no treatment allows to cure efficiently and durably skeletal muscle. We identified the transcription factor Forkhead box O3 (FoxO3a) as a potential target to alleviate skeletal muscle impairments through its key role on regulation of both autophagy-lysosomal pathway and glycogen homeostasis. Here, we search to analyze the effect of FoxO3a modulation in muscle impairments. The overexpression of FoxO3a was induced in newborn Pompe mice using a single intravenous administration of an AAV2/9-FoxO3a. Glycogen overload was analyzed using periodic acid-Schiff (PAS) stain and biochemical assay. Autophagic build-up characterization was performed using immunolabeling against LC3 and p62 corresponding to markers of autophagosome and of autophagic flux disruption, respectively. We showed a significative prevention of glycogen overload in treated mice. A decrease of the number and the size of LC3+-autophagic build-up were determined in treated mice. Moreover, p62 accumulation appeared reduced in treated mice, suggesting a restoration of a functional autophagic flux. A prevention of muscle fiber atrophy was observed. Overall, we provided the proof of concept of the key role of FoxO3a in the prevention of skeletal muscle impairments. Our results suggest that FoxO3a modulation could be used as a therapeutic target to alleviate both glycogen overload and autophagic build-up in Pompe disease.

P349

A toxicological assessment of *in vivo* lentiviral vector administration in a mouse model of liver injury

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A lentiviral vector (LV) expressing fumarylacetoacetate hydrolase (LV-FAH) intended for *in vivo* gene therapy for hereditary tyrosinemia type I (HT1) was evaluated for toxicity as a single intravenous dose in wild-type and chemically-induced liver injury mice. Mice were dosed with vehicle, DEN/CCl4, LV-FAH (109 TU/mouse), or the combination of LV-FAH and DEN/CCl4 and evaluated for general and hepato-toxicology endpoints. There were no changes in observational data in any group, but DEN/CCl4 and LVV-FAH caused slight decreases in body weight gain over the course of the study (76-88% of control). LV-FAH alone or with DEN/CCl4 was associated with reduced kidney weights (0.93-0.91x) with some animals exhibiting mild increases in BUN and/or creatinine. In the liver, exposure to DEN/CCl4 alone or with LV-FAH was associated with elevations in AST (2.3-2.7x, respectively) and ALT (3.2-4.2x, respectively), and in combination had increased in liver to body weight ratio (4.5x compared to 4.1x). Histologically, DEN/CCl4 showed increased Ki-67 staining in liver with diffuse hepatocellular hypertrophy/hyperplasia, karyocytomegaly, biliary stasis, and bridging fibrosis/fibroplasia, which were enhanced by combination with LV FAH, advancing to hepatocyte degeneration and necrosis. There were no tumors observed; however, DEN/CCl4 did cause increases in alpha-fetoprotein levels. These results indicate that systemic LV-FAH administration was unequivocally safe, with no adverse effects at this therapeutic dose. However, in the context of a chemically-injured liver, pretreatment with LV-FAH was associated with enhanced toxicity. These results support therapeutic development of *in vivo* lentivirus administration, and support the need for testing in both healthy and disease models.

P350

Liver-directed gene therapy for CBS deficiency in mice using a minicircle-based naked DNA vector

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Individuals with loss of function mutations in the cystathionine *β*-synthase (CBS) gene have classical homocystinuria, characterized by elevated total homocysteine (tHcy) in plasma and a variety of clinical phenotypes. Mice lacking Cbs die in the neonatal period, but can be rescued by expression of a transgene expressing patient-derived mutant human CBS. Here we use two mouse models, Tg-R336C $Cbs^{-/-}$ and Tg-I278T $Cbs^{-/-}$ to evaluate the potential of minicircle-based naked DNA gene therapy to treat CBS deficiency. A 2.3 Kb DNA minicircle containing the liver specific P3 promoter driving the human CBS cDNA (MC.P3-hCBS) was delivered into Tg-R336C $Cbs^{-/-}$ and Tg-I278T $Cbs^{-/-}$ mice via a single hydrodynamic tail vein injection (HTV). For Tg-R336C $Cbs^{-/-}$ mice we observed a 64% reduction in tHcy (326 to 121 μM, $P<0.0001$) after 8 days and a 34% reduction after 21 days (326 to 217 μM, $P<0.028$). For Tg-I278T $Cbs^{-/-}$ mice we observed a 51 % reduction (351 to 176 μM, $P<0.0007$) after 8 days and a 67% reduction (351 to 120 μM, $P<0.0001$) after 21 days. Analysis of liver lysates of Tg-R336C $Cbs^{-/-}$ mice showed presence of MC.P3-hCBS DNA at a level of 1-44 copies per genome. Western blot and enzyme activity analysis revealed significant vector-directed CBS expression. These data show that HTV injection of a CBS-expressing naked DNA minicircle vector can express CBS sufficiently to lower tHcy in CBS-deficient mice. These findings suggest that minicircle gene therapy may be feasible to treat CBS deficiency.

P351

Crigler-Najjar World Registry: shortcomings of current treatments underscore the potential benefit of gene therapy

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Crigler-Najjar syndrome (CN) is an ultra-rare, monogenic disorder of bilirubin conjugation that results in accumulation of neurotoxic unconjugated bilirubin (UCB). Current management aims to prevent bilirubin induced encephalopathy (kernicterus) by reducing serum UCB. Here we present data from the CN World Registry, aiming to compare the outcome of current treatment strategies. These results can serve as benchmark to validate adeno-associated virus(AAV) mediated gene therapy. A General Data Protection Regulation(GDPR) compliant registry platform was developed to collect anonymized retrospective data on clinical features, treatment and outcome of CN patients. Since September 2016 physicians from 12 centers in 10 different countries spread over 4 continents contributed life-time data of 141 CN patients, including genetic analysis in 89.4% of cases. The majority of patients (90.8%) received treatment, either with phenobarbital (7.8%), phototherapy (26.9%) or a combination (41.2%), leading to a mean serum total bilirubin of $289.7 \pm 105.6 \mu$mol/L and an incidence of encephalopathy in 12.1% of cases. Twenty-one patients (14.9%) received a liver transplant, all but one curative, but ten major complications (5/10 stenosis of biliary anastomosis) and three re-transplantations were recorded. At the time of analysis 54/141 patients (38.3%) were deceased. Within the deceased group a low age at death of 6.0 ± 4.5y and high serum total bilirubin of $495.2 \pm 120.2 \mu$mol/L suggest that treatment was insufficient. The CN World Registry is the largest retrospective data collection of CN patients reported so far, demonstrating that current treatment strategies are not always sufficient to prevent bilirubin induced encephalopathy. Gene therapy may offer an alternative treatment for CN patients.

P352

Estimated prevalence of aromatic l-amino acid decarboxylase (AADC) deficiency in the United States, European Union, and Japan

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AADC deficiency is a rare neurometabolic disorder resulting from dopa decarboxylase (DDC) gene mutations that cause profound monoamine neurotransmitter deficiencies. Disease prevalence rates may inform screening, diagnosis, and treatment. We estimated AADC deficiency prevalence using frequency of pathogenic allele variants in the DDC gene. Variants were identified through literature analyses (known) and an aggregate prediction model (predicted). The prediction model included 2 automated classifiers—the impact of missense mutations or intronic mutations on pathogenicity. Pathogenic allele frequency was evaluated using the Exome Aggregation Consortium and Genome Aggregation Database (US; EU) and the Human Genetic Variation Database (Japan). Birth prevalence rates of AADC deficiency were derived from pathogenic allele frequency using the Hardy-Weinberg equation. Population prevalence and mortality for individuals aged 0–19 years were estimated using known and predicted cases from the JAKE database. The aggregate model performed well (positive predictive value: 97%; sensitivity: 91%; estimated specificity: 89%). In the US and EU, 33 known and 183 predicted variants were identified; 3 additional predicted variants were identified in Japan. Estimated AADC deficiency birth prevalence rates were 44 children per year in the US, 45 in the EU, and 6 in Japan. Population prevalence rates of AADC deficiency in the US, EU, and Japan were 840, 853, and 124, respectively. Mortality rates for AADC deficiency in the US were 5–16 times higher than individuals of similar age; mean age of death was 5.5 years. These findings emphasize the need for increased awareness, diagnosis, and early treatment intervention of AADC deficiency.

P353

Naked-DNA minicircle-vector based gene-therapy for ornithine transcarbamylase (OTC)-deficient spf-ash mice

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Here we aimed at exploiting non-viral naked-DNA minicircle (MC)-based vectors for liver-directed gene therapy to target periportal hepatocytes in spf-ash mice, a model for the X-linked ornithine transcarbamylase (OTC) deficiency. OTC is an enzyme of the urea cycle that is only fully expressed in periportal hepatocytes. MC vector administration through vessels of the portal triad, i.e. portal vein, hepatic artery and bile duct, allows for periportal targeting. While injections through the portal vein were accompanied by high fatality in spf-ash mice (>75% lethality), administration via hydrodynamic retrograde intrabiliary infusion (HRII) was safer with a >95% survival rate ($n > 100$). Biliary infusion offers direct access to hepatocytes compared to

portal vein were the MCs need to pass the fenestrated endothelium. Luciferase-expressing MC vectors were used to establish delivery conditions with *in vivo* imaging (IVIS) in combination with quantitative immunohistochemical staining for pericentral (glutamine synthetase) hepatocytes. As therapeutic vector, we generated an expression cassette with a codon-optimized mouse Otc-cDNA and a truncated intron that is under transcriptional control of a liver-specific promoter-enhancer sequence. In pilot studies, we treated spf-ash mice based on elevated liver OTC activity, normalization of *in vivo* ureagenesis (using stable isotopes), and selective survival of severe hyperammonemia induced upon an shRNA-mediated knock-down of residual endogenous OTC expression. While this study proves that non-viral MC vectors have the potential to treat OTC deficiency in spf-ash mice, the HRII method still needs optimization.

P354

Mitochondrial DNA mutation causes nuclear chromatin changes and makes glutamine a substrate for histone acetylation

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Mitochondrial dysfunction has been recognized as a major driving force in human disease. We have previously shown that a progressive decline in mitochondrial function due to increasing mitochondrial DNA mutation load (heteroplasmy) causes distinct transcriptional changes in the nucleus, however, the mechanism of how mitochondrial dysfunction causes changes in nuclear gene transcription and, as a result, disease remains largely unknown. Here, for the first time, we examine the effect of a human pathogenic mitochondrial DNA mutation at various levels of heteroplasmy on cellular metabolism and nuclear histone modifications. We report that high levels of the mtDNA A3243G tRNA-Leu(UUR) cause depletion of key tricarboxylic acid cycle intermediates and acetyl-CoA, as well as changes in specific histone acetylation residues. We identify glutamine as a novel substrate for histone acetylation. We hypothesize that the induced global histone changes allow switching on and off whole transcriptional programs, thus efficiently regulating nuclear transcription changes based on the cell's bioenergetic status.

P355

Metabolic characterization of hiPSC-derived cardiomyocytes from patients suffering from infantile cardiomyopathy

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In this project, we are creating a model of the metabolism of the adult heart using human induced pluripotent stem cells (hiPSCs). We are studying ''combined oxidative phosphorylation deficiency 8 (COXPD8)'', an autosomal recessive metabolic disorder which is caused by mutations in the Alanine-tRNA synthetase 2 (AARS2) gene. The disease manifests primarily as lethal infantile hypertrophic cardiomyopathy, but different mutations within AARS2 are also known to cause encephalopathies and ovarioleukodystrophy. We have obtained two sibling hiPSC-lines from patients suffering from infantile hypertrophic cardiomyopathy and one healthy control-line from the mother. The patients have three unique compound mutations, of which one is known to be involved in infantile hypertrophic cardiomyopathy and the other to be involved in encephalopathies. Initial metabolic testing on hiPSC-derived cardiomyocytes showed no difference in the mitochondrial oxygen consumption and the glycolytic rate between the diseased lines and the related (mother) control. We, therefore, generated isogenic control-lines, using CRISPR/cas9-RNP, to uncover the specific effect caused by the mutations. Verification of successful correction lines was performed by ddPCR and resulted in a targeting efficiency of 2.2-3.4%. Currently, we're assessing the metabolic capacity and mitochondrial integrity of hiPSC-derived cardiomyocytes, cardiac-fibroblasts, and endothelial cells. Using the Seahorse-XF flux analyzer we will be able to measure both mitochondrial respiration and integrity, as well as the metabolic capacity of the different cell lineages.

P356

GLP-1 gene delivery stimulates cellular proliferation and trans-differentiation of pancreatic cells in type 2 diabetes

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Glucagon-like peptide-1 (GLP-1), which has been evaluated as a therapeutic agent for Type 2 Diabetes, stimulates glucose-induced insulin secretion (GSIS), and suspected to increase beta-cell mass through proliferation, neogenesis, and trans-differentiation.1 In order to produce stable GLP-1 synthesis and secretion, a GLP-1 encoding lentiviral vector (LentiGLP-1) was generated and its therapeutic efficacy was tested in experimental animal models of Type 2 Diabetes. Intraperitoneal injection of LentiGLP-1 into obese diabetic rats broke-down insulin resistance, improved glucose tolerance, and reduced plasma glucose levels without altering plasma lipid profile.2 Reduced plasma glucose level was correlated with increased pancreatic beta cell mass. Immunohistochemical analyses were performed on pancreatic sections dissected from LentiGLP-1 administrated rats to reveal the mechanism of beta cell expansion. In addition to appearance of small insulin positive cell clusters, Ki67 (+) cells were observed in the acinar regions of pancreatic tissue. These findings suggest that GLP-1 gene delivery promotes cellular proliferation and trans-differentiation of pancreatic cells. TUBITAK-112S114 1. Tasyurek MH, Altunbas HA, Canatan H et al. GLP-1-mediated gene therapy approaches for diabetes treatment. Expert RevMolMed 2014;16:e7. 2. Tasyurek HM, Altunbas HA, Balci MK et al. Therapeutic Potential of Lentivirus-Mediated Glucagon-Like Peptide-1 Gene Therapy for Diabetes. Human gene therapy, 2018.

P357

AAV-mediated liver directed gene therapy corrects the cholestatic phenotype in Abcb4$^{-/-}$ mice

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Progressive familial intrahepatic cholestasis type 3 (PFIC3) leads to severe liver injury during childhood with limited therapeutic options. PFIC3 results from mutations in the ABCB4 gene leading to reduced transport of phospholipids to bile. In the Abcb4$^{-/-}$ mouse absence of phospholipids in bile leads to bile salt–induced hepatotoxicity, which is aggravated by a cholate-supplemented diet, resembling the PFIC3 phenotype. We hypothesized that adeno-associated virus (AAV) mediated expression of hABCB4 in Abcb4$^{-/-}$ liver could correct the cholestatic phenotype by restoring the phospholipid content in bile. Ten weeks old Abcb4-/- mice received a single intra venous dose of AAV8-hABCB4 or -GFP (n = 8/8) under control of a liver specific promotor at 5×1013 vg/kg. All animals were challenged by a 0.1% cholate diet for 2 weeks at 8 or 24 weeks after vector administration. Bile duct cannulation enabled analysis of biliary phospholipids. All animals that received AAV8-hABCB4 showed complete normalization of plasma cholestatic markers 2 weeks after administration and were resistant to a cholate challenge, while the AAV8-GFP treated groups developed severe cholestasis (ALP wk10: 62 ± 8 vs. 919 ± 332; wk26: 73 ± 6 vs. 1152 ± 118U/L; Bilirubin wk10/26 < 1 vs. 208 ± 84 μmol/L). The phospholipid content in bile of treated animals was restored to wild type levels (wk10: 46.4 ± 12.7 vs. 5.7 ± 1.0; wk26: 13.3 ± 2.0 vs. 2.8 ± 0.4 nmol/min/100gr). Liver pathology scores were strongly reduced in treated animals versus controls. In conclusion, AAV-mediated expression of hABCB4 in the liver of Abcb4-/- mice is long lasting and completely corrects the cholestatic phenotype by restoring the phospholipid content in bile, underscoring the feasibility of gene therapy for PFIC3.

P360

Mitotic chromosome association predicts genome-wide transcription factor occupancy and impact on local chromatin accessibility

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Mammalian transcription factors (TFs) differ broadly in their nuclear mobility and sequence-specific/non-specific binding affinity for DNA. TFs search the genome for their specific binding sites not only by three-dimensional diffusion but also by one-dimensional scanning, which depends on non-specific DNA interactions. How differences in non-specific DNA binding affects the ability of different TFs to search the genome for specific sites and modify the surrounding epigenetic landscape is not understood. TFs also differ in their ability to bind mitotic chromosomes. While their physical association to mitotic chromatin is mediated by both specific and non-specific DNA binding, the colocalization of TFs with mitotic chromosomes visualized by microscopy essentially depends on non-specific DNA interactions. Here we combined live cell imaging of mitotic chromatin association of 507 TFs, fluorescence recovery after photobleaching (FRAP) measurements of TF mobility, single molecule imaging of TF- associated with slow TF mobility and co-localization with DNA-rich nuclear regions in interphase. Remarkably, mitotic chromosome binding correlated with the relative on- but not off-rate of specific DNA binding events, and predicted genome-wide specific site occupancy as well as TF ability to modify chromatin accessibility around bound sites over three orders of magnitude. Our study suggests that non-specific DNA interactions drive TF search for specific sites and largely determine their impact on local chromatin accessibility.

P361

A pre-senescence program in aged mesenchymal stromal cells contributes to inflammation in the hematopoietic compartment

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Hematopoietic stem and progenitor cells (HSPC) reside in the bone marrow (BM) niche serving as a reservoir for mature blood cells throughout life. However, during aging they become unable to guarantee tissue homeostasis. Mesenchymal stromal cells (MSCs) in the BM support HSC homeostasis. However, changes in MSCs biology and their contribution to HSPC dysfunction with age remain understudied. Cell senescence involves persistent DNA damage response activation, cell cycle arrest and activation of a senescent-associated secretory phenotype (SASP). Here, we conducted an extensive characterization of senescence features in MSCs derived from BM aspirates of young and geriatric healthy donors. Old MSCs displayed an enlarged senescent-like morphology, a delayed clonogenic potential and a reduced percentage of cells in active S-phase. Of note, the observed proliferation delay was associated with increased levels of SA-βgal in old MSCs at early passages in culture and a modest but consistent accumulation of physical DNA damage and activation of the DNA damage response pathway. Consistent with the establishment of a pre-senescence status in geriatric MSCs, we detected an increase of pro-inflammatory SASP factors, including IL1 and MCP1, both at the transcript and protein levels. Importantly, exposure of HSPC to factors secreted by old MSCs did not result in any skewing in the hematopoietic composition but induced pro-inflammatory cytokines in HSPC in a paracrine manner. Altogether, our results reveal that MSCs from geriatric healthy donors show features of pre-senescence *in vitro* and that their secretome could contribute to the inflammatory program observed in aged HSPC.

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A thiacalixarene-modified polydiacetylene-based DNA sensor for potential application in gene therapy

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Gene therapy is a new technique for treating many serious incurable diseases, where genes are used as medicine. The main problem limiting the application is the difficulty of transporting large, fragile and negatively charged molecules like DNA into the nucleus of the cell without degradation. Sensors based on the

sensitive polymer materials can be used as safe and efficient gene delivery vehicle as well as potential tools for detection of defective/damaged genes. The versatile and stable conjugated polymer - polydiacetylene (PDA) has useful structural and sensing properties. The unique and rapid chromatic change from blue to red can be directly perceived by the naked eye. Also, the functionalization of PDA surface leads to effective DNA binding and as a result for theirs delivery. In the present study, the formation of thiacalix[4]arene-PDA (TCA-PDA) vesicles was obtained by self-assembly of diethylenetriamine-modified thiacalix[4]arene and N-(3-aminopropyl)-10,12-pentacosadiynamide with subsequent photopolymerisation. Structure and size of TCA-PDA were determined with various physical methods such as dynamic/electrophoretic light scattering, UV/fluorescent-spectroscopy, TEM microscopy. Calf thymus DNA detection by TCA-PDA was shown both ''naked eye'' and using UV-VIS/fluorescent spectroscopy. The excellent sensitivity of modified colorimetric PDA sensor is explained by incorporation of positively-charged macrocycle, that provides multivalent binding of biopolymer. Importantly, TCA-PDA vesicles have no signal to DNA, cutted by restriction nuclease. Due to its technical simplicity, rapidity, high selectivity, and sensitivity, the new TCA-PDA vesicles have potential applications in the gene sensing. We thank the Russian Science Foundation for the financial support of this work (grant No. 14-13-01151).

P363

Triazole and pirazole p-tert-butylthiacalixarene derivatives - new DNA condensing agents for gene therapy

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One of the important challenges in gene therapy is to search safe and efficient gene carries capable of compacting, protecting, transporting, and delivering nucleic acids into the cell. Usually, condensing agents are functionalized on one side with appropriate cationic groups to act as DNA carriers, and, on the other side, with lipid-type chains that permit them to self-aggregate. Thiacalixarenes are versatile macrocycles that can combine the properties of both macrocyclic hosts and self-organizing systems, such as micelles and liposomes. In addition, their easy synthesis and low toxicity levels make them really promising vectors in gene delivery applications. Herein we demonstrate the synthesis and condensing abilities toward calf thymus DNA of new derivatives of p-tert-butylthiacalix[4]-arene containing O-alkyl lipophilic fragments (C4, C8, C14) and diethylenetriamine fragments or containing pirazole fragments in 1,3-alternate stereoisomeric form. The preliminary experiments using physical methods such as dynamic/electrophoretic light scattering, UV/fluorescent-spectroscopy, TEM microscopy showed that synthesized derivatives itself form stable aggregates in water solutions with average diameter within 50–100 nm and zeta potential around +70 mV. According to the dynamic/electrophoretic light scattering and fluorescent spectroscopy with ethidium bromide (EB) it was found that all macrocycles effectively interact with calf thymus DNA, causing EB release. Moreover, macrocycles with diethylenetriamine fragments were found to compress DNA 2–5 times into compact lipoplexes. The work was supported by the Russian Foundation for Basic Research (grant 17-03-00389) and the subsidy allocated to the Kazan Federal University for the state assignment in the sphere of scientific activities (4.1493.2017/4.6 and 4.5151.2017/6.7)

P364

Gold nanoparticles, capped by amphiphilic amidoamino calixresorcinarenes as a potential non-viral vectors for gene delivery

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Design of novel nanosystems for transfection of nucleic acids into cell is of current interest challenge. Gold nanoparticles (AuNPs) are promising objects in the field of gene therapy and perfect platform for formation of non-viral vectors due to their biocompatibility, stability, simplicity of the synthesis and highly tuned unique optical properties that can be used in visualization of various diseases. Recently, modification of AuNPs by synthetic macrocyclic molecules, such as cyclodextrins, pillararenes, calixarenes and calixresorcicarenes for creation of non-viral vectors are of particular interest. These macrocycles have great opportunities due to their structure. They equipped with hydrophobic cavity that can participate in CH-π, π-π, and cation-π interactions, and numerous hydrophilic and hydrophobic functional groups, capable of both AuNPs stabilization and multiple non-covalent interactions with DNA. Here, we propose using of AuNPs modified by water-soluble amphiphilic calixresorcinarenes with amidodimethylamine groups on the upper rim and alkyl substituents on the lower rim, to investigate their interactions with DNA with the aim of potential non-viral vectors creation. Hydrophilic substituents and positively charged calixresorcinarenes amino-groups are gladly able to bind negatively charged DNA molecules due to electrostatic and hydrogen interactions; alkyl substituents are capable of hydrophobic interactions and self-association. Creation of such hybrid multifunctional systems opens the outlook of controlled association of non-viral vectors with DNA with formation of small stable biocompatible nanoscale structures of definite morphology. This work was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (4.1493.2017/4.6 and 4.5151.2017/6.7).

P365

Influence of long-term cultivation and cryopreservation on phenotype of rat hepatic stellate cells

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Many organs and tissues have been reported to harbor regional stem cells. One of the candidates for this role in the liver is hepatic stellate cells (HSC). Cultivation of cells *in vitro* may lead to changes in their morphology and phenotype. The risk of changes increases with long-term cultivation (passage 5 and above), as well as with cryopreservation of cells. Freezing allows storage of isolated cell; however, the method can influence cells phenotype and properties. The aim of research was to study phenotype of rat HSC during long-term cultivation and cryopreservation. Three cultures of HSC (I – after cryopreservation,

II – long-term culture, passage 6, and III – control, passage 4) were stained immunocytochemically with antibodies to desmin, α-SMA, CK19, Ki-67; quantitative real-time PCR analysis was also performed to evaluate desmin and α-SMA gene expression. It was observed that morphology of cells was similar in all three groups. In groups with long-term cultivation and cryopreservation the expression of α-SMA was stable, desmin and Ki-67 increased. Results of real-time PCR confirmed results of immunohistochemistry. Expression of epithelial marker CK19 appeared only in cells with long-term cultivation (group II). Thus, if epithelial transdifferentiation of HSC is required, long-term cultivation is preferable. Cryopreservation does not have any negative influence on morphology and phenotype of HSC that remain similar to control cells passage 4.

P366

CD133-positive cells in prenatal development of human pancreas

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One of the markers of progenitor β-cells (PC) is CD133 (AC133, or prominin-1), which is detected in the pancreas of fetuses (18-22 weeks of gestation, WOG) and adult human. The exact time of gestation and origin of first $CD133^+$ cells in human pancreas remains unclear. Thus, the aim of the study was to identify time during gestation when $CD133^+$ cells appear and compare localization of $CD133^+$ with C-kit^+ (stem cell marker) PCs in human pancreas. The study was conducted on whole embryos (8 WOG) and fetuses (8.5-12.5 WOG) as part of self-inflicted miscarriages or legal medical abortions with Ethical committee approval and the patient's informed consent. Paraffin sections were stained immunohistochemically with commercial antibodies to CD133 and C-kit. We observed that first $CD133^+$ cells in human pancreas appeared in 11.5 WOG period. They surrounded the ducts and developing islets from outside and were absent in the epithelium of the ducts and inside of the developing islets. First C-kit^+ cells were found at 8.5 WOG in the duct epithelium and 11.5 WOG inside developing islets. Since first $CD133^+$ cells in ontogenesis appear later than C-kit+ cells, which are absent in the ductal epithelium and inside the islets, it remains unclear whether CD133 can be used as marker of β-cell PC. However, the close proximity to the forming islets and ducts does not exclude $CD133^+$ cells participation in the differentiation of β-cells and α-cells due to intercellular contacts or production of growth factors. Work supported by KFU Competitive Growth Program.

P367

VEGF-VEGFRs axis role in reparative osteogenesis in paraossal haematomas

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Reparative osteogenesis requires optimal conditions of tissue and cellular environment. The present work is aimed to study intercellular interactions during early stages of bone healing in the fracture of tubular bones from the position of the modern concept of regenerative histogenesis. Paraossal hematomas were obtained during reconstructive surgical treatment from 10 patients on days 1, 3-8, 10 12 after bone fracture. Histological methods were used to assess the pathomorphological changes. The haematomas on day 1-4 were represented by erythrocytes and macrophages located among the fibrin filaments. On day 4 blood vessels were found, proliferation index (PI) of fibroblasts was 59,50% ± 15,00%, endothelium – 77,53% ± 20,00%. Expression index (EI) of Flk-1 in endothelium – 92,97% ± 4,00%, in macrophages – 76,54% ± 14,00%; EI of Flt-4 in endothelium – 88,08% ± 9,00%, in macrophages – 93,86% ± 6%. On day 6-8 a loose connective tissue was found in the paraossal haematomas, PI of endotheliocytes was 88,55% ± 8,00%, the number of vessels reached a maximum on day 8. In this period the newly formed vessels produce cytokines, mostly VEGF, and its receptors. On the 10-12th day a loose connective tissue with the areas of the immature bone were observed. EI of the VEGF receptors was maximal in the osteoblasts (95,87% ± 1,00% for Flk-1 and 97,45% ± 1,00% for Flt-4) in the 10th day. In the early stages of reparative osteogenesis the regulatory axis of VEGF-VEGFRs plays a key role in the coordination of intercellular molecular interactions, which ensures a consistent replacement of cell-tissue differons. This work was supported by program of Competitive Growth of KFU.

P368

Amphiphilic octacationic calixresorcines as non-toxic potential non-viral vectors for gene delivery

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Gene therapy provides a unique approach to treatment of both inherited and acquired diseases of patients by delivering a therapeutic gene material and its associated regulatory elements into the nucleus. In contradistinction to viral vectors, non-viral vectors have important safety advantage, as well as low cost and ease of production. Unfortunately none of the currently available non-viral vectors fulfil ideal vector properties, which is why new compounds are constantly being proposed and investigated. One of the approach to production of non-viral vectors for gene delivery is the use of supramolecular amphiphiles, which efficiently interact with DNA due to numerous non-covalent interactions. As a rule, the amphiphilicity of such compounds leads to the compact of DNA into small nanoparticles with low polydispersity and high stability. Among synthetic macrocycles the calix[4]resorcinarenes have aromatic cavity which readily is functionalized by introducing hydrophilic groups at one rim and hydrophobic groups at the other rim to get macrocyclic amphiphiles. Aiming to obtain potential non-viral vectors for gene delivery we synthesized the of octaamidoammonium derivatives of calix[4]resorcines with different length of alkyl substituents on the lower rim. It was shown that obtained calixresorcines have low toxicity. The amphiphilicity of macrocycles and the sizes of their self-associates depend and are determined by the lower rim substituents length and can serve as a key to improve the size and stability of complexes with DNA. This work was

funded by the subsidy allocated to KFU for the state assignment in the sphere of scientific activities (4.1493.2017/4.6 and 4.5151.2017/6.7).

P369

Parenchymal and nonparenchymal cellular proliferation dynamics after partial hepatectomy with and without 2-acetylaminofluorene injection in rats

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Restoration of liver after partial hepatectomy (PH) depends on proliferation dynamics of parenchymal and nonparenchymal liver cell populations. To study separately proliferation impact of nonparenchymal cells it is recommended to inhibit hepatocytes proliferation by injection of acetoaminofluorene (AAF). The aim of the research was to study dynamics of parenchymal and nonparenchymal cells proliferation after PH w/wo AAF injection. Experimental groups were 1) PH; 2) PH with intraperitoneal AAF injection (0,2ml of 1.5g/100ml). Liver paraffin slices (1,5,7,14,21,28 days after transplantation) were stained with antibodies against proliferation marker Ki-67. The first days after PH 49,05 ± 6,14% of hepatocytes and 15,59 ± 1,46% of nonparenchymal cells proliferate in portal tract region, where cells were activation on 2nd and 14th days. In the region of central vein Ki-67+ hepatocytes and nonparenchymal cells increased only once on 2nd day (31,41 ± 4,72 and 39,70 ± 0,79 respectively). Injection of AAF inhibited proliferation of both cell populations. In region of central vein there were no Ki-67+ hepatocytes during the whole experiment, Ki-67+ nonparenchymal cells appeared later on 7th day and 5 times less than in PH group. In portal tract region proliferation dynamics of hepatocytes and nonparenchymal cells had 2 phases, but nonparenchymal cells activation was later (on 7th and 28th days). Ki 67+ hepatocytes were over 10 times less, nonparenchymal cells - 5-6 times less. AAF injection inhibits proliferation of parenchymal and nonparenchymal cells, has severe effect on central vein region, leads to reduction of liver regeneration (cellular dystrophy and degeneration on 28th days).

P371

Role of paracrine factors secreted by mesenchymal stromal cells in spermatogonial stem cell niche regulation

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A stem cell niche is a structural and functional microenvironment supporting and regulating a stem cell pool. Mesenchymal stromal cells (MSC), as a part of stem cell niche, maintain many types of tissue-specific resident stem cells particularly through the production of multiple bioactive factors and thus coordinating processes of tissue repair and regeneration. In spermatogonial stem cell (SSC) niche, cells functionally similar to MSC were also found, but their role remained uncovered. However, MSC can be crucial for SSC niche regulation as they produce paracrine factors affecting principal components of SSC niche by modulating cell proliferation, survival and differentiation. We studied the contribution of MSC secretome to SSC niche regulation within the development of a new MSC secretome-based drug for the treatment of male infertility. Using a model of rat bilateral abdominal cryptorchidism for reversible SSC niche injury we observed that MSC secretome components effectively restored spermatogenesis. By immunohistochemical analysis of testis sections we observed regeneration of the whole testicle, not only distinct seminiferous tubules. A direct correlation between number of tubules containing many proliferating cells and produced spermatozoa number, and an inverse correlation between the interstitial cell proliferation level and produced spermatozoa number were found. We also conducted *in vitro* evaluation of survival and proliferation of specific SSC niche cells to confirm *in vivo* data. Our findings suggest MSC could regulate SSC niche by paracrine signals and support spermatogenesis.

P373

PPMO treatment results in widespread muscle delivery and efficacy in mice and cynomolgus monkeys: a potential therapeutic platform for the treatment of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is caused by DMD gene mutations that disrupt the messenger ribonucleic acid (mRNA) reading frame and prevent biosynthesis of dystrophin. Exon skipping is a treatment strategy for DMD. Phosphorodiamidate morpholino oligomers (PMOs) can be designed to promote exon skipping, restore the mRNA reading frame, and facilitate production of an internally deleted dystrophin protein. Among DMD patients, ≈80% have genotypes amenable to exon skipping. We evaluated a novel treatment strategy using PMOs conjugated to cell-penetrating peptides (PPMOs) to increase intranuclear delivery. Mdx mice received single and repeated intravenous (IV) doses of a PPMO targeting exon 23. Exon skipping and dystrophin production levels were compared with those following administration of PMO. Additionally, nonhuman primates received repeated low, medium, and high IV doses of SRP-5051 and SRP-5053, PPMOs targeting exons 51 and 53, respectively. Results were compared with those for PMOs targeting the same exons. RT-PCR and Western blot analyses assessed exon skipping and dystrophin production. In mdx mice, PPMO treatment increased levels of exon 23 skipping and dystrophin biosynthesis in the quadriceps, diaphragm, and heart versus PMO. Restoration of dystrophin production with PPMO administration attenuated genetic expression of markers of inflammation and fibrosis in muscle and improved muscle function versus saline-treated mdx mice. In cynomolgus monkeys, PPMOs increased exon skipping in all relevant muscle groups,

including skeletal, cardiac, and smooth muscles. Together, these results indicate that PPMO may be a highly potent exon-skipping platform for DMD, with long-lasting, robust therapeutic effects in preclinical models.

P374

Current status of clinical development for gene therapy with Sendai virus vector expressing the human FGF-2 gene (DVC1-0101) to treat peripheral arterial disease

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A new gene product, namly DVC1-0101, which is based on an F-gene–deleted nontransmissible rSeV based on a Z-strain encoding negative-stranded full-length complementary RNA of human FGF-2 at the upstream of gene encoding N-protein (rSeV/dF-hFGF2) has been developed as a new gene product in clinical phase. DVC 1-0101 induces 'functional' angiogenesis as well as lymphangiogenesis and thereby expects the effectiveness for peripheral arterial disease (PAD) as a new gene therapy modality. The first-in-human phase I/IIa, open-label, four-dose-escalation clinical trial for 12 patients with severe PAD using the DVC1-0101 was completed. Single dose intramuscular injection of DVC1-0101 up to 5×109 ciu/60 kg/person demonstrated that this new RNA drug was safe, well-tolerated, and significantly improved walking performance. Subsequently, a phase IIb, randomized, double-blinded clinical trial with intermittent claudication patients began in 2014. The primary objective of this trial is to investigate the safety and clinical efficacy of DVC1-0101 (1 and 5×109 ciu/leg) and also aims to examine the dose-response relationship using the rate of improvement in walking as a primary indicator. DVC1-0101 is administrated at 30 sites (15 sites in each upper and lower leg) intramuscularly. An interim analysis will be finished in this summer, and this trial would be terminated with positive result if the effectiveness were confirmed by this analysis. The outcomes of this trial will provide valuable proof-of-concept data for DVC1-0101 in PAD patients, and will lead to a better understanding of the cutting edge of gene therapy in cardiovascular application using virus vector.

P375

Intranuclear inclusion body (INI) pathology and muscle atrophy in oculopharyngeal muscular dystrophy can be reversed by "silence and replace" AAV-mediated gene therapy

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Oculopharyngeal muscular dystrophy (OPMD) is a rare autosomal dominant late onset muscular dystrophy affecting approximately 1:100000 individuals in Europe. Phenotypically, OPMD is mainly characterized by progressive eyelid drooping (ptosis) and dysphagia although muscles of the lower limbs can also be affected late in life. Other features of OPMD include muscle fibrosis and atrophy. This muscle disease is due to a trinucleotide repeat expansion in the polyA binding protein nuclear-1 (PABPN1) gene. Patients express a protein with an 11-17 alanine tract that is misfolded and prone to form intranuclear inclusions (INIs) which are the hallmark of the disease. Currently no pharmacological treatments are available and OPMD patients can only be referred to surgeons for cricopharyngeal myotomy or corrective surgery to extraocular muscles to ease ptosis. We recently tested a new AAV-based gene therapy treatment in a mouse model of OPMD that overexpresses an expanded 17-ala PABPN1. This approach is based on co-delivery of 2 AAV vectors, the first providing complete knock-down of endogenous mutant and wild-type PABPN1, and the second expressing a replacement human codon optimized PABPN1 gene. This gene therapy approach reverts the amount of insoluble aggregates, partially rescues the muscle from atrophy, prevents the formation of muscle fibrosis and stabilizes the muscle strength to the level of healthy muscles. We show here that similar results can be obtained using a single vector that has one ''silence and replace'' cassette. These results further support the application of a gene therapy approach as a novel treatment for OPMD in humans.

P376

Single stranded AAV-mediated gene therapy corrects spinal muscular atrophy in mice.

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Spinal muscular atrophy (SMA) is an autosomal recessive disease of variable severity caused by mutations in the SMN1 gene. Deficiency of the ubiquitous SMN function results in spinal cord α-motor neuron degeneration and proximal muscle weakness. It is the most frequent genetic cause of infant death. Remarkable progress has been made in the last years on the development of therapeutic approaches for SMA. Gene replacement therapy by intravenous (IV) administration of self-complementary (sc) recombinant AAV9 vectors expressing SMN1 showed therapeutic efficacy in several animal models of SMA and a clinical trial in type 1 SMA patients. However, scAAV vectors have some limitations such as limited packaging capacity, low yield production and higher immunogenicity than single-stranded (ss) AAV vectors. In this study, we tested the therapeutic efficacy of a ssAAV9 vector expressing the human SMN1 transgene in the Smn2B/- mouse model of spinal muscular atrophy. Intracerebrospinal fluid (CSF) administration of a single dose of ssAAV9-hSMN1 at 8E12 vg/kg prolonged survival with a median lifespan of 228 days, compared to 26 days in non-treated mutant mice, ameliorated gait, muscle strength, and normalized pathological hallmarks of the disease such as neuromuscular junction morphology and spinal motor neuron number. These results demonstrate the feasibility of SMA gene therapy by administration of ssAAV vectors, which may represent a novel strategy for preclinical development.

P377

Biosynthesis of DYSF protein in Bla/J dysferlin-deficient mice after administration of recombinant adenovirus encoding dysferlin cDNA

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Limb-girdle muscular dystrophy type 2B (LGMD2B) is a group of recessive disorders called dysferlinopathies. This group of diseases is characterized by wide clinical heterogeneity. Currently, there is no approved therapy. We selected a recombinant adenovirus serotype 5 (Ad5) with high coding capacity and relatively low immunogenicity as vector for gene therapy treatment of dysferlinopathy. The aim of our study was to analyze biosynthesis of DYSF protein in Bla/J dysferlin-deficient mice injected with recombinant adenovirus carrying dysferlin cDNA (Ad5-Dysf). Adenoviruses Ad5-Dysf and Ad5-GFP were generated, amplified, concentrated and tittered according to ViraPower Adenoviral Expression System instructions. Evaluation of recombinant proteins expression *in vitro* was carried out in human myoblast cells transduced with recombinant adenoviruses. Retro-orbital administration of Ad5-Dysf, Ad5-GFP or physiological saline (solvent) as a control was performed using 9 Bla/J dysferlin-deficient mice and 3 C57Bl6 wild-type control mice. After two weeks, mice muscle tissues were harvested for further analysis. Protein lysates of Bla/J and C57Bl6 mice gastrocnemius muscles after administration of Ad5-DYSF, Ad5-GFP or saline were analyzed by western blotting (WB) for the presence of DYSF protein. Positive reaction was observed with rabbit polyclonal antibodies to dysferlin in C57Bl6 control mice as well as in two Bla/J mice after Ad5-DYSF administration. Hence, gene therapy treatment of dysferlin-deficient Bla/J mice model with recombinant adenovirus encoding codon-optimized dysferlin cDNA resulted in dysferlin protein biosynthesis in muscle tissues.

P378

Gene therapy for X-linked myotubular myopathy with AT132 (rAAV8-Des-hMTM1): preliminary results from the ASPIRO phase-1/2 study

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XLMTM is a rare monogenic disease caused by mutations in the MTM1 gene and characterized by profound muscle weakness, respiratory failure and early death. There is currently no disease-modifying treatment for XLMTM and patients are highly dependent on caregivers and technological support. AT132 is an investigational adeno-associated virus-mediated gene therapy, designed to correct muscle pathology by delivering functional MTM1 to skeletal muscle cells throughout the body. Safety and preliminary efficacy are being investigated in ASPIRO, a Phase 1/2, open-label, randomized, ascending-dose study in male XLMTM patients <5 years of age requiring ventilator support. Natural history data from the non-interventional run-in study INCEPTUS will provide longitudinal baseline and within-patient controls. As of 12 May, 27 patients have enrolled into INCEPTUS and 7 patients have transitioned into ASPIRO (Cohort 1; six treated patients, one delayed-treatment control). Treated patients received a single AT132 intravenous infusion of 1×1014 vector genomes/kg and oral prednisolone daily for 16 weeks. A total of 18 adverse events (AEs) were reported, of which six were considered serious AEs (SAEs). All four SAEs deemed probably or possibly related to drug occurred in Patient 3 and responded to intravenous steroids and supportive care. Three treated patients have completed 12-24 weeks' follow-up and demonstrate considerable improvements in neuromuscular (Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders [CHOP-INTEND] scale) and respiratory (maximal inspiratory pressure) co-primary endpoints; Patient 1 has achieved ventilator independence and oral feeding. Multiple motor developmental milestones have also been reached. Updated results will be presented.

P379

Gene therapy via trans-splicing for LMNA-related congenital muscular dystrophy

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We assessed the potential of Lmna-mRNA repair by spliceosome-mediated RNA trans-splicing as a therapeutic approach for LMNA-related congenital muscular dystrophy. This gene therapy strategy leads to reduction of mutated transcript expression for the benefit of corresponding wild-type (WT) transcripts. We developed 5′-RNA pre-trans-splicing molecules containing the first five exons of Lmna and targeting intron 5 of Lmna pre-mRNA. Among nine pre-trans-splicing molecules, differing in the targeted sequence in intron 5 and tested in C2C12 myoblasts, three induced trans-splicing events on endogenous Lmna mRNA and confirmed at protein level. Further analyses performed in primary myotubes derived from an LMNA-related congenital muscular dystrophy (L-CMD) mouse model led to a partial rescue of the mutant phenotype. Finally, we tested this approach *in vivo* using adeno-associated virus (AAV) delivery in newborn mice and showed that trans-splicing events occurred in WT mice 50 days after AAV delivery, although at a low rate. Altogether, while these results provide the first evidence for reprogramming LMNA mRNA *in vitro*, strategies to improve the rate of trans-splicing events still need to be developed for efficient application of this therapeutic approach *in vivo*.

P380

Integration-deficient lentiviral vectors for *in utero* gene therapy in spinal muscular atrophy

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Spinal muscular atrophy (SMA), the most common genetic disorder resulting in infantile death, is caused by defective production of Survival Motor Neuron (SMN) protein from *SMN1,* leading to degeneration of motor neurons (MNs) and neuromuscular dysfunction. Pre-symptomatic treatment may prevent development of the SMA phenotype and accompanying irreversible damage; therefore, we are attempting *in utero*delivery of *SMN1*. Integration-deficient lentiviral vectors (IDLVs) are highly efficient tools for delivering therapeutic genes to quiescent tissues. We have developed IDLVs expressing a novel version of *SMN1* under three transcriptional controls (CMV, hSYN, hPGK). Mouse E15 primary cortical neurons, rat MNs and fibroblasts obtained from type 1 SMA patients showed significantly increased SMN protein following transduction with IDLVs. Significant increases in gem numbers (an endpoint suggestive of functional *SMN1* expression) have been shown in neuronal cell lines Neuro2a and SH-SY5Y, as well as type 1 SMA fibroblasts. Since MNs are the primary pathological target of SMA, we have used our vectors in human induced pluripotent stem cell (iPSC)-derived MNs and shown up to 70% of cells can be successfully transduced, leading to a 23-fold increase in SMN protein levels. *In utero*delivery of IDLVs to E16 mouse fetuses has shown complete transduction of choline acetyltransferase-expressing spinal cord MNs and dorsal root ganglia neurons. Preliminary experiments of IDLV delivery of the murine *Smn*gene to E16 mice increased Smn protein at all levels of the spinal cord. Taken together, these results suggest that IDLVs could be efficient tools for SMA gene therapy during fetal development.

P381

Urine progenitor cells as an alternative source to generate iPSC-derived cardiomyocytes (iPSC-CMs) and electrical stimulus as an important mechanism for iPSC-CMs maturation

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The use of human-induced pluripotent stem cells (iPSC) is a promising strategy in cardiac therapy and disease modeling. However, the maturation of cardiomyocytes generated during differentiation need to be improved. The objectives of this work are: 1) to produce iPSC-CMs from urine progenitor cells (UPCs); 2) to investigate the effect of electrical stimulus (ES) on iPSC-CMs differentiation. iPSCs were reprogrammed from UPCs and fibroblasts and characterized: all iPSCs clones presented expression of pluripotent genes and protein markers and normal karyotype. Then iPSCs were differentiated into cardiomyocytes under ES from day 0 to 3. Spontaneously beating iPSC-CMs were observed at day 9 of differentiation. iPSC-CMs from UPCs displayed higher expression of GATA4, MYL2, HCN4, KCNJ3 and TNNI3 compared to iPSC and higher expression of KCNJ3 compared to iPSC-CMs from fibroblast. iPSC-CMs with ES presented contractile cells 71% of the time, while control iPSC-CMs only 57%. Moreover, iPSC-CMs with ES showed upregulated cardiac genes as GATA4, TNNI3, CALM3 and MYL2 compared to the control. Functional analyses show that frequency of beating cells, calcium (Ca) transient amplitude, time to peak and Ca decay rate were significantly higher in iPSC-CMs with ES compared to the control and in iPSC-CMs from UPCs compared to iPSC-CMs from fibroblast. These results showed that iPSC from UPCs can successfully differentiate into CMs and that ES can upregulate cardiac genes and promote functional CMs maturation. This study may contribute to use an alternative source of iPSC and to increase the differentiation process efficiency for therapeutic or disease modeling strategies.

P382

Heme oxygenase-1 modulates the properties of human induced pluripotent stem cells-derived cardiomyocytes

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Heme oxygenase-1 (HO-1, encoded by HMOX1) is a cytoprotective enzyme degrading heme into CO, Fe^{2+} and biliverdin which was demonstrated to affect cardiac differentiation of murine pluripotent stem cells, regulate metabolism of murine adult cardiomyocytes and influence regeneration of infarcted myocardium in mice. The effect of this enzyme, however, on human cardiogenesis and on the electromechanical properties of human cardiomyocytes has not been described. Thus, the aim of this study was to investigate the role of HO-1 in differentiation of human pluripotent stem cells (hiPSCs) into cardiomyocytes (hiPSCs-CMs). Footprint-free hiPSCs were generated from human fibroblasts and peripheral blood mononuclear cells using Sendai vectors. Upon confirmation that obtained hiPSCs lines expressed markers of pluripotency and differentiated *in vitro* into all 3 germ layers via embryoid bodies, CRISPR/CAS9 technique was utilized to inactive HMOX1. Lack of HO-1 protein was functionally confirmed by Western blot technique. Control and HO-1-deficient hiPSCs were further differentiated into hiPSCs-CMs using small molecules modulating WNT pathway with high efficiency of cardiac troponin T (cTnT)-positive cells generation (up to 95%). PCR, immunofluorescence and patch-clamp analysis of generated cells confirmed their cardiac properties i.e. expression of cardiac markers, synchronic contraction and electrophysiological potential. Importantly, lack of HO-1 resulted in significant shortening of depolarisation

phase in action potential of CMs. In line with this observation, HO-1-deficient hiPSCs demonstrated lower expression of potassium, sodium and calcium ion channels which suggests their more immature phenotype in comparison to isogenic controls. Thus, HO-1 is an important molecular target for modulation of human cardiomyocytes properties.

P383

AAV vector-based gene therapy for glycogen storage disease type III

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Glycogen storage disease type III (GSDIII) is a recessive disorder due to mutations in the glycogen debranching enzyme (GDE), the enzyme involved in the linearization of cytosolic glycogen. The lack of GDE leads to glycogen accumulation in all tissues. During childhood, GSDIII is mainly a metabolic disease characterized by hepatomegaly and fasting hypoglycemia. During adolescence, the metabolic manifestation of the disease becomes less prominent and a degenerative muscle weakness appears. At present, no curative treatment exists for GSDIII. A major limitation in the development of a gene therapy for GSDIII is the transgene size that exceed the AAV packaging size. We showed partial correction of the GSDIII phenotype in a mouse model of the disease using dual AAV vectors (Vidal, Mol. Ther. 2018). Here, we explored dual vector strategies based on recombination or trans-splicing of AAV vectors which were described as highly efficacious to deliver large transgenes. We compared different dual vector strategies to express GDE simultaneously in liver and muscle of GSDIII mice. In parallel, single vector strategies were also explored. While glycogen clearance and restoration of muscle strength was observed with dual AAVs, results obtained indicate that single vector strategies are viable and clinically relevant gene therapy strategies for GSDIII.

P384

Preliminary safety data from ADVANCE clinical study (ADVM-043-01) in alpha 1 antitrypsin (A1AT) deficiency

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A1AT deficiency is caused by mutations in the SERPINA 1 gene that result in production of a misfolded protein, typically a 5-fold reduction in plasma A1AT concentration, and an increased risk of COPD. The ADVANCE study is an ongoing multi-center, dose escalation Phase 1/2 study of a single intravenous or intrapleural administration of investigational gene therapy ADVM-043 to subjects with A1AT deficiency. ADVM 043 is an adeno-associated viral serotype rh.10 (AAVrh.10) vector encoding the functional M-A1AT protein cDNA. ADVM-043 is intended to deliver a functional gene to the liver of subjects with the Z-A1AT phenotype and induce functional M-A1AT protein expression. The primary endpoint of the ADVANCE study is safety and tolerability. Earlier liver-directed AAV gene transfer trials have suggested that capsid-specific immune responses may target transduced hepatocytes which can result in the decline in transgene expression. In the ADVANCE trial, a tapering course of oral prednisone was given to prevent potential development of cytotoxic T-cells directed against transduced hepatocytes. Hepatic transaminases and interferon-γ enzyme-linked immunospot (ELISpot) assays for detection of capsid-specific and A1AT specific cellular responses were monitored to assess the impact of prophylactic immunosuppression. Interim safety data through 03Jul2018 from 2 cohorts (N = 4) is presented. No AEs have been deemed related to ADVM-043 by the independent data monitoring committee. No patients exhibited cell-mediated response to A1AT or against AAVrh.10 and no clinically-significant transaminase elevations were observed. Preliminary clinical data suggests that ADVM-043 has an acceptable safety profile and is well tolerated.

P385

Comparison of immunodetection based-technologies for HCP testing for gene therapy products

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AAV vectors are highly promising tools for treating monogenic diseases and have been recently enrolled in a large number of clinical trials. As a biopharmaceutical product, AAV vectors are manufactured using a host cell system and characterization of the product includes testing for impurities including Host cell Proteins (HCP). For our AAV vectors, HCPs are derived from the HEK293 cell line used for the production. Currently, a traditional sandwich-type ELISA is used for their quantitation. ELISA is referred as the gold standard method due to accurate HCP quantitation. However, ELISA is labor intensive, have short dynamic range and in-process samples show interference with this method. For these reasons, new technologies based on immunodetection have been evaluated as they may offer advantages for HCP monitoring: Meso Scale Discovery (MSD), Forte Bio Octet and the Gyros Gyrolab platform. Comparison between these methods in terms of range of detection, precision, accuracy, linearity are presented. More practical aspects such as cost, ease of use, high throughput are also compared. Futhermore, a HCP clearance study was performed in order to evaluate the performance of each technology for HCP quantitation of in-process samples.

P386

Impact of the inclusion of the Dystrophin C-terminal domain in a micro-Dystrophin transgene designed for gene therapy of Duchenne muscular dystrophy

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Duchenne Muscular Dystrophy (DMD) is an X-linked disease caused by mutations in the gene encoding for the Dystrophin

protein and is characterized by progressive loss of muscle function. Dystrophin establishes a mechanical link between cytoskeletal actin and the extracellular matrix of muscle fibers through the Dystrophin-Associated Protein Complex (DAPC). Recombinant Adeno-Associated Virus vectors (rAAV) hold great promise and allow efficient transduction of skeletal and cardiac muscles. However, full-length dystrophin cDNA exceeds the packaging capacity for a single rAAV gene-delivery cassette. Therefore, shorter versions named micro-Dystrophins (MDs) have been designed to contain important domains of the Dystrophin protein. In particular, a MD variant called MD1 was described as a promising candidate. However, MD1 does not contain the Dystrophin C-Terminal (CT) domain, which interacts with DAPC components such as Dystrobrevin and Syntrophins and that could thus be important for the optimal efficacy of MD. A new MD sequence, called MD4, which contains the CT domain, was recently designed. In order to compare the therapeutic potential of MD1 and MD4 *in vivo*, our first challenge was to optimize the length of the MD4 expression cassette, knowing that the initial one exceeds the maximal encapsidation capacity of rAAV vectors and generated poorly infectious vectors. Being able to obtain MD1 and MD4 protein expression in muscles of rAAV-injected DMDmdx rats, our goal is now to compare the DAPC composition between muscle and heart samples expressing MD1 or MD4, using mass spectrometry. The recent results and the specific developments of this project will be presented.

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Targeting lung diseases with pseudotyped lentiviral vectors

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Lung diseases such as cystic fibrosis and other monogenic disorders are attractive candidates for gene therapy. The challenge is to achieve high levels of transduction in the target lung epithelia in order to obtain efficacy in preclinical and clinical studies. Lentiviral vectors pseudotyped with the VSV-G envelope protein have resulted in low transduction efficiency of the airway system due to a number of reasons including lack of VSV-G cellular receptors on the apical surface of respiratory epithelia, the mucosal barrier and ciliary movement. The use of alternative viral envelopes derived from viruses that naturally target the airway system is crucial for efficient vector entry to the lung as demonstrated by *in vivo* delivery using vectors pseudotyped with Sendai, Influenza A and Baculovirus gp64 envelopes in a variety of models. We are currently evaluating lentiviral vectors pseudotyped with envelopes derived from multiple viral families including Paramyxoviridae and Orthomyxoviridae. We have selected envelopes with broad tropism for the respiratory tract and assessed their ability to transduce fully differentiated respiratory Air Liquid Interface (ALI) cultures derived from primary human bronchial epithelial cells. These vectors transduced ALI cultures from the apical surface more efficiently than VSV-G pseudotyped vectors and were able to transduce multiple epithelial cell types. Further *in vitro* and *in vivo* studies are ongoing to identify the best candidate for high efficiency gene transfer in the respiratory system. Utilising Oxford BioMedica's advanced LentiVector® platform and serum free bioreactor scale-up capabilities will allow large scale GMP production for use in therapeutic respiratory indications.

P388

Regulatory T cells and effector T cell exhaustion are essential to establish and maintain liver-mediated immunological tolerance to muscle-expressed antigens

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Limb-Girdle Muscular Dystrophy type 2D (LGMD2D) is a recessive muscular disorder caused by deficiency in α-sarcoglycan (SGCA), a transmembrane protein part of the dystrophin-associated complex. AAV-mediated gene transfer demonstrated safety and efficacy in animal model. However, an anti-SGCA specific immune response was reported in Sgca deficient mice that can lead to a loss of transgene expression in muscle fibers. Given its crucial role in the development of transgene-specific peripheral tolerance, we hypothesized that liver transduction may control the anti-SGCA immune responses. To validate our hypothesis, mice were treated simultaneously with an intramuscular injection of AAV6-SGCA vector to induce an immune response against the transgene and with an AAV9-SGCA vector expressing the same transgene in the liver. We demonstrated that liver transduction resulted in both prevention and eradication of anti-SGCA immune responses. To further characterize the cytotoxic immune response toward the transgene product we fused the CD8-specific SIINFEKL epitope with SGCA. We demonstrated that the simultaneous administration of AAV vectors expressing this highly immunogenic transgene in muscle and liver resulted in the complete reduction of anti-SGCA-SIINFEKL immune responses. In addition, we provided evidence that both T cell inhibitory receptors and CD4+ regulatory T cells contribute to establish and maintain liver-mediated peripheral tolerance. In conclusion, this study confirms the robustness of liver peripheral tolerance and demonstrates the importance of liver gene transfer to reduce anti-transgene immune responses in peripheral tissues.

P389

Evaluation in mice of two genetic modifiers of muscular dystrophy on the phenotypic severity of FKRP deficiency

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The last decade, genetic modifiers modulating the clinical presentation of muscular dystrophies (MD) have been identified including variants in the Latent TGFβ Binding Protein 4 (LTBP4) and Cytidine Monophosphate-sialic Acid Hydroxylase (CMAH) genes. The former was associated with an increased severity of the phenotype in a mouse model of Limb-Girdle-MD (LGMD) type 2C on the DBA-2J/JAX (DBA) background, and an earlier loss of ambulation in Duchenne MD (DMD) patients. The latter has been inactivated in humans during evolution and reduces the severity of phenotype in mdx mice, a model of DMD, thus suggesting that this inactivation could underly the more severe phenotype observed in patients. To study LGMD2I, we developed a FKRP knock-in model (FKRPL276I). Characterization showed a mild impairment and slow progression of dystrophic features. To get a more severe model, we backcrossed

FKRPL276I mice onto the DBA and the Cmah-/- backgrounds and investigated the effects of these modifiers on the level of impairment. The effects were evaluated by histological examination and measurement of the expression levels of dystrophy-related genes and compared to those obtained with the mdx mice, a more severe MD model. We observed that the LTBP4 modifier weakly affected regeneration and fibrosis processes in the FKRPL276I mouse while the CMAH one did not have any effect. In parallel, the mdx phenotype worsened on the DBA background whereas the CMAH deletion showed an impact on the regeneration process only. In conclusion, the LTBP4 and CMAH modifiers did not drastically worsen the phenotype of the FKRPL276I mouse model.

P390

Transcriptional activation of mutant dysferlin gene expression in human skin fibroblasts using CRISPR/Cas9 SAM technology

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Dysferlinopathies represent a group of neuromuscular diseases caused by absence or the disruption of dysferlin protein functionality due to mutations in corresponding gene. One of the reasons for slow progress in treatments development is lack of a model that most accurately reproduces the pathological processes of the disease in humans. Currently, gene therapy is a promising approach for dysferlinopathies treatment. Model systems based on human cell lines that are defective in dysferlin are required for testing new gene therapy drugs. Dysferlin is expressed abundantly in muscle cells, however, due to the difficulties in isolating and cultivating of myoblasts scientists are searching for alternative sources of cellular material. In our work we took human fibroblasts (HFs) and activated expression of dysferlin using CRISPR/Cas9 SAM transcriptional activation system. HFs were isolated from patient carrying 26th exon mutation in DYSF gene and immortalized by shRNA knockdown of p53 oncosuppressor mRNA. The lack of p53 expression in genetically modified fibroblasts was confirmed by western blot analysis and real-time PCR. For transcriptional activation of immortalized fibroblasts, recombinant lentiviruses encoding transcription factors MS2-P65-HSF1, dCas9-VP64 and DYSF specific guide RNA were used. Dysferlin mRNA expression was confirmed by qPCR. Our results confirm that patient-derived HFs can be transcriptionally activated to express mutant DYSF mRNA and can be used in further studies of DNA and RNA editing as new therapeutic approached for treatment of neuromuscular diseases.

P391

Gene therapy for the treatment of injuries of the superficial digital flexor tendons and branch of suspensory ligament lesions in horses

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Regenerative medicine is a fast developing area with gene therapy being one of promising new approaches. However development of veterinary gene therapy applications using species-specific genes has been limited. In our study, we used gene therapy technology to treat ten horses that had gone lame due to injuries affecting the suspensory ligament and superficial digital flexor tendon (SDFT) in the lower leg. Plasmid DNA encoding two therapeutic species-specific (Equus ferus caballus, horse) protein growth factors: vascular endothelial growth factor A (VEGFA164) and fibroblast growth factor 2 (FGF2) was used to treat ten lame horses. Seven horses had naturally occurring injuries of the superficial digital flexor tendon (SDFT) and three additional horses with suspensory ligament branch desmitis. Follow-up data revealed significant clinical improvements in all animals. Eight horses with SDFT and suspensory ligament branch lesions returned to their pre-injury level of performance 2-6 months after receiving the treatment. Significant ultrasonographic improvements were not observed in one horse with severe suspensory ligament branch desmopathy within the first 2 months after treatment, but did improved clinically and became less lame. One horse with severe SDFT had significant clinical improvements but was re-injured 6 months later. This data and study is highly promising in the treatment of injuries and restoration of tissues; however, it requires further research using experimental models to evaluate equine tendons and ligaments after application of the plasmid DNA encoding VEGF164 and FGF2 genes.

P392

BB-301: a single "silence and replace" AAV-based vector for the treatment of oculopharyngeal muscular dystrophy (OPMD)

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OPMD is an autosomal dominant disease mainly characterized by weakness of the eyelid and pharyngeal muscles, leading to ptosis and dysphagia. The disease is caused by an abnormal expansion of alanine-encoding trinucleotide repeats in PABPN1 gene. Previously, we described a gene therapy approach to treat OPMD using two AAV vectors tested in the A17 mouse, which recapitulates most of the features of human OPMD patients including muscle atrophy and weakness, associated with insoluble nuclear aggregates of mutant PABPN1. Here we now describe the development of BB-301, a single vector ''silence and replace'' therapeutic comprised of an AAV9 capsid and a muscle-specific promoter to produce a bifunctional RNA expressing shRNA against PABPN1 as well as a codon-optimized shRNA-insensitive wildtype PABPN1. In a 20-week experiment, treatment of TA muscles with BB-301 at 6e10 vg/muscle results in robust inhibition of mutant PABPN1 expression by up to 87% and restores wildtype PABPN1 levels up to 91% of endogenous levels. Concomitantly, BB-301 treatment resulted in correction to near wildtype levels of aggregates, fibrosis, and muscle strength. A follow-on dose ranging experiment was performed

from 4e8 to 7.5e11 vg/muscle. Mid-ranged doses of BB-301 resulting in 75% inhibition of mutant PABPN1 and 26% restoration of wildtype PABPN1 produce full phenotypic correction of muscle strength and weight, suggesting a broad therapeutic window. On-going safety studies are being performed by injection into throat muscles in sheep to support a first-in-man study, where BB-301 will be injected directly into the pharyngeal muscle for treatment of OPMD-related dysphagia.

P395

Indirect treatment comparison of AVXS-101 to nusinersen for the treatment of type 1 spinal muscular atrophy (SMA1)

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Until recently, SMA1 patients faced a poor prognosis with no approved treatments. Nusinersen is currently the only FDA-approved SMA treatment; however, onasemnogene abeparvovec (AVXS-101), a gene replacement therapy candidate, has been evaluated in a Phase I clinical trial with promising results. To compare the relative efficacy and safety of AVXS-101 relative to nusinersen in the absence of a common comparator, a Bayesian naïve indirect treatment comparison between AVXS-101 and nusinersen (ENDEAR trial) was conducted. The outcomes of interest were event-free survival (EFS), CHOP-INTEND response (≥4-point improvement), and overall and treatment-specific adverse events (AEs and TEAEs, respectively). Relative treatment effects were expressed as relative risk (RR), risk difference (RD), and number needed to treat/harm (NNT/NNH). At 12 months, 100% of patients on AVXS-101 were alive and did not require permanent ventilation, compared to 61% of patients on nusinersen, representing a 77% greater EFS likelihood (RR of being alive and event-free 1.77, 95% CrI 1.49-2.20; RD 0.44, 95% CrI 0.33-0.55; NNT 2.60, 95% CrI 2.02-3.59). AVXS-101 also showed a more favourable CHOP-INTEND response (RR 1.40; 95% CrI 1.23-1.65; RD 0.28, 95% CrI 0.18-0.40; NNT 3.52, 95% CrI 2.53-5.42). AVXS-101 and nusinersen showed similar safety profiles and TEAE rates. Of the most frequently reported TEAEs (vomiting, upper respiratory tract infection, cough, and constipation), none were considered AVXS-101-related, with most related to patients' underlying illness. Elevated serum aminotransferase levels with AVXS-101 were without clinical sequelae. AVXS-101 offers promising efficacy compared to nusinersen regarding survival, reduced requirement for permanent ventilation, and improvement in motor function.

P396

Novel gene therapy approaches for whole brain delivery of the lysosomal GCase enzyme for wide protection from alpha-synuclein toxic aggregates

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Approximately 5–8% of PD patients are carriers of a heterozygous GBA1 mutation, causing enzyme reduction in GCase global activity. Conversely, stimulating GCase activity has been shown to fuel lysosomal activity to stimulate degradation of alpha-synuclein (alpha-Syn) toxic aggregates. Furthermore, increasing GCase levels can be an effective therapeutic strategy for alpha-Syn-related disorders. We have established two novel gene therapy approaches to widely express GCase in the whole brain and, thereby, reaching a global protection from alpha-Syn-dependent toxicity. First, we showed that a single intravenous injection of the brain penetrant GBA1 expressing AAV-PHP.B is sufficient to provide robust and long-lasting protection from alpha-Syn deposits in a mouse model of synucleinopathy. AAV-PHP.B delivered GCase resulted in a significantly diminished accumulation of insoluble alpha-Syn species in all the forebrain regions. As an alternative gene therapy approach, we conceived a different AAV serotype able to exploit the cell-to-cell spreading of the GCase enzyme. In fact, we showed that GCase can be released by brain endothelial cells and re-uptaken by surrounding neurons and astrocytes. Thus, we generated GBA-expressing AAV2-BR1 virus able to specifically target the brain microvasculature and allow GCase to diffuse throughout the central nervous system. Remarkably, hA53T-alpha-Syn transgenic mice subjected to this treatment exhibited a widespread reduction of alpha-Syn deposits. We propose that the AAV2-BR1-based gene therapy is a crucial strategy to convert the brain microvasculature in a stable source of supplemental GCase enzyme for the long-term protection of neural tissue from accumulation of alpha-Syn aggregates.

P397

Spheroid formation and evaluation of human induced pluripotent stem cell-derived hepatocytes through self-assembly process

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One of the most intriguing human induced pluripotent stem cell (hiPSC) applications is the generation of a new, safe and reliable source of mature hepatocytes that possess the potential to overcome many of the limitations of primary human hepatocytes (PHH), such as their restricted availability, the rapid deterioration of their functionalities in culture and their viability after cryopreservation. Despite the numerous protocols available, still today the hiPSC-derived hepatocytes (iHeps) do not often show mature features. In this work, a homemade 3D culture system was used to promote the differentiation of hiPSC-derived hepatoblasts (iHBs) into iHeps in a highly reproducible manner. Briefly, iHBs were generated following a well-established differentiation protocol and harvested cells were let self-aggregate in inert microwells. Cell differentiation, apoptosis and cell motion were assessed; two days after seeding, spheroids with regular shape and smooth surface were generated. Immunofluorescence staining upheld in the spheroids the precocious appearance of albumin and the expression of hepatocyte markers such as the apical membrane proteins responsible for the Phase III transport of conjugated xenobiotics into the bile canaliculi. The formation of hepatic-specific structures in spheroids was also recorded. Above all, in 3D versus 2D

cultures, the specific secretion of proteins revealed a fivefold significant increase in albumin secretion together with, after only four days of 3D culture, the nearly complete disappearance of α-fetoprotein, signature for the acquisition of adult hepatocyte phenotype. Thus, the self-assembling into spheroids enhances differentiation of iHBs into mature and functional iHeps by mimicking the *in vivo* 3D cell-cell signalling.

P398

shRNA-mediated knockdown of prothymosin α ameliorates experimental colitis in mice

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Inflammatory bowel disease (IBD) is a group of inflammatory condition of the colon and small intestine. Two major types of IBD are ulcerative colitis and Crohn's disease. Prothymosin α (ProT), an acidic nuclear protein, is involved in a variety of cell functions, such as proliferation, chromatin remodeling, and anti-apoptosis. We have shown that ProT can enhance Smad7 acetylation and inhibit TGF-β signaling, which may be involved in the pathogenesis of inflammatory diseases. Matrix metalloproteinases (MMPs) play roles in the pathophysiological processes of various diseases, including IBD. In this study, we investigated whether overexpression of ProT contributes to the pathogenesis of IBD. Analysis of microarray data revealed that ProT, MMP-7, and MMP-12 were overexpressed in patients with IBD. Furthermore, there were positive correlations between the expression levels of ProT and MMP-7, as well as ProT and MMP-12. In the dextran sulfate sodium (DSS)-induced colitis mouse model that resembles human IBD, ProT transgenic mice expressed higher levels of TNF-α and Smad7, as well as exhibited shorter colon length and lower body weight compared with their wild-type counterparts. By contrast, knockdown of ProT expression in the intestine ameliorated colitis symptoms and reduced the levels of TNF-α and Smad7 in C57BL/6 mice with DSS-induced colitis. In conclusion, our results indicate that excess expression of ProT may play an important role in inflammatory conditions during the IBD process. Furthermore, silencing of ProT expression may be a novel therapeutic strategy for IBD.

P399

Deactivation of hepatic stellate cells by down-regulation of exosomal miRNAs in HCV-induced liver fibrosis

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Hepatitis C virus (HCV) is a major cause of chronic liver diseases, including liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC). However, the mechanism by which HCV cause fibrosis and other chronic diseases is poorly understood. The objective of this study was to develop anti-hepatitis C virus (HCV) genetic agents that can prevent chronic liver injury and fibrosis by HCV. Previously, we validated HCV infection-induced miR-192, one of which upregulated TGF-β1 in hepatocytes. In the present study, we determined the molecular mechanisms underlying chronic liver injury and fibrosis by HCV-induced miR-192 and the role of exosomes in HCV infection. Remarkably, miR-192 is secreted and transmitted through exosomes from HCV-infected hepatocytes into hepatic stellate cells (HSCs) as well as the level of exosomal miR-192 can be modulated by cellular miR-192 levels. Moreover, exosomal miR-192 stimulates the expression of fibrogenic markers in HSCs through upregulation of TGF-β1. Anti-miR-192 treatment of HCV-infected hepatocytes efficiently reduced miR-192 levels in exosomes, downregulated miR-192 and fibrogenic marker levels in HSCs, and impeded transdifferentiation of the cells. Notably, transdifferentiation of exosome-exposed HSCs is reversed following down-regulation of miR-192 into the HSCs. This study revealed a novel mechanism of HCV-induced liver fibrosis and exosomal miR-192 would be a diagnostic marker for HCV-induced fibrosis and a critical target for attenuating the viral pathogenesis.

P400

Addressing challenges in meeting chemistry, manufacturing and control regulatory requirements for cell and gene therapy products

ABSTRACT WITHDRAWN

P401

Anc80-mediated gene therapy rescues the hearing and balance dysfunctions in the Pjvk mutant mice

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Genetic hearing loss is a common disease entity of diverse etiologies which may involve pathologies anywhere along the auditory/vestibular pathways, including sensory cells of the inner ear, auditory/vestibular nerves, and brainstem nucluei. Recently, several novel adeno-associated virus (AAV) serotypes, such as Anc80, have been confirmed as a promising delivery system for restoring the function of inner ear sensory cells. However, the efficiency of these new AAVs in targeting other pathological changes of the auditory/vestibular pathways remains unclear. We have previously established a mouse model with a common missense PJVK mutation (p.G292R) in the Asian population. The Pjvk p.G292R mice exhibited both hearing and balance dysfunctions. In addition to the sensory cells of the inner ear, pathological changes were also observed in the neurons of auditory/vestibular nerves and brainstem nucluei. We delivered wild-type PJVK into the inner ear of Pjvk p.G292R mice at P0-P3 using the Anc80 vector. At P45, the treated mice showed lower auditory brainstem response thresholds and improved vestibular function as compared to the control group. These findings suggest that the Anc80-directed gene therapy is an efficient delivery system for simultaneously introducing genes to

both the sensory cells of the inner ear and the neurons of the central auditory/vestibular pathways.

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Improved analysis of transgenes in blood for gene therapy and gene doping testing

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Vector persistence in blood is commonly examined during the development and use of gene transfer medicine. Reliable and standardised analysis of transgenes in blood also forms the basis of detecting the misuse of gene transfer technology in sport, called gene doping. The current study improves vector detection in blood through increased sensitivity, making the analysis cheaper, easier and quicker, while maintaining reliability. As a model system, we used nonviral and AAV vectors carrying the human erythropoietin transgene, transgene-specific probe-based PCR assays and certified reference material with a unique design that allows identification of false positives due to cross-contamination. To identify false positives, in addition to improved analysis of amplicon size, an alternative method was developed that examines amplicons' melting profiles by including a suitable intercalating dye in probe-based assays. Sensitivity of transgene analysis in plasma was increased by ultrafiltration of extracted DNA. We compared detection of cell-free and cell-associated transgenes using whole blood, plasma or white blood cells as DNA extraction matrix. Using the developed protocols, reliable transgene detection was achieved by extracting DNA from whole blood. With this extraction matrix, blood samples kept frozen at -80°C for at least three months were suitable for reliable transgene analysis. The adoption of the described protocols may facilitate generation of more informative and reliable results from vector clearance studies in gene therapy and consistent inter-laboratory comparisons. With regard to gene doping detection, the improvements will increase utility and operational robustness of the existing test, facilitating its implementation and use in doping control.

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Evaluation of multiple adherent and suspension 293-based cells for production of AAV

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Production of AAV for early-phase clinical use requires manufacture utilizing hundreds to thousands of liters of upstream cell culture. Production facilities for early-phase trials have often relied upon adherent cell-based processes, though suspension cells offer greater flexibility and far superior scalability. Transitioning from adherent to suspension cells can be challenging, however, especially with respect to matching viral titers on a volume basis. Here, we evaluated four types of adherent and two types of suspension 293-based cells for their ability to produce an AAV8 vector packaging a self-complementary Factor IX genome. Adherent cells tested included HEK-293 cells (ATCC); two 293-based derivatives optimized for AAV production: AAV293 cells (Stratagene) and 293AAV cells (Cell Biolabs); as well as 293T/17 cells that incorporate the SV40 large T antigen. Suspension cells tested included HEK-293F (Gibco) and Viral Production Cells (VPCs; Gibco), a derivative of the 293F cell line, both without the SV40 large T antigen. Adherent cells were maintained and transfected in DMEM with 10% FBS supplemented with glutamax, and suspension cells were grown and transfected in serum-free LV-MAX Production Medium. Cells and culture media for all cell types were harvested six days post-transfection. Results measured with AAV8 capsid ELISA and ddPCR showed that among adherent cells, 293T/17 cells provided 2-6-fold higher production than the other cell types. Among suspension cells, VPCs provided the best production, up to 2-fold higher than 293F cells. Importantly, VPCs produced AAV titers on a volume basis comparable to or greater than adherent 293T/17 cells.

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Early diagnosis and speed to effect in type 1 spinal muscular atrophy (SMA1)

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SMA1 is marked by rapid, precipitous motor neuron loss; thus, achieving immediate biological effects is crucial. In the nusinersen phase 3 study (ENDEAR), ~10% of subjects died or required permanent ventilation by 2 months, the time required to complete four loading doses, and almost one-third of subjects died or required permanent ventilation by 6 months, suggesting a non-immediate therapeutic impact related to the loading dose schedule. This study explored the rapidity of onasemnogene abeparvovec (AVXS-101) therapeutic effect by evaluating early changes in CHOP-INTEND compared with the nusinersen response (ENDEAR, ≤5-point increase at 2 months post-dosing). SMA1 patients received a one-time intravenous AVXS-101 injection (NCT02122952; cohort 2; N = 12; followed up to 24 months). The outcomes were survival (death or ≥16 hours ventilation/day for >2 consecutive weeks) and motor function improvements (changes in CHOP-INTEND from baseline). All 12 patients survived to end of study. Rapid increases in CHOP-INTEND of 9.8 (SD = 3.91) and 15.4 (SD = 6.36) points were observed at 1 and 3 months post-dose, respectively. AVXS-101 appears to improve survival (100% vs. 61% for nusinersen) and induce a more rapid improvement in motor function (9.8 points at 1 month) as measured by CHOP-INTEND score relative to nusinersen (≤5 points at 2 months, ENDEAR), which leads to rapid restoration of SMN expression in motor neurons with a single-dose administration. Advances in the understanding of SMA, available and investigational pharmacologic treatments, and the gene replacement therapy, AVXS-101, underscore the importance of early diagnosis and treatments with a near-immediate onset of action to maximize clinical improvements.

P405

Optimization of transfection conditions to maximize AAV production in suspension 293-based cells

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Production of AAV for early-phase clinical use requires manufacture utilizing hundreds to thousands of liters of upstream cell culture. Production facilities for early-phase trials have often relied upon adherent cell-based processes, though suspension cells offer greater flexibility and far superior scalability. Here, we have optimized transfection conditions to maximize production of an AAV8 vector packaged with a self-complementary FIX genome from commercially available HEK-293F cells (Gibco) and Viral Production Cells (VPCs; Gibco), a derivative of the 293F cell line. Cells in shake flasks were maintained and transiently transfected using PEI in FreeStyle 293 Expression Medium (FEM), CD FortiCHO Medium (CDF) or LV-MAX Production Medium (LVM) and several critical parameters were identified over the course of experimentation. These include selection of culture medium, the concentration of DNA in culture medium, the ratio of PEI to DNA, cell seeding density and seeding time relative to transfection, day of harvest, and the concentration of DNA in the transfection mixture. Optimizing these conditions in 293F cells led to a greater than 10-fold increase in AAV titer, from less than 3E10 capsids/mL to greater than 3E11 capsids/mL in combined medium and lysate samples, as measured by AAV8 capsid ELISA. Optimization of conditions in VPCs provided even greater titers of over 6E11 capsids/mL. Digital drop PCR analysis of samples from both cell types demonstrated a similar ratio of genome-containing particles for this product.

P406

Novel human iPSC-derived hepatocytes with advanced functionality and long-term 2D cultures of human primary hepatocytes for metabolic disease studies

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Our new version of highly homogenous cryopreserved hPS cellderived hepatocytes with a novel maintenance medium shows multiple mature liver features. They secrete albumin and urea during a 2-weeks window and show mRNA expression levels of albumin and urea cycle enzymes that are comparable to those of human primary hepatocytes (hphep). In addition, genes involved in both glucose and lipid metabolism are expressed on the same levels as in hphep. The hPS cell-derived hepatocytes respond to insulin by phosphorylation of AKT and show capacity to take up low-density lipoproteins and become steatotic if incubated with fatty acids. Moreover, activity of cytochrome P450 enzymes and expression of genes essential for the drug metabolizing machinery are stably detected during a 2-weeks window and on similar levels as in hphep. The novel maintenance medium developed for hPS cell-derived hepatocytes was tested, with minor modifications, on hphep in order to see if similar improvements in long-term viability and functionality could be achieved. Surprisingly, the novel culture medium allowed maintained morphology, viability and functionality of hphep for 4 weeks post-thawing and, thus, could prevent the typically observed rapid loss in functionality and cell viability of hphep in conventional 2D cultures. This is in sharp contrast to existing hepatocyte maintenance media. Both the novel generation of hPS cell-derived hepatocytes with mature hepatocyte functions and the new maintenance medium for hphep will empower the usage of hepatocytes in the area of metabolic diseases.

P407

Antifibrotic environment of stromal cells drives scar-free healing of human endometrium during menstruation

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Endometrium of a woman of reproductive age undergoes damage and healing without scar during every menstruation. It is an obvious yet unique example of scar-free healing after significant injury of soft tissue in human. Investigation of mechanisms of this phenomenon can be an impact in regenerative medicine. We suggest that scar-free healing of endometrium during menstruation happens due to either unique characteristic of stromal cells or due to putatively antifibrotic environment. We isolated and characterised endometrial stromal cells (eSC) from menstrual blood of two healthy donors. They were CD73 (98%), CD90 (99%), CD105 (99%), CD146 (73%) and vimentin (99%) positive indicating clear MSC lineage. We also isolated soluble part of menstrual blood (menstrual fluid, MF) from the same donors and simultaneously obtained samples of serum of peripheral blood (SPB) to compare its ability to induce profibrotic myofibroblast phenotype in eSC *in vitro*. ESCs cultured in DMEM/F12 with SPB during 5 days acquired myofibroblast-like morphology and expressed -smooth muscle actin (-SMA) localised in stress fibres and ED-A fibronectin deposited outside cells indicating myofibroblast phenotype. eSCs cultured in DMEM/F12 with MF became smaller and did not have -SMA stress fibres. Quantity of ED-A fibronectin and -SMA in lysates of this cells was two-fold lower than in eSC treated by SPB. We concluded that scar-free healing of human endometrium is mediated by antifibrotic environment rather than by intrinsic characteristic of endometrial cells. The research was carried out within the state assignment of Lomonosov MSU and conducted using biomaterial collected under RSF grant #14-50-00029.

P408

Planning for successful and efficient ATMP development

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1: XENDO

As the face of modern medicine is changing, so should development strategies of new medicines, including advanced therapy medicinal products (ATMPs). Here, we present essential steps in ATMP development; how to design a valuable project plan, set critical milestones, and the identification of development gaps that can be intercepted without compromising on safety and efficacy, all to smoothen and speed up the process from ATMP development to marketing authorization. Innovative and often complex ATMPs are frequently being developed in academia and startups for a wide range of disorders. As science and technology frontiers are translated to medicinal product development these future therapies will further add to the complexities and challenges of drug development. Academia and startups are usually more focused on getting the science right but are often less experienced regarding the development of a medicinal product. As a guide through the development maze, it is of utmost importance to create a development plan and identifying all the interdependencies between non-clinical, CMC,

and clinical development, from an early stage on. Furthermore, a tailor-made regulatory strategy should be developed. This regulatory strategy should provide guidance and focus, especially in early development where engagement with regulatory agencies supports to align development milestones and assure regulatory compliance in the end. Although each ATMP is unique and needs a tailored development and regulatory strategy, critical steps can mostly be identified and anticipated on beforehand.

P409

Number needed to treat in spinal muscular atrophy type 1 with AVXS-101 relative to nusinersen

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This study assessed the number needed to treat (NNT) to prevent death and use of permanent ventilation and improve motor function with onasemnogene abeparvovec (AVXS-101) compared to nusinersen in spinal muscular atrophy type 1 (SMA1) patients. Patients with SMA1 were treated with AVXS-101 (NCT02122952; study cohort 2; N = 12; up to 24 months) or nusinersen (NCT02193074; N = 80; up to 13 months). NNT with AVXS-101 compared to nusinersen was assessed for survival and event-free survival (absence of death and permanent ventilation) at last visit, and for motor function (≥4-point increase in CHOP-INTEND score from baseline) at last visit and at a median of 9 months. The NNT to prevent one death, one event (death or use of permanent assisted ventilation), or for one patient to improve motor function relative to nusinersen was calculated as the reciprocal of the difference between AVXS 101 and nusinersen in event rates or motor function achievement rates. Patient mean age at first dose was 3.4 (0.9-7.9) and 5.3 (1.7-7.9) months in the AVXS-101 and nusinersen trials, respectively. NNT analyses suggests that treating 6.2 patients with AVXS-101 instead of nusinersen would prevent 1 more death by the last visit; treating 2.6 patients with AVXS-101 versus nusinersen would prevent 1 more event; and treating 3.5 patients with AVXS-101 versus nusinersen would allow 1 more patient to improve motor function (at last visit and at a median of 9 months). Efficacy in preventing death and use of permanent ventilation and improving motor function is much higher with AVXS-101 versus nusinersen.

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One-step monolith purification of oncolytic Influenza virus produced in Vero cells

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Chromatography is frequently used in purification of biological products, such as proteins, viruses and antibodies. It provides an easily scalable platform with a wide selection of interaction modes (ion exchange, hydrophobic, affinity, etc.), which makes it suitable for molecules with very diverse properties. Convective Interaction Media (CIM®) monolithic columns contain large open channels suitable and are dedicated for larger particles (viruses, exosomes, plasmid DNA). The large channels offer high surface accessibility of binding sites and eliminate the negative effects caused by diffusion, typical for beaded chromatography resins. The result is a flow-independent performance. The aim of the study was to develop Influenza virus purification platform applicable to multiple virus strains. The number of purification steps was minimised by omitting Tangential Flow Filtration (TFF) and other pre-treatment and concentration steps. The result is a process consisting of clarification and a single chromatography step, resulting in high host-cell DNA and protein removal. The binding capacity of the chromatography media and the productivity of the process were measured. The concentration factor achieved with chromatography yielded a higher concentration than the final formulation requirement, leaving only dilution to achieve final formulation.

P411

Economic burden of infant-onset (type 1) spinal muscular atrophy: a retrospective claims database analysis

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Type 1 spinal muscular atrophy (SMA1) is a rare genetic, life-threatening neuromuscular disease. Data on the cost burden of SMA1 for U.S. health plans are limited. This retrospective analysis estimated the economic burden of SMA1 using QuintilesIMS's PharMetrics Plus Health Plan Claims Database. Infants with ICD-9 codes for SMA ≤1 year old were classified as SMA1 (N = 119) and matched (1:1) with a random sample of infants by age, gender, index year, and Charlson Comorbidity Index. Healthcare resource utilization (HCRU) and costs (pharmacy, outpatient, and inpatient/hospitalization) incurred between February 2011 and November 2016 during the post-index/follow-up period (≥30 days up to 360 days) were compared. Significantly more SMA1 patients (98.32%) received ≥1 pharmacy, outpatient, or in-patient services (54.62%; $P < 0.0001$). Mean per-patient-per-month (PPPM) all-cause HCRU was significantly higher for SMA1 infants: pharmacy (1.43 vs. 0.37 prescriptions); outpatient (14.10 vs. 2.17 services); in-patient (0.23 vs. 0.003 admissions) (all, $P < 0.0001$). Mean PPPM hospital admissions (0.23 vs. 0.003), length of hospital stay (6.93 vs. 0.09 days), procedures per admission (1.49 vs. 0.03), and readmissions (0.04 vs. 0.00) were also significantly greater for SMA1 infants (all, $P < 0.0001$). Pharmacy, outpatient, and inpatient costs PPPM were significantly greater in SMA1 infants ($371 vs. $20; $4,192 vs. $232; and $22,500 vs. $22, respectively [all, $P < 0.0001$]), resulting in extrapolated all-cause total annual costs of $324,751 (SMA1 cohort) vs. $3,294 (matched cohort). The economic burden of SMA1 is substantial; a treatment that alters the early natural course of the disease might result in long-term cost savings.

P412

Prevalence of AAV 1, 2, 3, 5, 6, 8, 9 and 10 neutralizing factors in non-human primates

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Recombinant adeno-associated virus (rAAV) is the most largely used viral vector for *in vivo* gene therapy. However, despite promising results in preclinical and clinical studies, pre-existing

immunity against the viral capsid remains one of the major hurdles to the efficiency of rAAV-based strategies. In particular, anti-AAV pre-existing neutralizing factors at low titers were shown to prevent *in vivo* successful gene transfer in animal models and even patients. Although largely used as models, Non-Human Primates (NHP) are subjected to AAV natural infections, and develop pre-existing neutralizing responses similarly to humans. Screening of NHP individuals before vector delivery is necessary during preclinical studies using rAAV from proof of concept and dose finding steps to toxicology phase. In addition, NHP models are useful to understand the biology of AAV infection and the impact of neutralizing factors on gene transfer, as well as the assessment of immunomodulation strategies aiming to decrease or inhibit AAV neutralization. In this study, we report the seroprevalence of neutralizing factors against AAV serotypes 1, 2, 3, 5, 6, 8, 9 and 10 in a cohort of cynomolgus monkeys, the most commonly NHP model used in rAAV-based gene therapy studies. These results will give an insight on the frequency of natural AAV infection in cynomlogus macaques and could help the design of AAV-based gene therapy strategies.

P413

(r)evolution of AAV2 titration ELISA – from monoclonal to recombinant

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PROGEN's AAV2 Titration ELISA is well known to provide reliable and accurate quantification of rAAV2 total capsid titers for many years. It uses the monoclonal antibody A20. This antibody is produced in hybridoma cells with specificity for AAV2 intact particles and is employed for capturing and detecting the capsids in a sandwich assay format. The antibody A20 is not trivial to produce as it shows variable expression levels during production, has the tendency to oligomerize and is very sensitive to storage conditions at low temperatures. Therefore, the production conditions of the A20 antibody need thorough monitoring in order to achieve the required high lot-to-lot consistency. To overcome these obstacles, PROGEN produced a new, recombinant variant of the A20 antibody, called A20R. In contrast to A20 it contains the variable antigen binding region in an IgG1 context instead of the original murine IgG3. The new antibody A20R improves processing, handling and storage conditions during the manufacturing process. Here we demonstrate that A20R shows the same binding specificity to AAV2 compared to A20. Moreover, applying the A20R antibody in the well-established AAV2 ELISA results in the same consistent sensitive and reproducible total capsid titer measurements in comparison to the original A20 antibody.

P414

HSC transduction protocol improvement – RetroNectin or no RetroNectin, that is the question

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Ex-vivo hematopoietic stem cell (HSC) gene therapy has shown great promise for cure of multiple genetic disorders. This field of gene therapy have passed through many changes and optimisations to get to the current state. Studies using HSCs started with transduction protocols using gamma-retroviral vectors, although patient gene correction was achieved, adverse effects of insertional mutagenesis occurred. Modifications were made to make these vectors safer, nevertheless, most of ex-vivo HSC gene therapy clinical studies moved to the use of self-inactivating lentiviral vectors, which have the advantage of transducing dividing and non-dividing cells. With the change of vector came several changes to the HSC transduction protocol like cytokines concentrations and HSCs pre-stimulation time, however parts of this protocol were left unchanged like the use of RetroNectin coated plates/bags during transduction. While RetroNectin has proven to be of great importance for transduction with gamma-retroviral vectors, its effect on lentiviral vectors has been controversial. The aim of this work was to study RetroNectin effect on HSC transduction with lentiviral vectors. Results show that regarding transduction efficiency and vector copy number the use of RetroNectin coated plates has no advantage over non-RetroNectin coated plates. HSC viability, clonogenic capacity and immunophenotype were also not impacted with presence of RetroNectin. These results are very important as removal of RetroNectin from the transduction protocol leads to reduction of process costs and process simplification. The human biological samples were sourced ethically and research use was in accord with the terms of the informed consents under an approved protocol.

P415

RFX1 and RFX3 transcription factors interact with the D sequence of adeno-associated virus inverted terminal repeat and regulate AAV transduction

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Adeno-associated virus (AAV) transduction efficiency depends on the way in which cellular proteins process viral genomes in the nucleus. In this study, we have investigated the binding of nuclear proteins to the double stranded D (dsD) sequence of the AAV inverted terminal repeat (ITRs) by electromobility shift assay. We present here several lines of evidence that transcription factors belonging to the RFX protein family bind specifically and selectively to AAV2 and AAV1 dsD sequences. Using supershift experiments, we characterize complexes containing RFX1 homodimers and RFX1/RFX3 heterodimers. Following transduction of HEK-293 cells, the AAV genome can be pulled-down by RFX1 and RFX3 antibodies. Moreover, our data suggest that RFX proteins which interact with transcriptional enhancers of several mammalian DNA viruses, can act as regulators of AAV mediated transgene expression.

P416

AAV titration ELISA for standardized gene delivery

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1: Progen Biotechnik GmbH

A growing number of academic and industrial labs are using AAV vectors for the development of gene therapies leading to an increase in the demand for effective and reliable analytical AAV tools for R&D and manufacturing. To enable safe and effective AAV gene therapies, a dependable and reproducible quantification of accurate rAAV titers is needed to ensure safe and reliable gene transfer. Current quantification methods for rAAV vector preparations include qPCR, digital droplet PCR (ddPCR) for measuring DNA, Dot Blot and ELISA for measuring intact viral capsid protein. Key factors such as accuracy, time and cost determine which technique is ideal for standardized therapy protocols. Most of the AAV preparations contain a significant number of empty capsids which can be a critical factor for dosage determination in gene therapy. So, there is a need to measure the total capsid titer (empty & full) and ensure a reliable and reproducible determination of AAV titers. Here we demonstrate the procedure for calibration of the AAV5 ELISA, measuring total capsid titers, and that this method currently appears to be the best format for the quantification of rAAV preparations in comparison to other quantitative techniques in terms of interassay variability and ease of use.

P417

A universal analytical tool for protein identification and quantification in gene or cell therapies development

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1: ANAQUANT

Through the development of new therapies such as gene or cell therapies, the need to identify and quantify residual proteins considered as impurities for final product is critical. Indeed such proteins can present a safety risk to patients. These proteins can be come from the production of the viral vector and known as Host Cell Proteins or Host Related Proteins. But they could also come from reagents used in the full cell therapy process (any serum or proteins added during the process). Protein impurities identification and quantification must be monitored for consistency purpose. In order to develop a generic approach completely independent from the therapy process to protein monitoring, mass spectrometry (MS) coupled to liquid chromatography appears as an interesting tool. A global approach based on label-free MS absolute quantification strategy was thus developed. This strategy consists in performing protein identification and quantification evaluation within the same analysis. Protein identification is performed thanks to classic proteomics workflow while quantification evaluation is based on internal standards spiked in all samples. This internal standard generates a titration curve within the sample of interest and then allows quantification evaluation of all detected proteins. Thanks to this analytical tool, completely independent from any biological reagents, residual proteins can be detected and quantitate to have full data completeness through the whole R&D and manufacturing development.

P418

Chromatography purification of adenoviral vectors

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Numerous clinical studies have demonstrated the efficacy of Adenovirus-based vaccine vectors for the treatment of infectious diseases or cancer. Further progress and eventual use of these Adenoviral vectors as marketed drugs requires the establishment of efficient manufacturing processes that can deliver maximum yields of purified and highly potent viral vectors. Our group has focused on monolithic column chromatography due to the continuous network of large-sized pores and channels that are ideally suited for the purification of large biomolecules such as virus particles. Gradient and step elution patterns were tested using lysates from a 100 mL infected cell culture that were then purified on a 1 mL CIMmultus Quaternary Amine (i.e. strong anion exchanger) column (BIA Separations). A step elution method was identified which resulted in >80% virus recovery and >90% removal of total protein content in the purified virus fractions. Scale up to an 8 mL CIMmultus column using lysates from a 500 mL infected cell culture was performed and fractions corresponding to the virus peaks were pooled, concentrated and buffer exchanged to formulation buffer used for clinical lots. Overall recovery compared to the initial sample injected in the column was 53%. Particle to PFU ratio of the concentrated virus was comparable to the ratios achieved during Adenovirus purification by CsCl density gradient ultracentrifugation. Further work will focus on characterizing the amounts of other contaminants such as host cell DNA and Benzonase, and on further scale up to 4L of virus-infected cell culture containing approximately 5e12 infectious units.

P419

Paramagnetic immunobead isolation of human adipose-derived stem cells: a proof of concept study

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Human adipose-derived stem cells (ASCs) exist in an undifferentiated state, with the ability to differentiate along several mesodermal lineages. They hold great promise for cell therapy and tissue regeneration. Enzymatic methods of ASC isolation reliably define the stromal vascular fraction (SVF). However, enzymatic methods require a proteolytic reagent and a digestion time of 20 minutes or more. Paramagnetic immunobeads (PIBs) have been shown useful in cellular immunoprecipitation. We present a technique for the isolation of SVF from lipoaspirate using PIBs with a head-to-head comparison to isolation of SVF with a highly purified collagenase. Fresh human lipoaspirate was used for this study. 15 ml of lipoaspirate underwent digestion with Corase® (Reviticell, Inc, Jacksonville, FL) for SVF isolation. PIBs (Dynabeads™ Protein G Kit, ThermoFisher) initially underwent antibody binding using rabbit anti-mouse IgG (H+L) Superclonal™ Antibody (ThermoFisher). Secondary mouse anti-human antibodies (Human MSC Analysis Kit, BD Biosciences) were then conjugated: CD90, CD44, CD105, and CD73. The bead suspension was then incubated with 15 mls of lipoaspirate for 10 minutes at room temperature. After magnet precipitation, the supernatant was removed. The enzyme SVF and the PIB resuspension underwent subsequent culturing. Total live cell count for the enzymatically digested lipoaspirate was 5.4×105 and for the PIB lipoaspirate was 1.44×106. Both sets of cells underwent ASC-phenotypic plastic adherence and colony formation. This study shows that more than twice the quantity of SVF nucleated cells was isolated from lipoaspirate by PIB assay than enzymatic digestion.

P420

Automated analysis of quality attributes of pluripotent stem cell morphology in adherent and suspension culture

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Manufacturing development of cell-based therapy products derived from pluripotent stem cells (PSCs) usually starts with differentiation protocols that often involve manual handling unit operations and assessment of quality attributes based on visual observations and operator judgement. One of the challenges facing scale-up and manufacturing of PSC-derived cell therapy products is the development of automated, quality-assessment tools that can provide quantifiable data about critical process parameters. Here, we have adapted image processing software designed for scientific multidimensional images to develop an automated, in silico, image analysis strategy that can accurately measure both the 2-D confluency and 3-D size of aggregates of PSCs from standard cell culture phase-contrast microscopy images. This approach greatly reduces the variation observed between multiple process operators and vastly increases the potential for successful PSC-derived, cell therapy manufacturing. Image analysis tools can integrate automated analytical platforms to monitor, control and forecast process performance and product quality throughput the manufacturing process.

P422

Strategic planning and regulatory landscape for the development of complex biologics

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It is an exciting time for companies developing complex and new biologics. As we get more experienced to apply more advanced technologies, as well as new production processes for biologics, the regulatory landscape and recent strategies for the development of biotech products are rapidly evolving at the same time. In the development of biologics, it is not easy to keep track on best development practices and rapidly evolving regulatory expectations. Additionally, trends can be observed within leading health agencies to become more open for discussions and accept new scientific- and product-tailored development strategies. As usual, it's all about risk assessment, impact evaluation and scientific-based justifications, but agencies are actively paving the way by new regulatory procedures and guidelines.

P425

Endogenous fluctuations of OCT4 and SOX2 bias pluripotent cell fate decisions

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The SOX2 and OCT4 transcription factors are key regulators of embryonic stem (ES) cell self-renewal and differentiation, but how temporal fluctuations in their endogenous expression levels bias lineage commitment is unknown. We generated knock-in reporter fusion ES cell lines allowing to measure endogenous SOX2 and OCT4 protein fluctuations and determine their impact on mesendodermal and neuroectodermal commitment. Surprisingly, small differences in endogenous SOX2 and OCT4 levels impacted cell fate commitment in G1 but not in S phase. While SOX2 fluctuations had a minor impact on neuroectodermal commitment, elevated OCT4 levels at the onset of differentiation strongly biased ES cell towards both neuroectoderm and mesendoderm at the expense of self-renewal and primitive endoderm. Genome-wide measurements of chromatin accessibility revealed OCT4 level-dependent priming of differentiation-associated enhancers. Finally, CRISPR/Cas9 knock-out of an OCT4 binding site in a key Eomes enhancer abolished the ability of OCT4 to promote mesendodermal differentiation. Our study demonstrates how small endogenous fluctuations of transcription factors prime cell fate decisions in a cell cycle-specific manner by modulating chromatin accessibility at regulatory regions, and thus represent a major source of heterogeneity in the ability of individual ES cells to respond to differentiation cues.

P426

KRAB-ZFPs and transposable elements rewire early human embryonic development transcriptional networks

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The LTR12/ERV9 family of transposable elements has colonized primate genomes over the past 30 million years, contributing new enhancers and promoters. In particular, those insertions have rewired transcriptional networks specific to early human embryonic development, creating new isoforms of previously existing genes. These isoforms are normally kept repressed at all times in adult cells, but their expression can also be induced by the inhibition of DNMTs or HDACs, for example in the context of a cancer treatment. Through an analysis of the genomic binding sites of all KRAB zinc-finger proteins, we identified two candidate repressors for this transposable element family. We follow the evolution of these KZFPs in primate genomes and describe their functional relationship with the LTR12/ERV9 family.

P427

Interrogation into the molecular role of p62 in iPS reprogramming

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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 to produce iPSC is a major roadblock to realising the full potential of this powerful cellular tool. The p62 protein is a highly conserved scaffolding protein with roles in nutrient sensing, autophagy, inflammation and disease. p62 has

at least six functional domains with a range of binding partners including Nrf2 and LC3 as well as ubiquitinated proteins. There is a well-established role for autophagy in reprogramming, and emerging evidence to suggest that p62 could play a role in modulating the efficiency of reprogramming. I have created a library of p62 mutants with deletions in various functional domains allowing for investigations into the molecular role of each of them. I have started to investigate the expression and localisation of p62 and other autophagy related genes/proteins in various mouse and human cell types including p62-/- and p62 overexpressing cell lines. Future work will focus on the utilisation of these deletion mutants to determine the mechanistic role of p62 in iPSC reprogramming. Transcription factor activated reporters will be utilised investigate the molecular pathways involved in iPSc reprogramming in the presence and absence of wild type and mutant p62. Overall, I aim to define processes by which p62 modulation can improve the efficiency of iPSC reprogramming.

P428

Efficient expansion of immunophenotypically primitive, genetically modified haematopoietic stem cells derived from mobilized peripheral blood using small molecules

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Lentiviral vector (LV) ex-vivo gene therapy (GT) of hematopoietic stem cells (HSCs) has shown therapeutic potential for genetic disorders. However, the challenge of obtaining high yields of engraftable HSCs under culture conditions generates the need for further process optimization. Certain small molecules (SMs) have been identified that improve the expansion of unmanipulated cord blood-derived HSCs, while retaining self-renewal and inhibiting differentiation. Nevertheless, the expansion capacity of SMs on genetically-modified, adult HSCs remains largely unexplored. We investigated whether LY2228820-LY, pyrimidoindole derivative-UM171 or Stem-Reginin-SR1, alone or in combination (X3), can sufficiently expand mobilized $CD34^+$ cells transduced with a LV-GFP vector. SMs were added either from the initiation of transduction (5-day culture) or after its completion (7-day culture) and their effect on expansion, immunophenotype, transduction, differentiation and clonogenicity of HSCs was assessed. The 7-day culture resulted in higher expansion of total CD34+ cells over the 5-day culture, without any quantitative differences among tested SMs and the control (DMSO). Importantly however, UM171 and X3 expanded up to 5 fold, HSCs bearing a primitive phenotype (% $CD34^+CD38^-$, $CD34^+CD38^-CD90^+$ and $CD34^+CD45RA\text{-}CD133^+$) and expressing higher GFP levels over control. In methylcellulose and erythroid cell differentiation cultures, UM171 and X3 combination led to increased transduction efficiency and GFP expression. We here provide evidence for significant expansion of phenotypically primitive, genetically-modified mPB HSCs. Should these data be solidified by successful *in vivo* xenograft studies, the incorporation of UM171 or X3 in cell manufacturing will advance the ex-vivo genetic engineering of HSCs and the overall outcome of GT.

P429

Transient OKSM expression in cardiomyocytes induces reprogramming towards pluripotency *in vitro* and *in vivo*

P430

IPSCs derived from inducibly-immortalised adult stem cells as a source for clinical-scale manufacture of allogeneic cell therapies

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Induced Pluripotent Stem Cells (iPSCs) derived from somatic cells by epigenetic reprogramming have great potential in cell therapy. However their teratoma potential, the inefficiency of differentiation protocols and the fact that the target clinically-relevant cell type is often a progenitor cell unstable *in vitro* mean that clinical application of iPSC derivatives is challenging. CTX is a neural stem cell line in clinical trials for stroke and limb damage. It is immortalised by an integrated transgene, with cell cycle progression controllable with the estrogen derivative 4-hydroxytamoxifen. We have reprogrammed CTX to pluripotency using episomal vectors encoding the transcription factors OCT4, L-MYC, KLF4, SOX2 and LIN28. CTX-iPSCs

display many features characteristic of hPSCs. After reprogramming, cell morphology changes from a neuronal phenotype with extended processes to densely packed ''islands'' of undifferentiated cells with prominent nucleoli. They express the enzymatic marker alkaline phosphatase, are positive for the pluripotency transcription factor OCT4 and the antigens TRA-1-60 and SSEA-4, but do not express the early differentiation marker SSEA-1. CTX-iPSCs represent a useful clinical resource. They may be differentiated like normal iPSCs, but subsequent activation of the immortalising transgene can allow routine, scalable production of clinically-relevant but difficult or impossible-to-culture *in vitro* derivatives, providing allogeneic off-the-shelf treatments whilst obviating the issues with iPSC-derivatives noted above. Furthermore, as CTX-iPSCs are derived from a cell line which has already passed clinical phase safety trials, their application to new indications is likely to be accelerated.

P431

The phylogenetically distinct early human embryo

ABSTRACT WITHDRAWN

P435

Exploring the potential of extracellular vesicles as drug delivery systems

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Extracellular vesicles (EVs) are nano-vesicles produced by cells and key players of intercellular communication. Given their natural internalization capabilities, they have a strong potential as tunable, bio-inspired drug-delivery systems (DDS). However, the rationale of using EVs still requires improvements (drug loading yield, pharmacokinetics). Our team aims at overcoming these hurdles by using its pharmaceutical and physico-chemical skills to perform post-production modifications on EVs, to create a potent DDS. We produced and isolated EVs from murine mesenchymal stem cells (differential centrifugation) and characterized them: dynamic light scattering and nanoparticle tracking analysis (NTA) (size, concentration), protein quantification, western blot and proteomics (presence of specific proteins), cryoTEM (structure), lipidomics. After fluorescent labeling, EVs were incubated with the parent cells or foreign cells (NIH3T3), in the presence of endocytosis inhibitors, and cells were analyzed by flow cytometry. All experiments were also performed on liposomal commercial standards as a comparison. Drug-loading modifications were performed on EVs and their internalization was monitored. EVs were 94 ± 10nm (NTA, $n = 20$) with a production yield of 2.2 μg protein and 9.7.10^8 particles / 10^6 cells ($n = 20$), and expressed EV-specific markers such as TSG101, CD81 and ADAM10 (western blot, proteomics). EVs were internalized to a greater extent than their liposomal counterparts and they followed different endocytic routes ($n = 4$). Among the processes evaluated for drug loading, EVs were extruded (50 nm membranes) and freeze-dried without damage, and the modifications did not impact their internalization ($n \geq 3$). Overall, our work provides evidence that EVs hold great promise for the delivery of exogenous biomolecules.

P436

Bacterial artificial chromosomes for rapid generation of high titre stable suspension producer cell lines for lentiviral vector manufacture

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Supply of lentiviral vector at the large scales required to treat patient populations remains a significant challenge for ensuring patient access to gene therapy medicines. To date most clinical grade lentiviral vector manufacturing has used transient transfection of plasmid DNA into adherent 293T cells grown in cell factories. This manufacturing process entails high costs and long lead times for sourcing clinical grade plasmid DNA and is limited by poor scaleability of adherent cell factory processes beyond low tens of litres. To meet the need for large scale lentiviral vector manufacture GSK has established a method to rapidly generate stable lentiviral vector producer cell lines. This process is based on stable transfection of a single Bacterial Artificial Chromosome (BAC) construct encoding all lentiviral vector components as individual expression cassettes. We have demonstrated that when grown in 50L stirred tank bioreactors these cell lines produce volumetric titres equivalent to current widely-used cell factory processes. Through extended cell culture we have demonstrated that the cell lines are sufficiently stable to be expanded up to manufacturing processes at 2000L bioreactor scale. We are currently testing the performance of these cell lines at 200L stirred tank bioreactor scale.

P437

A novel non-viral, non-integrative nano-DNA vector system for T-cell engineering

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Adoptive immunotherapy based on the functional introduction of Chimeric Antigen Receptors (CARs) into naïve Human T Cells represent a promising avenue for the treatment of cancer. Currently, the genetic engineering of these cells is achieved through the use of integrating vector systems such as lentiviruses and sleeping beauty transposons which present a potential risk of genotoxicity associated with their random genomic integration. We have invented a novel DNA Vector platform for the safe and efficient generation of genetically engineered T-Cells for Human Immunotherapy. This sysyem contains no viral components, and it replicates autonomously and extrachromosomally in the nuclei of human cells. These DNA Vectors offer several advantages over currently used vector systems; they are cheaper and easier to produce, and they can more quickly genetically modify human cells without the inherent risk of integrative mutagenesis. In preclinical studies, we have successfully generated genetically engineered human CD3+ cells with a range of transgenic CAR

receptors against several known cancer cell epitopes, demonstrating their viability and capability in the targeted killing of human cancer cells. We showed *in vitro* and *in vivo* that CART-Cells generated with our technology killed more efficiently target cells when compared to T-Cells engineered with the current state of art integrative lentivirus. We believe that this novel DNA Vector system provides a unique and innovative approach to this strategy for cancer therapy, and we estimate that this methodology will provide a simpler method of CAR T-cell manufacturing, resulting in a 10-fold reduction in the cost of the CART-product.

P438

Axonal transport of a modified AAV2 in the non-human primate brain

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Axonal transport of adeno-associated virus (AAV) is an essential feature of neurological gene therapy. We previously reported that a modified AAV serotype 2, incapable of binding to its receptor heparan sulfate proteoglycan (AAV2-HBKO), distributes more extensively after thalamic injection than parent AAV2. The present study characterized axonal transport of this AAV2 variant after putaminal injection in the non-human primate. AAV2-HBKO encoding green fluorescent protein (GFP) was coinfused into the putamen (1.2×10^13 vector genomes mL^-1) with an (MR) imaging contrast reagent by convection-enhanced delivery. The efficient dispersion of infusate throughout the target region was confirmed by real-time MR monitoring. Infusions of AAV2-HBKO resulted in widespread central nervous system transduction in the cortex, deep cerebellar nuclei, and several subcortical regions. GFP expression was found in substantia nigra pars compacta neurons that project to putamen, indicative of retrograde transport of AAV2-HBKO. Meanwhile, GFP-positive fibers found in the substantia nigra pars reticulata, a region that receives projections from putamen, indicated anterograde transport of AAV2-HBKO. A separate group of animals received a 10-fold lower dose of the vector in the left hemisphere compared with the contralateral hemisphere. By means of GFP immunohistochemistry, we observed a titer-dependent effect on axonal transport directionality where the high dose resulted in an enhanced expression of transgene to anterograde-linked rather than to retrograde-linked structures. These data advance our understanding of AAV2-HBKO distribution in the primate brain and support its use in the treatment of neurological disease with a substantial cortico-striatal pathology.

P439

Tolerogenic nanoparticles enhance transgene expression and intracellular vector copy number after initial and repeated administrations of AAV-based gene therapy vectors through immunological and non-immunological mechanisms

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Achieving clinically active and stable expression of therapeutic transgenes is a key challenge for gene therapy. In particular, transgene expression following liver-directed systemic delivery of AAV vectors in paediatric patients is expected to wane over time and the formation of neutralizing antibodies prevents AAV re-administration. It has been previously shown that tolerogenic nanoparticles encapsulating rapamycin (SVP-Rapamycin) have the ability to block the humoral and cellular responses against the capsid, thereby enabling repeat administration of AAV vectors. Here we further demonstrate that co-administration of SVP-Rapamycin with AAV-based vectors also enhances transgene expression after the first dose of AAV vector in naïve mice. This first dose enhancement of transgene expression is immediate, dose-dependent and not mouse strain-specific; it requires direct interaction of SVP-Rapamycin particles with AAV virions and is characterized by increased vector copy number in liver cells. Moreover this activity appears to be independent of rapamycin's immunomodulatory effects on adaptive immunity, as it is also observed in beta 2-microglobulin- and Rag2-deficient mice. Our data suggests that SVP-Rapamycin enhances autophagy *in vivo*, which has been reported to increase AAV transduction and transgene expression (Hősel et al. Hepatology, 2017). This two-pronged mechanism of SVP-Rapamycin action makes it an attractive candidate to enhance systemic gene therapeutic applications, particularly in those clinical indications where repeat vector dosing may be necessary.

P440

Comparable efficiency of photoreceptor transduction by AAV2tYF, AAV5 and AAV8 in non-human primates

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AAV gene therapy vectors are currently in Phase I/II clinical development for treatment of patients with X-linked retinitis pigmentosa (XLRP). This study compared the photoreceptor transduction efficiency of subretinally delivered AAV2tYF, AAV5 and AAV8 capsids in a head-to-head non-human primate (NHP) experiment, using a GFP expression cassette under the control of the photoreceptor-specific GRK1 promoter. Cynomolgus macaques received a bilateral subretinal injection of 0.1 mL AAV vector (animals received a different vector in each eye) at a concentration of 4.5E+11 vg/mL and were followed for 13 weeks. Safety parameters included ocular exams, clinical observations, clinical pathology, and anatomic histopathology. GFP expression was evaluated by quantitative analysis of fundus fluorescence, and by qRT-PCR and immunohistochemistry (IHC) (post-mortem). A direct comparison between AAV2tYF-GRK1-GFP ($n=12$), AAV5-GRK1-GFP ($n=4$) and AAV8-GRK1-GFP ($n=8$) revealed that the AAV2tYF capsid showed a statistically higher GFP fluorescence intensity in fundus images relative to both AAV5 and AAV8. By qRT-PCR, AAV2tYF ($n=3$) showed equivalent GFP mRNA expression in the retina compared to AAV8 ($n=3$). By IHC, AAV2tYF ($n=9$) showed equivalent GFP protein expression in photoreceptor cells compared to AAV5 ($n=4$) and AAV 8 ($n=5$). In conclusion, AAV2tYF was comparable to or superior to both AAV5 or AAV8 in transduction of photoreceptors in NHPs when delivered subretinally. AAV2tYF represents an attractive therapeutic choice for human XLRP gene therapy.

P441

Application of a synthetic adeno-associated viral vector for the delivery of anti-hepatitis B virus gene therapeutics

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Chronic hepatitis B virus (HBV) infection remains a global health problem with over 800 000 annual deaths reported globally. Inadequacy of current anti-HBV drugs prompted efforts to develop a more potent therapy for chronic carriers of HBV. RNA interference (RNAi) based gene silencing and gene editing tools are amongst the most promising anti-HBV gene therapeutics. Lack of safer and efficient delivery systems of these nucleic acid sequences hampers their progress into advanced stages of clinical trials. Naturally occurring serotypes of adeno-associated viral vectors (AAV) have shown an impressive safety profiles and efficiency in transducing multiple cell types. However, pre-existing immunity against these vectors limits their application in the treatment of chronic diseases such as chronic hepatitis B. Recently, a synthetic AAV (Anc80L65) was designed and shown to transduce a variety of tissues including the liver. The suitability of this vector for anti-HBV gene delivery has never been explored. In this study, Anc80L65 vectors carrying anti-HBV RNAi activator or transcription activator-like effector nuclease (TALEN) were designed and characterised *in vivo* and/or *in vitro*. Anc80L65 expressing an RNAi activator or a TALEN transduced liver derived cells and effected significant HBV replication inhibition. A single dose of RNAi activating Anc80L65 resulted in a sustained HBV gene expression inhibition over the four months period in HBV transgenic mice. Most importantly, anti-HBV Anc80L65 did not result in obvious liver toxicity in mice. These observations demonstrate that Anc80L65 is a promising tool for anti-HBV gene therapy development.

P442

Novel insights into wild-type and recombinant AAV mitochondrial integration

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Previous studies showed that recombinant (r)AAV-1 integrates within human and murine skeletal muscle upon intramuscular administration. To further characterize AAV mitochondrial integration, we investigated wild-type (wt) and rAAV-2 integration profiles in mitochondria-rich cell types by LAM-PCR. Sequences obtained from infected human immortalized cardiomyocytes were aligned to the mitochondrial genome yielding 5,496 sequences corresponding to 55 and 21 wt- and rAAV-2 mitochondrial integration sites (IS), respectively. Remarkably, IS located within the ND2 and ND5/ND6 mitochondrial genes were identified for both wt- and rAAV-2, these genes correspond to the OR4F29 and PCBD2 genes located within nuclear mitochondrial DNA segments (NUMTs) which were previously described as mitochondria-related integration hotspots. Human primary skeletal muscle cells yielded similar results with 61 and 14 mitochondrial IS (130 and 726 sequences) upon wt- and rAAV-2 infection, respectively. Current efforts focus in defining the relationship between AAV mitochondrial integration frequency and the cellular mitochondrial content. Mitochondria-associated membranes (MAM) connect the endoplasmatic reticulum and the mitochondria and we currently investigate these contact sites as players in rAAV mitochondrial trafficking. To determine whether modified numbers of MAMs correlate with divergent rAAV mitochondrial integration frequencies, HEK293T cells overexpressing proteins known to alter the number of MAM (CypD, Grp-75, MFN-2) were infected with rAAV2 for subsequent LAM-PCR. Preliminary data show increased numbers of mitochondrial IS in cells overexpressing MAM-related proteins when compared to the negative controls, further confirmation and characterization experiments are still ongoing. Therefore, our data suggest that the mitochondrial genome should be considered when evaluating rAAV targeting mitochondria-rich tissues.

P443

Reconfiguration of AAV Rep-Cap coding sequences significantly increases viral vector yield and enables inducible AAV production in HEK293 cells

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AAV vectors are demonstrating significant clinical benefit in patients for a range of disease indications, however the ability to produce large quantities of functional AAV particles in a scalable manufacturing system remains challenging. The majority of current AAV production systems exploit the native Rep-Cap expression configuration found in the viral genome. Here, we re-designed the wild-type configuration of the AAV coding sequences using heterologous promoters and a range of alternative DNA elements. Interestingly, configuration of the Cap and Rep genes in the reverse order to their normal position using a CMV driven internal ribosome entry site (IRES) system improved AAV particle yields by upto 6-fold, as well as significantly increasing the full:empty capsid ratio. This core optimised design also enabled inducible AAV production in HEK293 cells, something previously unachievable due to the transactivating effect of adenoviral components present in HEK293 cells on the native Rep-Cap promoters. We demonstrate the effectiveness of this system in both adherent, and suspension serum-free, HEK293 cells. Yield and quality may be further enhanced by addition of an over-expressed form of the AAV assembly-activating protein (AAP) cassette to the re-configured plasmid. Furthermore, this work underpins the basis for generating inducible stable packaging and producer cell lines.

P444

Evaluating central nervous system promoters using double strand adeno-associated virus 6 triple mutants (T492V-Y705F-Y731F) virus

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Adeno-associated virus (AAV) is a versatile gene therapy tool that can transduce many cell types found in the central nervous system (CNS). Two approaches have been developed to increase transgene expression delivery by AAV: (1) self-complementary (scAAV) also known as double-stranded (dsAAV) and (2) viral capsid mutants. dsAAV provide higher efficiency transgene expression by circumventing the single to double DNA conversion. AAV capsid mutations of the tyrosine or threonine external residues provide higher transgene expression by inhibiting the capsid phosphorylation and preventing virus degradation. Current study evaluated the tropism of pseudotyped dsAAV using a previously described AAV6 triple mutant capsid (T492V/ /Y705F/ Y731F) [AAV6-M3] in combination with dsAAV having CNS specific promoters, mouse minute virus intron (MVMi), humanized green fluorescent protein (hGFP), and the bovine growth hormone poly A signal (BHG pA). Promoters used: (1) CBA – target all cells, (2) CamKII, MAP2, and SYN – neurons, (3) GFAP104 and BLBP – astrocytes, (4) MBP and MOG – oligodendrocytes, and (5) CD68, F4/80, and CD33 – microglias. Several primary murine CNS derived 2D and 3D culture were used to validate the CNS cell specific transgene expression of new dsAAV6-M3 viruses. Primary mix neuroglia was used as the 2D culture system. Organotypic brain slice and spinal cord slice were used as 3D cultures system. Virus transduction efficacy in culture system was evaluated by direct observation of hGFP. The new dsAAV6-M3 CNS specific were injected intracerebroventricular of neonatal mouse (p0 injection) and transgene expression evaluated after 15 days by immunohistochemistry.

P445

New lentiviral pseudotypes based on murine syncytin-A or -B are efficient tools for the transduction of murine and human B cells

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Recombinant lentiviral vectors derived from HIV-1 (rHIV) enable stable gene transfer in multiple cell types. The cellular tropism of rHIV is conferred by its envelope glycoprotein pseudotype enabling cell entry via receptor-mediated recognition and membrane fusion. We explored the use of syncytins as rHIV pseudotype. Syncytins are fusogenic placental glycoproteins derived from endogenous retroviruses. Syncytins mediate several fusogenic biological processes including the development of syncytiotrophoblast and the generation of skeletal myofibers. These self-proteins also possess an immunosuppressive domain. So far, attempts to use syncytins as retroviral pseudotypes have been unsuccessful. For the first time, we report the possibility of generating infectious rHIV vectors pseudotyped with murine syncytin-A or -B. These lentiviral particles are poorly infectious for most cell types *in vitro* except for murine or human CD19+ B lymphocytes. High levels of transduction are achieved *in vitro* in the A20 murine B lymphoma cell line in the presence of Vectofusin-1® (88 ± 10% transgene+ cells). Transductions of both human (64 ± 5%) and murine (89 ± 4%) primary CD19+ B cells are obtained without pre-activation. In murine bone marrow, vectors transduce B cell precursors including pro-B, pre-B, immature B cells with increasing intensity throughout differentiation up to mature B cells. Evaluations *in vivo* are ongoing in mice following intravenous administration of a syncytin-A Luc2 rHIV vector to track transduced cells in bone marrow and lymphoid organs. This is the first report that syncytins can efficiently pseudotype rHIV and interact with B cells. Ongoing studies explore entry mechanisms and possible therapeutic applications of these pseudotypes.

P446

Recruitment of MRN complex to the ITRs limits permissivity of the cells to scAAV transduction

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MRN (Mre11, Rad50, Nbs1) protein complex is a major factor responsible for sensing and resolution of DNA double-strand breaks in proliferating cells. Although indispensable for DNA repair machinery, MRN can negatively influence permissivity of the cells to transduction by adeno-associated viral (AAV) vectors. It was previously shown that Mre11 binds to the inverted terminal repeat (ITR) region of single-stranded AAV genomes and may interfere with the synthesis of the second strand of viral DNA. Interestingly, there is limited evidence that MRN influences also the transduction efficiency of self-complementary (scAAV) vectors, pointing out different aspects of ITR-MRN interaction. To investigate the mechanism of scAAV transduction we used 3T3-L1 MBX fibroblasts since they lose Mre11 expression during their differentiation to adipocytes. Even though only in differentiated cells the transgene was efficiently expressed, viral genomes were maintained for a relatively long time (over a month) in the nuclei of undifferentiated fibroblasts exposed to scAAV. Moreover, transgene expression from such genomes was rescued during the differentiation process, what was associated with a rapid decrease of Mre11 level. A similar effect was observed after Mre11 silencing by shRNA in scAAV-transduced 3T3-L1 MBX cells. Additionally, our coimmunoprecipitation analysis revealed that Mre11 directly interacts with the ITR of scAAV. Our findings underline the importance of MRN action in scAAV transduction and processing of viral DNA. Currently, our attempts to improve the efficiency of scAAV transduction are focused on the construction of the modified vectors with a lowered affinity for Mre11 binding.

P447

Insertion of a liver-specific promoter in an SV40 gene delivery vector results in improved expression of therapeutic genes in hepatoma cell lines

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SV40 vectors have been reported to efficiently transduce liver, without inducing neutralizing antibodies to the vector capsid. The latter would allow effective re-administration rendering this non-integrating vector a potential candidate to treat disorders that require treatment early after birth. In all *in vivo* studies expression of the encoded gene was controlled by the

endogenous SV40 early promoter. This ubiquitous promoter does not prevent expression in other tissues, including antigen presenting cells, which may induce an immune response towards the transgene and possibly prevents long-term correction. A tissue-specific promoter could overcome such an unwanted response. The luciferase expression of the liver specific SV40HLP-Luc was compared to SV40-Luc in human hepatoma cell lines, HepG2 and Huh7. In both cell lines, luciferase expression upon transduction by SV40HLP-Luc was much higher than the expression seen upon transduction with SV40-Luc. In non-hepatoma cell lines, HEK293T, CV-1 and COS-1, no effect of the presence of the HLP promoter was seen. Since these results indicate insertion of a tissue-specific promoter does provide hepatocyte specific expression, an SV40 carrying the human UGT1A1 gene controlled by the HLP promoter was generated. Transduction of this SV40HLP-UGT1A1 in human hepatoma cell lines also resulted in an increased expression of the therapeutic protein compared to the SV40-UGT1A1 vector, confirming tissue-specific expression using SV40 vector is feasible. This is the first study to show that the specificity of SV40 vectors can be improved by inserting a tissue-specific promoter. *In vivo* testing in a relevant animal model is performed to demonstrate its potential applicability.

P448

The influence of AAV shuffled library platform design on the outcome of directed evolution

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The power of AAV Directed Evolution to identify novel vector variants with improved properties has been well established, as evident by high-impact publications reporting on numerous synthetic AAV variants developed over the last decade. In this study we compared two previously published Directed Evolution platforms and a novel recently developed selection platform, hereafter referred to as Platforms V1, V2 and V3, for screening of capsid libraries generated using DNA family shuffling. Platforms V1 and V2 were developed in the laboratory of Prof Mark Kay and are based on replication-competent design, leading to selections of capsid variants able to enter target cells and replicate in the presence of Adenovirus. Platform V1 contains modifications flanking the cap gene, introduced to create unique cloning sites facilitating convenient insertion of capsid libraries into the recipient construct, but impairing expression of Rep/Cap proteins. Reversion of these modifications to an AAV2 wild-type-like conformation in Platform V2 restored wild-type levels of Rep/Cap expression. Importantly, both libraries are not designed to select variants based on transgene expression, which remains the primary objective of subsequent applications involving selected variants. Instead libraries based on Platforms V1 and V2 require co-infection with Adenovirus and may bias selection in favour of efficient replication, rather than functional transduction. To address this limitation, we have designed a novel AAV selection platform technology permitting evaluation based on transgene expression (Platform V3). We will report results of a direct comparison between the three platforms analysing the selection process of two libraries on human liver cells.

P449

Post-transcriptional fine-tuning of AAV vector gene expression for hemophilia A gene therapy

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As partners in the European research network SMART-HaemoCare, we develop an innovative hemophilia A gene therapy by targeting natural anti-coagulants using single-domain antibody fragments (nanobodies) expressed from liver-specific recombinant (r)AAV vectors. To hone target selectivity, we exploit tissue- or cell-specific expression patterns of mi(cro)-RNAs. Incorporation of miRNA binding sites in the 3′UTR of a transgene can induce mRNA destabilization, decreasing transgene expression in a cell-/tissue-specific manner (switch-OFF strategy). Moreover, miRNAs can be used to downregulate bacterial repressor proteins that bind to an operator sequence in a promoter, activating transgene expression in the targeted tissue (switch-ON strategy). Here, we present a toolbox that significantly extends these previous systems by combining OFF- and ON-switches in a single rAAV. We first validated the switch-OFF system in mice by injecting rAAV8 vectors carrying a luciferase reporter tagged with different permutations of up to 10 multiplexed miRNA binding sites. We then applied this strategy to hemophilia A treatment by tagging an anti-antithrombin nanobody with a combination of miRNA binding sites to silence off-target expression in numerous cells/tissues including skeletal muscle and heart. In parallel, we implemented the switch-ON system by comparing different repressor systems *in vitro*. We chose to further optimize the vanillic acid-responsive transcriptional repressor VanR and tagged it with binding sites for the liver-specific miR-122, to restrict transgene expression to liver cells. The robust and customizable switch-OFF/ON rAAV system developed here will be pivotal not only for hemophilia A treatment, but for numerous other human diseases requiring efficient and selective on-target gene expression

P450

Deciphering the role of hepatic cell microRNAome during AAV infection

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Cellular microRNAs (miRNA) are known to modulate the life-cycle of different viruses that infect the liver such as Hepatitis C Virus. Surprisingly, no data exists on AAV- induced changes to the cellular microRNAome in hepatic cells, in particular. The study of hepatocyte specific barriers to successful virus transduction is an important pre-requisite to develop AAV based vectors with improved transduction and lower immunogenicity. We reasoned that inducible miRNA in response to an active AAV infection may regulate immediate and long-lived cellular responses necessary for the cell's own survival as well as its ability to control several aspects of viral life cycle. To

study this, we performed a global small RNA sequencing analysis in AAV serotype 2 infected hepatic cell line model. This screen identified 7 differentially expressed miRNAs, in AAV2 infected HuH-7 cells. An enrichment and pathway analysis of these miRNAs predicted its possible effects on gene targets involved in multiple biological processes including transcriptional regulation and post-translational modifications. Our data showed that 5 out of 7 miRNAs had an impact on multiple pathways such as the amino sugar /nucleotide sugar metabolism and glycan biosynthesis. Based on these findings, we have further validated the role of some of these novel pathways/targets on two important aspects of AAV life-cycle- its transduction and packaging ability. Further studies to delineate the specific biological functions of the many hepatic miRNAs identified in our study here, and their interactions with AAV2 is likely to be rewarding to improve its hepatic gene transfer efficiency.

P451

Challenges and successes in high concentration AAV drug product formulation development

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As the gene therapy field matures, development scientists are increasingly facing manufacturing and ''drug-ability'' challenges common to traditional biopharmaceutical manufacturers. As the development of therapies progress, careful consideration should be given to the final form provided for both pre-clinical and clinical studies. All biological drug products, including vector-based therapies, require attention to formulation, final drug product container closure presentation, and stability at intended storage conditions as well as those required for manufacturing and administration. Gene therapy drug products, however, face additional challenges versus typical biological products. Often, the availability of reliable and validated assays is limited, and those available may have high variability, be non-quantitative, or have long lead times. Adding to the complexity is the nature of the gene therapy vectors themselves. They have limited stability in the liquid state and a high propensity for large-scale aggregation at low concentration. In addition, they are often delivered directly to treatment areas; which requires excipients and formulation parameters be compatible with tissue function, microenvironment, and volume restrictions. This work describes the challenges faced in developing a successful AAV-vector mediated gene therapy drug product formulation. Using current tools and *in vivo* restrictions, we have developed a stable high concentration AAV vector formulation for clinical use. This work demonstrates the importance of investing early time and effort in pre-clinical development on formulation and drug product design to ensure an optimized clinical presentation and ease of manufacturing.

P452

Adenoviral-mediated lactoferrin gene therapy for abscesses of the maxillofacial area in rat model

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Pyo-inflammatory diseases of maxillofacial area is often complicated by a broad range of pathological conditions because of ineffective antimicrobial therapy. One of the promising innovative approaches includes gene therapy employing recombinant genes encoding bacteriocidal or bacteriostatic factors. This study investigates the efficiency of *in vivo* gene therapy using adenoviral-mediated lactoferrin gene delivery in rat with phlegmon of maxillofacial area. The original model of maxillofacial area phlegmon was employed. After incision and drainage of mature abscess, all animals received antibacterial therapy with Ceftriaxone intramuscularly (1000mg/kg/day). Rats in gene therapy-treated (GT) group were injected with recombinant adenovirus Ad5 carrying Lactoferrin gene subcutaneously at five points around the abscess. Evaluation of gene therapy efficacy was based on survival, blood testing and histological analysis of immune system. During the experiment control rats were euthanized at 4-5 days. In GT-group the wound was closed by secondary intention and recovered rats were sacrificed at 7-8 days for histological examination. Blood analysis of control rats revealed leukocytosis due to pronounced monocytosis against lymphocytopenia. In GT-group the tendency to leukocytopenia with less pronounced lymphocytopenia was observed. Histological examination of thymus and spleen did not show pathologic changes in both groups. Meanwhile, the acute purulent lymphadenitis was documented in regional lymph nodes of control rats and preserved morphology of lymph nodes in GT-group. Thus, our results suggest that Ad5 carrying Lactoferrin gene may transduce cells around phlegmon and overexpress bacteriostatic molecules enhancing the action of antibiotic therapy.

P453

Development of AAV full/empty capsid ratio analysis using semi-automated transmission electron microscopy

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Adeno-associated virus (AAV) has gained increasing interest because of its potential as an efficient and safe gene therapy vector. To ensure quality of product for clinical and pre-clinical use, continuous development of the manufacturing processes and characterization of AAV is needed. One key parameter of AAV products to be determined is the ratio of full and empty vectors. This characterization is currently done by analytical ultracentrifugation, traditional or cryo transmission electron microscopy (TEM), comparing genome copy number with particle titre or chromatographic separation, all which can be laborious and/or affected by various buffer components and host cell derived contaminants. Faster analytics are needed for on-line determinations, enabling measurements even on the same day independent of sample type. For this purpose we have started the development of a highly automated TEM assay for rapid AAV full/empty ratio evaluation. By using the highly automated MiniTEM system we have imaged recombinant AAV produced with and without transgene as well as their mixtures. The analysis of full/empty ratio is done manually and automatically, and

the accuracy of automatic measurements is determined. This project is on-going and aims to achieve a fast method for approximation of transgene packaging success in AAV production.

P454

Comprehensive comparative evaluation of the qualitative attributes of AAV5 batches produced in mammalian and insect cells

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Scalable and robust production of clinical grade AAV material is an integral component en route to delivering successful AAV based gene therapies to patients. To date, two production platforms have emerged as the predominant modi operandi to deliver research and clinical grade rAAV material. One approach relies on the chemical transient transfection of Hek293 cells to deliver relevant genetic components while in the other Baculovirus expression vectors deliver the AAV genetic material to a suspension culture of invertebrate cells. Because of the fundamental differences between the two producer systems one can posit plausible differences in AAV quality and performance. We hypothesize that there is no difference in the quality and potency of the produced rAAV between the two platforms. Three rAAV5 batches were produced per production platform and purified with an identical downstream process. The rAAV material was not only compared quantitatively and qualitatively but also on their comparative performance in *in vitro* transduction and *in vivo* infection experiments. Productivity of rAAV5 per cell proved to be similar between the two platforms while the *in vitro* and *in vivo* efficacy of the recombinant material demonstrated that the optimized insect cell based rAAV5 material had a slight performance lead over the Hek293t produced rAAV5. We conclude that the biochemical, molecular and biological quality attributes of the AAV produced in the insect cells are comparable in most and surpassing in some assays to mammalian cell produced rAAV5. Moreover, insect cell based rAAV production is significantly more scalable at lower cost in comparison.

P455

Protein transduction of genome editing enzymes with new lentivirus vector derived particles

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Genome editing enables a plethora of applications that can benefit the fields of gene and cellular therapies. Enzymes used for genome editing range from natural or engineered endonucleases and recombinases to the CRISPR/Cas system. These enzymes are generally delivered into target cells by expression from viral vectors or through transfection of plasmid DNA, mRNA or ribonucleoprotein complexes. However, transfection is not always possible or as efficient as transduction with viral vectors, but integrating nuclease or recombinase genes into the genome by chance is also not desirable. We have previously described lentivirus vector (LV) packaging plasmids that enable the incorporation of foreign proteins into vector particles. These particles can be used for simultaneous protein transduction and transgene expression, and proven to be useful in delivering small dimeric endonucleases and transposases into cells. Here we describe new versions of the packaging plasmid that were designed to incorporate larger proteins into LVs than previously tested. Another objective was to package proteins that are encoded from genes with repetitive elements, because such sequences interfere with reverse transcription and are not well tolerated as transgenes in LVs. To test the latter objective, the red fluorescent tandem dimer (Td)Tomato protein was cloned into the new packaging plasmids and used in LV production. TdTomato became correctly processed and incorporated into LV particles, and was functional upon transduction. Subsequent experiments involved the packaging of enzymes relevant for genome editing, and the most recent results of these studies will be presented.

P456

Efficient pseudotyping of different retroviral vectors using a novel, codon-optimised gene for chimaeric GALV envelope

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The Gibbon Ape Leukaemia Virus envelope protein (GALVenv) has been shown to mediate efficient transduction of human cells, particularly primary T lymphocytes, and is therefore of great interest in gene therapy. Since the original glycoprotein did not efficiently pseudotype lentiviral vectors, chimaeric GALVenv proteins using internal domains from murine leukaemia viruses (MLV) were derived and shown to readily incorporate into lentiviral particles (e.g. GALV-C4070A). We developed a codon-optimised (co) gene variant of GALV-C4070A, to investigate whether i.) the expression efficiency can be improved and ii.) the potentially higher expression level leads to increased vector titres. We found that coGALVenv mediated efficient pseudotyping not only of γ-retroviral and lentiviral vectors, but also α-retroviral vectors. The titres obtained were equal to those with the classical GALVenv, as determined on HEK293T cells. Notably, the required plasmid amounts for transient vector production in HEK293T cells were for all vector types 20 to 30 times lower than with the commonly used GALVenv plasmid. Finally, we tested coGALVenv-pseudotyped vectors on K562, but also primary human T cells. For all three vector types, transduction rates were identical to those with the standard GALVenv. In conclusion, we propose that the novel codon-optimised coGALVenv gene will be very useful for the efficient production of high-titre vector preparations, e.g. to equip human T cells with novel specificities using transgenic TCRs or CARs. Especially in large-scale vector productions using transient transfection the considerably lower amount of plasmid needed may represent a significant cost advantage.

P457

Lentiviral/retroviral vector large scale manufacturing

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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 (LVV), produced with transient quadri-transfection in 293T cell line and retroviral vectors (RVV), 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 two different systems with cells in adhesion using Pall iCELLis® fixed-bed disposable bioreactors and with suspension cells in a culture system without animal derived reagents. The optimized parameters included seeding cell density, DNA concentration, transfection agents, timing and volumes of harvest. Final vectors were characterized for infectious viral titre (TU/mL), particle content (ng p24 or ng p30), process related impurities and then compared to vectors produced by current GMP processes based on Cell Factories (CFs). Downstream process was appropriately scaled up and designed in order to accommodate larger volumes of vectors from bioreactor. 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. A full scale run (200L) in Pall iCELLis® bioreactor system has been already performed and results will presented.

P458

Human codon-optimization of GaLV-pseudotyped semi-retroviral replicating vector system improved genomic stability and anti-cancer activity

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The spRRV system is composed of two trans-complementing replication-defective retroviral vectors, sRRVgp and spRRVe, encoding MuLV gag-pol genes and GaLV env gene, respectively. In this study, we introduced two suicide genes, yeast cytosine deaminase gene (sRRVgp-sEF1α-CD) and HSV-1 thymidine kinase gene (spRRVe-sEF1α-TK), into the spRRV for double-suicide gene therapy. We have optimized the spRRV system for minimizing non-homologous recombination during viral vector spread by codon-optimization of the yeast CD and the HSV-1 TK genes for the genetic stability of the virus and the CD activity in transduced tumor cells. The spRRV systems encoding human-codon optimized therapeutic genes were tested genetic stability and therapeutic activity through prodrug (5-fluorocytosine & ganciclovir, 5-FC & GCV) susceptibility. We report here the selection of one optimized combination vector system, sRRVgp-sEF1α-CD8/spRRVe-sEF1α-TK encoding an optimized CD and wild-type HSV-1 TK genes, shows increased prodrug-mediated therapeutic effect and markedly improved genetic stability. We also examined the potential for mutual enhancement of TK/GCV and CD8/5-FC systems *in vivo* under our conditions. The results demonstrated that the intratumoral injection of sRRVgp/spRRVe particles encoding suicide genes into pre-established U-87 MG glioblastomas resulted in, if three outlier mice harboring secondary tumors formed by early metastatic process or from small numbers of tumor cells injected accidentally at nearby primary tumor site were discarded from analysis, a 100% survival of treatment group over a follow-up period that lasts longer than 150 days, while all mice in control groups were expired between 30 ~ 45 days after tumor injection.

P459

Improving viral and non-viral vectors: adopting exosome biology for efficiency and hydrogels for controlled release

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Viral vectors are the tools of choice for the delivery of transgenes but for a variety of reasons also non-viral vectors and delivery systems receive more and more attention. Lentiviruses (LV) and Adeno-associated virus (AAV) are frequently used because of their long-lasting, stable transgene expression and capability to transduce non-dividing cells. Most human cell types and tissues secrete small from intraluminal endosome-derived membranes (exosomes) with sizes of appr. 30-120 nm in diameter. Here, we demonstrate that enhanced levels of the exosomes-enriched tetraspanin CD9 led to increased levels of secreted exosomes in the respective vector producing cell lines and in turn to both a drastic increase of lentiviral infectivity and a boost of exosome associated AAVs (exo-AAV) production. The resulting viral vectors acted superior to standard vectors for cell and gene therapy in terms of transferring genetic material to many target cells. A broader range of therapeutic cargos, including synthetic RNA or linear DNA cassettes can efficiently and cell type specifically be delivered in engineered virus-like particles of the human polyoma JC virus (VLP). These non-viral carrier complexes have successfully been used *in vitro* and *in vivo*, e.g. for systemic RNAi against endogenous targets. In addition, we successfully tested chitosan hydrogels for a continuous release of the different vectors and observed efficient transduction with LV, AAV and VLP. Finally, the utilization of injectable hydrogel as carrier matrix for VLPs resulted in drastically increased efficiency in systemic siRNA delivery and extended target gene silencing of >75% for at least 14 days.

P460

Generation and characterisation of cGMP-compliant suspension HEK293-TetR cell line for adenoviral vaccine manufacture

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Large scale manufacture of Adenoviral vectors remains a challenge for the clinical development of vaccine candidates in which the transgene exhibits cellular toxicity. Here, Oxford Genetics, working in collaboration with Vaccitech, have developed a cGMP-compliant cell line stably expressing the Tetracycline repressor (TetR) gene, based on a fully-suspension, animal component free process. Characterisation of the cell line (and additional clonal derivatives) using a variety of assays demonstrated the ability to efficiently repress expression of Tet-regulated

reporter molecules, support Adenoviral transduction and to facilitate preferential replication of 'toxic' human or simian adenoviral vectors. The cell line has now been expanded for MCB generation and full characterisation in preparation for cGMP-viral vector manufacture

P461

AAV vectors are going viral in gene and cell therapy

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Although gene and cell therapy are promising tools for the treatment of genetic diseases, progress has been slow in developing effective clinical approaches. The issue lies in the difficulty to develop safe and efficient gene-delivery systems. Amongst other applications, plasmid DNA is often used as starting material in the GMP-compliant production of recombinant viruses, antibodies and RNA. Therefore, it also represents a solid start for adeno-associated virus (AAV) vector manufacturing, where these vectors are the active pharmaceutical ingredients (API) used in clinical trials. There are several opportunities to produce efficient and pure AAV vectors. For example, the choice of the serotype and of the applied plasmid system is important. Replacing the plasmid system by minicircle DNA allows to reduce contaminations and to increase transduction efficiencies of scAAV preparations significantly (Fig. 1). We will give an overview regarding the first important steps of AAV vector manufacturing.

P462

Analysis of the impact of NAbs on the transduction efficiency of the synthetic hepatotropic AAV-Anc80 vector in non-human primates

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In AAV-based gene therapy, the presence of circulating neutralizing antibodies (NABs) against AAV vector is a major challenge for achieving a successful transduction. Patients can present with NABs due to naturally acquired infections with wild type AAV or after a previous treatment with AAV vector. New serotypes are on development with the goal of avoiding pre-existing immunity while maintaining or improving transduction efficacy. Recently, Zinn et al have reconstructed in silico a putative ancestral virus that is highly effective at delivering gene therapies to different organs and that has the potential to overcome pre-existing immunity. In the present study, we have developed a highly sensitive assay to measure NAbs titers; we have subsequently assessed AAV-Anc80 vector transduction efficiency and biodistribution in NHP and evaluated the effect of the presence of NAbs on liver transduction. Male and female NHP received a single intravenous dose of AAV-Anc80 expressing human secretable Alkaline Phosphatase (hSEAP) under the control of a liver specific promoter. Long term SEAP expression was detected in the majority of the animals. Biodistribution studies showed a remarkable hepatotropism of AAV-Anc80 vector with a very reduced presence in other tissues. We also found that low NABs titers can significantly reduce AAV-Anc80 transduction in NHP andwe also detect a rapid dissapearence of the vector from circulation in the presence of NABs. In conclusion, AAV-Anc80 vector represent a highly promising vector for the treatment of hepatic disorders, however, strategies to circumvent the effect of Nabs effect should applied in seropositive patients.

P463

Generation of helper virus-free adeno-associated viral vector packaging/producer cell lines based on a human suspension cell line

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The emerging number of clinical trials in the gene therapy field poses the challenge to the industry to produce viral vectors in a scalable, reproducible and cost-efficient manner. In particular due to moving away from mainly targeting ultra-rare diseases and taking more common indications into focus, significant improvements concerning productivity and consistent quality of AAV vector production are needed in order to ensure supply. CEVEC has developed a helper virus-free packaging cell line that can easily be turned into a producer cell line by only one additional step of cell line development. The basis of the packaging cell line are CAP-GT cells which grow to high density in serum free conditions and which enable reproducible and high titre production of viral vectors. These cells were genetically modified to stably express Rep proteins under a Tet-inducible promoter. Extensive screening of Rep expressing single cell clones resulted in clonal cell lines, which produced high AAV titres upon induction and transfection of the missing components. In a next step, the adenoviral helper functions E2A, E4orf6 and VA RNA were introduced. Finally, capsid function and GFP as transgene flanked by the ITRs were integrated resulting in a proof of principle producer cell line. This approach enables a consistent quality production of AAV vectors that abolishes the drawbacks of transient transfection concerning reproducibility, consistency and high costs for GMP-grade DNA.

P464

Relative infectivity as a reliable method for quantifying differences in the infectivity of AAV vectors *in vitro*

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The absolute quantitation of infectious titer by limiting dilution endpoint analysis (also known as TCID50 infectious titer assay) has been the standard method for measuring the infectivity of AAV vector preparations *in vitro*. While the TCID50 infectious titer assay has been useful for confirming that AAV vector preparations are infectious, its large assay variability (up to 200% geometric coefficient of variation) limits its applicability for supporting product conformity, comparability, and stability. We have developed an *in vitro* relative infectivity

method capable of detecting and quantifying small differences in the infectivity of AAV vectors. The method involves infection of adherent cells grown in 96-well plates with a dilution series of the AAV vector test sample in parallel with a dilution series of a AAV vector reference standard. Following the infection period, the cells are washed, collected, and subjected to ddPCR to quantify the vector DNA present in the cells. The amount of vector DNA recovered at each dilution are used to calculate the infectivity of the sample relative to the reference standard. We demonstrate the application of this method for comparing the infectivity of AAV vectors across different batches, as well as for quantifying changes in infectivity due to degradation. The method is easily adapted to compare the ability of AAV vectors to infect different human cells, assess improvements in infectivity for engineered AAV capsid variants, probe AAV infection kinetics, or assess the activity of different AAV capsids across different projects as a platform method to support process and formulation development.

P465

Anti-tumor activity of GM-CSF and IL-12 expressing oncolytic HSV-1

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Oncolytic herpes simplex virus type 1(oHSV-1) is suited for cancer treatment owing to capability of large therapeutic gene insertion and the ability of infiltration into the tumor. We generated ΔICP6 oHSV by deleting the UL39 (ICP6) gene which is a large subunit of ribonucleotide reductase, plays a role in maintaining dTTP pools in infected cells by converting ribonucleoside diphosphates to the corresponding deoxyribonucleotides. To confirm the deleted boundaries, the genotype of ΔICP6 was confirmed by PCR, restriction enzyme digestion. We conducted MTT assay and oncolytic assay to evaluate cancer cell killing efficacy. ΔICP6 oHSV showed a similar cancer cell killing ability compare with the wild-type HSV-1 (Wt-HSV). However, in the multi-step progeny virus growth assay, ΔICP6 oHSV was attenuated by 10- 10000-fold viral yield compared with Wt-HSV control virus in normal cells. To induce the more effective anti-tumor immune response, we inserted murine GM-CSF or IL-12 into the ΔICP6 oHSV and then measured tumor growth in B16F10 melanoma tumor model. We observed ΔICP6-GM-IL12 expressing GM-CSF and mIL-12 simultaneously diminished tumor growth more efficiently compared to the activity of ΔICP6-GM or ΔICP6-IL12 expressing singe cytokine, respectively.

P466

Generation of cGMP-compliant stable packaging and producer cell lines for inducible lentiviral vector production

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Lentiviral-mediated gene transfer remains the method of choice for stable, long-term genetic manipulation of a range of cell types, as exemplified by ex-vivo modification of T-cells for CAR-T therapy. Despite recent positive clinical data in this field, broader adoption of these technologies is limited due to cost and lack of reproducibility associated with fully transient plasmid transfection methods currently employed for vector production. Here, we have generated a stable packaging cell line producing high-titre (mid-10^6 TU/mL) lentiviral vector (LV) with single plasmid transfection. LV production from the packaging cell line is tightly controlled by Oxford Genetics' proprietary Tet inducible system and no LV is produced in absence of inducer. Process development with this cell line, using the Sartorius AMBR platform, is currently underway to further optimise production parameters, including media, feeding strategies, induction and transfection conditions. The packaging cell line, derived from Oxford Genetics' cGMP suspension HEK293 cell bank using a fully-defined, animal component free process, retains stability without antibiotic selection and offers direct scalability for LV manufacture. cGMP-compliant LV producer cell lines have also been developed from the packaging cell line and characterisation is in process.

P467

Production of Caltech AAV capsids with BEVS: challenges and solutions

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Recombinant Adeno Associated Virus (AAV) is a growing vector of choice for gene therapy, and clinical trials are ongoing for multiple diseases. The lack of efficient and cost-effective methods of production was a bottleneck, until a baculovirus /Sf9 cells expression system was developed for AAV. The baculovirus/S9 system is fully scalable, enabling the production of AAV from 25ml flask up to 10,000L with very good reproducibility and at high quality. Nevertheless, the widely used bacmid-based Baculovirus Expression Vector System (BEVS) is not optimized for AAV production. Aberrant proteolytic degradation of viral proteins in the bacmid-based BEVS is an emerging and unexpected issue, precluding the large-scale production of uniformly uncleaved AAV capsids using the baculovirus/Sf9 system. We have identified the source and pathway of this protein degradation and have developed a system which eliminates degradation during production of AAV capsids as well as capsid variants in the baculovirus/Sf9 system. This new system enables not only the mitigation of proteolytic degradation but increases yield thereby providing a wider harvest window to support large-scale AAV manufacturing for clinical trials.

P468

While, no barrier insulator function is required for stable transgene expression from Sleeping Beauty transposon, 6CTCF enhancer blocker improves its biosafety for therapeutical applications

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The currently used Sleeping Beauty (SB) transposon-based non-viral gene delivery vectors support stable expression, are relatively safe, as they provide no alternative transcription signals, and exhibit an almost unbiased integration profile. Here, we aimed at optimizing the therapeutic vector for efficient transgene expression and further improve biosafety. We asked if insulators in the context of SB would be beneficial to ensure long-term transgene expression in retinoid pigment epithelium (RPE) cells. First, we set experiments to clarify whether transgene silencing is an issue using the SB vector in RPE cells. We also investigated the potential genotoxicity of SB-mediated integrations in genic regions of cultured RPE cells (ARPE-19), and asked if using insulators would improve the biosafety features of SB. Finally, we investigated if incorporating the 6CTCF into the SB vector would affect transgene expression. Our results suggest that SB requires no insulators to support long-term transgene expression. Regarding genotoxity of genic integration sites, we found that while antisense intronal integrations are potentially genotoxic, the sense intronal integrations, even if they initiate robust downstream transcription, are mostly filtered out at translation level. Incorporating enhancer-blocker insulator into the 6CTCF insulators could improve the biosafety feature of the SB vector. The 6CTCF motif flanking the therapeutic gene provides perfect shielding, but negatively interferes with transgene expression in bulk. Nevertheless, using selection, stable, insulated clones can be established that support stable, high transgene expression from low copy number integrations.

P469

Development of a plasmid construct encoding HGF and VEGF165 for gene therapy

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One of promising areas of regenerative medicine is ''therapeutic angiogenesis'' - gene delivery *in vivo* to express angiogenic growth factors and treat cardiovascular disease. Earlier we have 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 was administered alone. This time we set out to develop a single-molecule plasmid encoding two growth factors as a potential new gene therapy drug. For co-expression of two growth factor's genes, we developed six different plasmid constructs carrying internal ribosome entry sites (IRES) of the encephalomyocarditis virus (EMCV) or eukaryotic proteins: Bip, FGF1 and eIF4G. To evaluate the production of VEGF165 and HGF, HEK293 cells were transfected and concentrations of VEGF165 and HGF were evaluated by ELISA. The biological activity was evaluated using HUVEC cells in tube-formation assay. Only two plasmids: HGF-IRES_EMCV-VEGFopt and HGF-IRES_Bip-VEGFopt provided synthesis of sufficiently high concentrations of the target proteins that caused formation of capillary *in vitro*. Vectors were injected to mice for *ex vivo* analysis of VEGF165 and HGF production by explanted muscles yet trace production of VEGF165 was detected. Further work will focus on design of new plasmids with two different or bi-directional promoters for each growth factor. Currently, there are no drugs based on bicistronic vectors available on the market that urges us to complete the initial goal. The research was carried out within the state assignment of Lomonosov MSU

P470

Inclusion of HR arms into LVs improves rDNA-directed integration with an IN-I-PpoIN119A fusion protein

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Potential adverse effects related to random transgene integration raise questions about the safety of lentivirus vector (LV) mediated gene therapy. These concerns could be alleviated by targeting integration to genomic safe harbors (GSHs), positions in the genome where transgenes could be inserted without compromising the expression of endogenous genes. We have been studying the 28S ribosomal RNA gene as a GSH candidate. The gene contains a recognition sequence for the I-PpoI endonuclease which we joined to the viral integrase (IN) protein for integration targeting purposes. A major advantage of this construct is that it can be packaged into the same vector particle as the transgene. Instead of the wild-type I-PpoI, we used the inactivated I-PpoIN119A as it allows for integration targeting without adversely affecting the transduced cells. In our previous integration site analysis study, the IN-I-PpoIN119A fusion protein was found to target ribosomal DNA with an efficiency of 2.7%. We now tested the effect of target site sequence homology on integration targeting. A 500 bp homologous region (HR) matching the sequence flanking the I-PpoI recognition site in the 28S rRNA gene was added to either side of the transgene. The HR was found to enhance both the efficiency of transgene integration and the efficiency of integration targeting into the 28S rRNA gene when using IN-I-PpoIN119A LVs. Control LVs with a wild-type IN did not target integration into the 28S rRNA gene. The overall efficiency of integration targeting and the ratio of homologous recombination to non-homologous end-joining will be discussed.

P471

Large-scale optimisation and production of rAAV vector encoding VEGF in iCELLis bioreactors

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Large-scale production of recombinant Adeno-Associated Virus (rAAV) vectors remains a critical step to achieve in the gene therapy field. In this work, we addressed large-scale manufacturing

of rAAV encoding Vascular Endothelial Growth Factor in iCELLis 500/333m2 fixed-bed bioreactor in GMP-like conditions. We were able to produce a total of 5 × 10^16 viral genome (vg) by transfection of mammalian cells and purify the recombinant vector. In parallel to the large-scale development, we have optimised the plasmids used for rAAV production and compared the viral vectors produced using iCELLis Nano bioreactors. With optimised plasmids, we were able to enhance the rAAV vg titers obtained in the cell culture. In parallel, we reduced contaminating empty capsids levels and contaminating intra-capsid DNA enhancing chromatography process along with final product quality.

P472

UPV: production of customized high quality viral vectors at a 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 it has been dedicated to the design, production and purification of more than 1200 viral vectors for basic research and gene therapy pre-clinical studies for both public and private laboratories. Key products and services: - AAV vectors: Concentrations starting at 2e12vg/mL. Titres higher than 1e13vg/mL are frequently obtained. - AAV pseudotypes: AAV1, AAV2, AAV5, AAV6, AAV8, AAV9, AAVrh10. Contact us to request information of other pseudotypes. - Adenovirus vectors: Concentrations starting at 1e12pp/mL. Serotypes: Ad5, Ad5/40, Ad5/52, CAV2. - 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. Funding: UPV work is developed in the context of TECNIO (TECDTP18-1-0006) and ADVANCE(CAT) (COMRDI-15-1-0013-16) with the support of ACCIÓ (Generalitat de Catalunya) and the European Regional Development Fund 2014-2020. JP is partially supported by the PTA programme (#09357) of the ''Ministerio de Economía y Competitividad''.

P473

Comparison of recombinant AAV vector yield using transient transfection of producer cells with 2- and 3-plasmid systems

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Transient production of recombinant AAV vector requires transfection of a producer cell line with plasmids containing AAV rep/cap, the helper virus genes and the recombinant transfer vector. Each element can be provided on a separate plasmid (3-plasmid system), or rep/cap can be cloned in tandem with the helper virus genes in a single plasmid (2-plasmid system). The 2-plasmid system can reduce cost of vector production by reducing plasmid production costs. However, the larger size of the combined rep/cap and helper gene plasmid used in the 2-plasmid system could potentially reduce transfection efficiency of producer cells, reducing yield compared to the 3-plasmid system. We compared the 2-plasmid system of recombinant AAV vector production to the 3-plasmid system to determine if there is a negative effect on yield that negates the benefit of reduced plasmid production cost.

P474

Variability assessment of vector genome titer for AAV vectors: implications for future development

ABSTRACT WITHDRAWN

P475

Minicircle DNA as starting material for development of ATMPs

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Minicircle DNA is gaining increasing importance for clinical research applications in gene therapy and genetic vaccination. For direct gene transfer into human, GMP DNA is mandatory whereas for GMP production of e.g. mRNA or viral vectors, in many cases High Quality Grade (HQ) DNA is accepted as a starting material. If the therapeutically active substance is a genetically modified cell, e.g. for an Advanced Therapy Medicinal Product (ATMP), the situation is currently controversially discussed, here the DNA may either serve as a starting material or be part of the drug substance. Based on results clearly showing the advantages of minicircle DNA in direct comparison to the corresponding plasmid, minicircle DNA shows a great benefit on the one hand avoiding the presence of unnecessary bacterial backbone sequences, on the other hand due to higher transfection efficiencies. Hence, these constructs provide a striking benefit especially for production of AAV vectors and CAR-T cells and both are currently the most promising tools moving forward to clinical applications. Consequently, in close collaboration with the leading experts in the fields of AAV and CAR-T cell-based therapies, we are developing a process for the production of High Quality Grade minicircle DNA, meeting the requirements of most regulating agencies.

P476

Construction of plasmid vectors encoding pro-angiogenic factors for gene therapy using picornoviral structural elements

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Selection of optimal vector for delivery of various therapeutic genes is an important question in gene therapy. Plasmids seem to

be one of attractive vectors due to their cost-effectiveness, simplicity and low immunogenicity. In our study we used clinically approved plasmid vector pVax1 (Thermo Scientific) for construction of monocistronic recombinant plasmids pVax1-VEGF-P2A-RFP, pVax1-FGF2-P2A-RFP and pVax1-VEGF-P2A-FGF2-P2A-RFP which contain picornoviral ''self-cleaving'' 2A-peptide sequences, separating codon-optimized sequences of human vascular endothelial growth factor 165 (VEGF), basic fibroblast growth factor (FGF2), and red fluorescent protein (RFP) in one open reading frame. Vector design allows equimolar expression of all encoded cDNAs in single cell which might be important for synergetic effect of combinations of growth factors. To confirm functional activity of generated plasmids HEK293 cell line was transfected and evaluated by fluorescent microscopy after 24 hours. Immunofluorescent assay with specific antibodies against VEGF and FGF2 demonstrated expression of recombinant proteins in transfected cells. ELISA assay confirmed secretion of target recombinant proteins into culture medium of transfected cells. Secretion levels of target proteins were significantly higher in supernatants of genetically modified cells in comparison with untransfected cells and cells transfected with pVax1-DsRed. Thereby generated recombinant plasmids provide expression of therapeutic proteins and could become an attractive therapeutic system for regenerative medicine applications which require stimulation of angiogenesis and/or neuroregeneration.

P477

iVector, a core facility for bioproduction of viral vectors (lentivirus, AAV & CAV-2) used in neurosciences research at the Brain and Spine Intitute.

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Lentiviral vectors, Adeno-associated virus vectors (AAV9, AAV2retro, AAV9-PHPeB & PHP-S serotypes) and Canine Adenovirus (CAV-2) vectors are all particularly promising tools for *in vivo* research mainly on brain and central nervous system. The Vectorology core facility produces batches of highly concentrated viral gene transfer vectors of controlled quality under different scales in order to meet the needs for any *in vitro* and *in vivo* applications. iVector implements continuously in his services portfolio gene transfer in innovative technologies (ie: CRISPR, Split-Cas9, split-reporters, …) to allow new approaches in basic research and lead to new applications. We have developed a large collection of viral expression backbones ready to accept any gene of interest to provide the best solution for every *in vitro* or *in vivo* application encompassing fundamental research, cell engineering, gene therapy, cellular therapy, CAR-T and vaccine. Our laboratory BSL2 and BSL3 biocontained suites allow us to satisfy every demand of viral production whatever the transgene type (unknown function, toxic, oncogenic …). Our viral vectors tools are intensively used for: Cell and tissue transductions (Brain, CNS, …); *In vivo* viral transgenesis (mouse and other animal models); Primary cells immortalization; Stem cells, iPS transduction; Knock-in, knock-out, cell labeling, gene silencing, CRISPR; Constitutive and inducible expression, cell or tissue specific expression. iVector is part of the ICM core facility network which facilitates any translational research projects.

P478

uniQure down stream purification process shows excellent viral clearance capabilities

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Prior to conducting clinical trials, viral clearance studies must be performed to ensure the downstream purification process is capable of removing endogenous and potential adventitious viruses from the product stream. uniQure's platform downstream purification process includes four orthogonal viral reduction steps i.e. viral inactivation (VI), affinity chromatography (AC), ion-exchange (IEX) chromatography and nanofiltration (35nm). uniQure rAAV are produced by means of baculovirus expression vector system (BEVS), which uses baculovirus as delivery vehicles. These vectors must be removed from the process stream prior to generation of a drug substance. In this presentation, we demonstrate significant removal of baculovirus (> 16 logs) and other model viruses, which shows excellent capabilities of uniQure's DSP platform to effectively remove varaiety of viruses with different physico – chemical properties.

P479

Addressing large-scale manufacturing of clinical grade viral vectors using an optimized PEI-based transfection process

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With the progress in developing new viral vector systems guided by safety, specificity and potency considerations, several gene and cell-based therapies are now more than ever closer to being clinically approved and commercially available to treat genetic diseases. Viral vector delivery systems, of which mainly adeno-associated viruses (AAV) and lentiviruses are produced by transient transfection of mammalian producer HEK-293 cell lines. Virus vector production using the right transient transfection method is crucial to provide the flexibility and reproducibility that is needed to scale-up from initial process development to manufacturing of high-quality grade viral vectors. Here, we describe an optimized PEI-based virus production process for high-yielding viral vector production, compatible with different cell culture adherent and suspension systems. We further demonstrate the robust viral vector production yields, as well as the adaptability and reliability of the PEI-based transient gene expression approach to efficiently manufacture GMP-grade viral vectors at a sufficiently large scale for more advanced clinical trials, and in fine to drive commercialization of therapeutic vectors.

P480

No evidence of ectopic bone formation in soft tissues of rats injected with plasmid DNA encoding human VEGF165 and BMP2 cDNA

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Ectopic bone formation is a process of manifestation of bone tissue outside their usual origins, for example in soft tissues such as muscle. It is a pathological process, which is thought to be caused by local inflammation or hereditary diseases such as fibrodysplasia ossificans progressiva. This disease is a result of a mutation in ACVR1 gene that causes upregulation of bone morphogenetic proteins (BMP). It was previously reported that transfection with plasmid DNA (pDNA) containing mouse BMP4 cDNA can induce *in vivo* ectopic bone formation in mouse skeletal muscles. Previously we developed pDNA encoding human BMP2 and vascular endothelial growth factor 165 (VEGF165) for osteoregeneration applications. The purpose of this work was to evaluate safety of such pDNA for soft tissue injections. We injected pDNA expressing VEGF165 and BMP2 into anterior bulbous muscle, sciatic nerve and spinal cord of rats at 200μg/10μl. In control group, 0,9% NaCl was injected. The histology of anterior bulbous muscle, sciatic nerve and spinal cord of rats 4 weeks postoperatively showed absence of new bone formation in control and experimental groups at the place of injections. Thus, injecting pDNA expressing human VEGF165 and BMP2 into soft tissue of rats did not led to the formation of ectopic bone.

P481

A novel suspension-based lentiviral production platform to achieve cost-effective clinical manufacturing

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Lentiviral vectors have gained increasing momentum as the preferred genetic delivery vehicle for cell and gene therapy applications. Manufacturing lentivirus for advanced therapeutics has long centered around the transient transfection of adherent cultures for vector production, but these methods invariably suffer from scale-up, labor, cost and consistency challenges. To circumvent these issues, a comprehensive Design of Experiment methodology was used to identify key components enabling lentiviral yield improvements to be made. This presentation will provide an overview of a new serum-free lentiviral production system comprised of a robust suspension HEK293 cell line, a chemically-defined culture medium, a PEI-alternative transfection reagent, and a novel enhancer. In addition, we will provide a cost analysis of the different lentiviral production systems available to determine the most economical option.

P482

Analytical characterization of plasmid DNA for viral vector

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Recent years have seen huge growth in the use of plasmid DNA as a starting/critical raw material for the production of viral vectors for cell and gene therapy. The recent success of these therapeutic products creates new manufacturing and regulatory challenges, with viral vector products requiring multiple (3 or 4) plasmids for the production of each vector. Plasmid DNA is classified as a ''starting/critical raw material'' when used in these applications under EU clinical guidelines. As such, less stringent requirements apply than those for a drug substance. In this context, literature is lacking of consensus on the quality panel required to release the plasmid produced under the so-called High-Quality standard. We recently implement a new ISO9001:2015 compliant High- Quality manufacturing strategy. Briefly it consists in E.coli banking, fermentation (up to 50L), harvest, cell lysis, purification, TFF and fill and finishing Here we will describe the analytical panel for both banking and product steps, including methods and limits. In conclusion, we proposed a strategy to produce and characterized plasmid according to the EMA guidelines for the highest quality standards, and in a way very similar to in GMP (SOP, traceability /documentation, pharma grade and animal free reagents, IQ/OQ on all instruments etc.) the only differences are: - the product is not release as a drug substance by the QP. - it is manufacture in a non-classified areas as of reagents requirements.

P483

Exploiting adenovirus mechanisms for the enhanced production of AAV vectors

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Adeno-associated virus (AAV) has shown great promise as a vector for gene therapy. However, current manufacturing strategies are unable to scale to support the considerable demands for rAAV vectors for clinical and pre-clinical applications. Here we describe a new strategy for the manufacture of AAV using Adenoviral vectors based on the modulation of the major late promoter (MLP). We show that by strategic insertion of tetracycline repressor binding sites into specific loci of the MLP and encoding the tetracycline repressor under transcriptional control of the virus MLP, we enabled doxycycline-dependent controlled expression of Adenoviral structural proteins, with enhanced adenoviral DNA amplification in cells and reduction of adenovirus contamination by up to $\sim$2.5 million-fold. Using this negative feedback self-repression system, we demonstrated delivery of adenoviral 'helper' functions and AAV DNA to yield >4-fold increases in AAV production without any adenovirus or small drug contamination. Moreover, by infecting AAV packaging cells this novel approach allows for a scalable, single agent, rAAV manufacture system that is free of plasmid DNA and contaminants, with considerable advantages over standard production approaches.

P484

Identification of new muscle-tropic adeno-associated virus (AAV) capsids for treatment of rare hereditary muscular disorders

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AAV vectors are very promising tools for therapeutic gene delivery, especially owing to the amenability of their capsid for engineering and repurposing. Our aim is to create specific and efficient AAV capsids that mediate gene delivery in the heart, skeletal muscle and diaphragm, which are affected by rare

hereditary muscular disorders. To this end, we created two AAV libraries composed of serotypes AAV1/6/8/9 or AAV1/6/8/9/po.1 through DNA family shuffling. These libraries were subsequently cycled multiple times in C57BL/6 mice, by generating secondary libraries from enriched viral genomes that were rescued from the on-target tissues. We noticed a striking enrichment of AAV9 sequences in the C terminus of the vast majority of clones from both libraries. DNA/RNA barcoding followed by next-generation sequencing (NGS) was used to validate the efficiency and specificity of 33 preselected candidates. AAV9 was included as golden standard next to a peptide-modified capsid that has recently been identified as very potent in another screen by our group. Notably, we thereby identified four new shuffled AAV capsids with a markedly improved tropism in muscle tissues. Their further modification by peptide insertion and subsequent NGS-based analysis of *in vivo* biodistribution covering all major organs yielded two lead candidates. Additional quantification of reporter gene expression validated their superiority in terms of robustness and specificity of expression in the on-target muscle tissues. These two unique AAV capsids will now be harnessed within the EU consortium MYOCURE and combined with muscle-specific promoters prior to further validations in small or large preclinical animal models.

P485

Tandem promoter design confers tolerogenic and persistent transgene expression to AAV gene therapy in neonate Pompe mice

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Hepatocyte-restricted, AAV-mediated gene transfer is being used to provide sustained, tolerogenic transgene expression in gene therapy. However, given the episomal status of the AAV genome, this approach cannot be applied to pediatric disorders where hepatocyte proliferation may result in significant loss of therapeutic efficacy over time. In addition, many multi-systemic diseases require widespread expression of the therapeutic transgene that when provided with ubiquitous or tissue-specific non-hepatic promoters often results in anti-transgene immunity. Here we have developed tandem promoter monocistronic expression cassettes that, packaged in AAV, provide combined hepatic and extra-hepatic tissue-specific transgene expression and hepatic-mediated transgene immune tolerance. We validated our approach in infantile Pompe disease, a prototype disease caused by the lack the ubiquitous enzyme acid-alpha-glucosidase (GAA), presenting multi-systemic manifestations and detrimental anti-GAA immunity. We showed that systemic AAV gene therapy in immunocompetent Gaa-/- mice using the tandem promoter design confers sustained immune tolerance to the GAA transgene. Combined with the persistent transgene expression in non-dividing extra-hepatic tissues, this resulted in whole-body therapeutic efficacy upon vector delivery in neonate Pompe mice. In conclusion, tandem promoter design provides tolerogenic, long-term transgene expression following AAV vector-mediated gene transfer which can be beneficial when treating multi-systemic pediatric conditions.

P486

A novel genomics-based platform for the creation of synthetic promoters for cell and gene therapy

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The wealth of genomic data generated by high-throughput sequencing methods (RNA-Seq, ChIP-Seq, CAGE, etc.) makes possible to mine the genome for gene regulatory elements that can be used to design synthetic promoters. These synthetic promoters can be tuned to deliver exquisite expression control of the GOI in combination with the gene therapy vector, thereby increasing the safety profile of the therapeutic product. We have developed a gene promoter design platform that integrates data from large-scale functional genomics datasets and employs machine learning algorithms in data analysis to identify functional gene regulatory elements. These regulatory elements are then ranked according to many distinct criteria and subsequently used as component parts in synthetic promoter construction, using engineering biology principles. Here we introduce the platform and present results detailing how we have employed it to create liver and muscle selective promoters and to generate novel inducible promoters responsive to different chemical and biological stimuli, whose activity is stimulated solely with the addition of an inducer, without the requirement for the co-expression of a trans-activator. We present data showing their activities, highlighting the tightness of gene regulation that they mediate and showing how multiple layers of regulation (transcription, splicing, translation, etc.) can potentially be combined to create expression cassettes optimised for different therapeutic applications. In summary, we have developed a novel genomics-based platform that enables the rational design, synthesis and testing of mammalian gene regulatory elements, and whose output can help support the construction of the next generation of cell and gene therapeutics.

P487

FVIII expression driven by its native promoter allowed phenotypic correction in hemophilic mice

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A possible strategy to overcome inhibitors formation in haemophilia is represented by the restriction of FVIII expression in specific and tolerogenic cell type, preferably the natural FVIII-secreting cells. Thus, we studied the F8 promoter (pF8) sequence to drive transgene expression in LV to verify the feasibility of expressing FVIII under its natural promoter for gene therapy approaches. Analysis of FANTOM5 collection of human libraries was performed using the Zenbu browser genomic tool and the software PROMO 3.0 used to predict transcriptional factor (TF) consensus sequences on pF8. FANTOM5 data confirmed the usage of previously described F8 TSS, which promotes the transcription of the annotated reference sequence (NM_000132). In silico analysis of TF consensus sequences predicted the presence of several myeloid-specific TF, in addition to hepatocytes- and endothelial-specific TF. After

LV.pF8.GFP administration *in vivo*, GFP expression was mainly restricted to liver sinusoidal endothelial (LSEC) in the liver, while in spleen and bone marrow GFP was mainly expressed in myeloid cells. Injection of LV.pF8.hFVIII in hemophilic mice resulted in therapeutic FVIII activity levels (up to 12%) without inhibitors up to 1 year after LV administration and using more active forms of FVIII cDNA we improved FVIII activity levels up to 25%. LV pF8.hFVIII injection in previously FVIII-immunized HA mice resulted in therapeutic FVIII levels and reversion of inhibitor titers, while Treg depletion resulted in inhibitors formation. Our data suggest that using the pF8 FVIII is mainly secreted by LSEC and Tregs are involved in tolerance induction to FVIII after gene transfer.

P488

MicroRNAs, overlooked contaminants of AAV vector batches

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Adeno-associated viruses (AAV) are excellent vehicles for targeted gene delivery to a variety of cells and tissues. More than one hundred clinical trials are ongoing with AAV vectors and two products have been commercialized. Concurrent with this success, regulatory agencies have enhanced the quality control requirements to ensure the efficacy and safety of these novel drugs. Among process-related impurities, undesirable DNA sequences have already been shown to be internalized into AAV particles during the production. Curiously, among nucleic acid contaminants, the presence of small (cell-derived) RNAs, such as micro-RNAs, has never been investigated. Here, we have quantified a set of miRNAs by RT-qPCR in AAV2 and 8 vector lots produced in HEK293 or Sf9 cells. Our data demonstrated that miRNA are co-purified with AAV vectors in a proportion that is correlated with their abundance in the producer cell line. In particular, miR-19b, an oncogenic miR, and miR-30c, known to suppress cell apoptosis, are among the most represented miRNAs found in HEK293-derived vectors. The miRNAs profile is independent of the serotype and of the type of AAV particles, i.e. empty versus full. However, the amount of miRNAs varies depending on the purification process (CsCl gradient density or immunoaffinity column), which suggests that these miRNAs are external to capsids. Although further investigations are required, our study raises the concern of inadvertently co-delivering small RNAs with AAV vectors and consequently modifying cellular pathways and/or lower transduction efficiency, if these RNA are detected by the host cell sensors.

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Cyclosporine H overcomes IFITM3-mediated innate immune restriction to lentiviral transduction and gene editing in human haematopoietic stem cells

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Innate immune factors may restrict haematopoietic stem cell (HSC) genetic engineering and contribute to broad individual variability in gene therapy outcomes but specific effectors have not yet been identified. We show here that HSC harbor an early, constitutively active, innate immune block to lentiviral transduction that can be efficiently overcome by cyclosporine H (CsH). CsH increased LV gene transfer up to one log in cord-blood (CB)-derived CD34+, rendering them almost as permissive as the highly transducible 293T cell line. In a more clinically relevant setting, CsH enhanced transduction more potently than any other compound described thus far, yielding significantly higher gene marking in long-term repopulating HSC and deriving progeny *in vivo* with one single LV dose as compared to the current standard protocol consisting of two subsequent rounds of transduction. Because CsH enhances also integrase-defective (ID)LV transduction, it enhanced donor DNA insertion by homology-directed recombination by two-fold achieving a compelling 20% of edited long-term repopulating HSC *in vivo*. Underlying these effects is the CsH-mediated inhibition of interferon-induced transmembrane protein 3 (IFITM3), which potently restricts VSV glycoprotein-mediated vector entry into HSC. Importantly, individual variability in endogenous IFITM3 levels correlated with HSC permissiveness to transduction, suggesting that CsH treatment will enhance gene transfer and editing as well as abrogate donor variability in *ex vivo* gene therapy. Overall, our work unravels the involvement of innate pathogen recognition molecules in immune blocks to gene correction in primary human HSC and highlights means to overcome them for the purpose of innovative cell and gene therapies.

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DNA family shuffling has no detrimental impact on the functionality of the assembly-activating protein (AAP) from AAV

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Currently, increasing efforts in the AAV field focus on improving our understanding of the biology and role of the assembly-activating protein AAP in the AAV life cycle. Recently, we have verified the requirement of AAP for capsid stabilization and particle assembly for 12 different AAV serotypes, and have observed a high degree of interchangeability of AAPs to rescue AAP knockouts in most of these serotypes. Here, we additionally asked whether AAP functionality is perturbed during DNA family shuffling of the AAV cap gene, i.e., a predominant method for AAV capsid evolution. This concern was implied by the overlap of the cap and AAP open reading frames in the AAV genome, which inherently results in AAP chimerism from cap shuffling. To this end, we performed a set of complementary experiments in which we (i) assessed the ability of

AAP from chimeric AAV-DJ to rescue its parental serotypes AAV2, 8 and 9; (ii) determined the potential of 60 chimeric AAPs from five different libraries to rescue an AAV2 AAP knockout; (iii) tested whether excess AAP during recombinant vector production increases transduction efficiency; and (iv) studied the rescue potential of 12 chimeric AAPs isolated from an *in vivo* selected library. Together, our consistent findings that (i) all studied AAPs preserved their functionality although not all corresponding capsids were able to transduce the cell lines tested, and that (ii) AAP overexpression during production did not increase transduction efficiencies or library titers alleviate the long-standing concern that AAP chimerism is limiting AAV capsid shuffling technology.

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Combination of an engineered AAV vector Anc80 and tolerogenic nanoparticles encapsulating rapamycin enables efficient transgene expression in mice with pre-existing neutralizing antibodies and provides a therapeutic benefit in a mouse model of methylmalonic acidemia

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Two immunological barriers to gene therapy using adeno-associated virus (AAV)-based vectors stem from the high prevalence of pre-existing neutralizing antibodies (Nabs) in the human population and the inability to re-dose patients due to de novo formation of vector-induced Nabs. We have developed tolerogenic nanoparticles encapsulating rapamycin (SVP-Rapamycin), which if co-administered with AAV-based vectors provide a dose-dependent and long-term suppression of humoral and T cell responses against AAV and therefore allow for productive AAV vector re-dosing. Although SVP-Rapamycin benefit is limited in the presence of high titres of pre-existing antibodies, this impediment may be partially circumvented by utilizing Anc80, a rationally engineered AAV vector. Anc80 vector expressing secreted embryonic alkaline phosphatase (SEAP) combined with SVP-Rapamycin enabled efficient transgene expression in mice that were previously exposed to AAV8 and developed AAV8 Nabs. The level of SEAP expression was similar to that observed in naïve mice administered Anc80-SEAP vector alone. In addition, SVP-Rapamycin enhanced transgene expression when co-injected with an Anc80 vector in mice receiving passive transfer of sera from human donors with low titres of pre-existing antibodies to AAV. Moreover, co-administration of Anc80 and SVP-Rapamycin suppressed de novo generation of antibodies against Anc80 thus enabling its repeat administration to deliver reporter transgenes in wild type mice and the MUT gene in a mouse model of methylmalonic acidemia. The combination of SVP-Rapamycin and Anc80 is promising approach to mitigate the detrimental impact of both pre-existing and de novo formed Nabs on gene therapy.

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Liver-directed gene therapy in newborn mice with lentiviral vectors

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Liver-directed gene therapy with adeno-associated viral (AAV) vectors delivering a functional copy of a clotting factor gene into hepatocytes has shown successful results in adult patients 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. On the contrary, lentiviral vectors (LV) integrate into the target cell chromatin and are maintained as cells divide. We developed LV that achieve stable transgene expression in the liver of adult mice, dogs and non-human primates. We now set out to evaluate the fate of LV-modified liver cells during growth. We administer increasing doses of LV expressing marker genes (GFP or luciferase) under the control of a hepatocyte-specific expression cassette to newborn mice. Exploiting 3D imaging of cleared livers and bioluminescence, we show that transduced hepatocytes are maintained over time and proliferate locally. We unexpectedly observed an initial decrease in transgene-expressing hepatocytes around 3-4 weeks of age followed by stabilization and maintenance, suggesting different growth phases supported by different cell populations within the mouse liver. Preliminary data support *in vivo* LV transduction of bile duct cells and their capacity to generate LV-positive liver organoid cultures *in vitro*. Our work will inform about the extent and mechanism underlying long-term maintenance of LV-transduced hepatocytes in newborns, provide a rational for application of LV-mediated liver gene therapy to pediatric patients and will also address the role of different cell populations involved in post-natal liver growth.

Late-Breaking Abstracts

P219

AAV-mediated gene therapy to treat ocular failure in NCLs caused by transmembrane protein deficiencies

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The neuronal ceroid lipofuscinoses (NCLs) are inherited lysosomal storage disorders characterised by severe neurodegeneration, vision loss and premature death. At least 13 genes were linked to NCL with 5 genes encoding membrane-bound proteins. A major obstacle to developing gene therapies for NCLs - particularly for transmembrane protein deficiencies - is the challenge to deliver transgenes throughout the brain. In the past, we demonstrated that the photoreceptor degeneration in Cln6nclf mice, modelling a variant form of NCL deficient in the membrane-bound protein Cln6, can be prevented by AAV gene therapy. Based on these data, we investigated the ocular phenotype of two more NCLs caused by membrane-bound protein defects using Cln7 KO mice, mimicking another variant form of NCL, and Cln3Δex7-8 mice, modelling the most common transmembrane protein deficiency of NCL. We found that Cln7 KO mice showed significantly reduced electroretinography (ERG) a-waves and severe loss of photoreceptors. Subretinal delivery of AAV.CLN7 lead to significantly increased a-waves and a preserved photoreceptor layer in treated mutant eyes. In contrast, Cln3Δex7-8 mice presented with a mild but significant decrease in the ERG b-waves accompanied by a moderate loss of inner retinal cells. Intravitreal delivery of AAV.CLN3 resulted in significantly higher b-waves and preserved retinal morphology in treated eyes. We developed ocular gene therapies for three forms of NCL caused by three different gene defects, each requiring a specific gene therapy strategy. This work highlights the importance of thorough pre-clinical assessments of gene therapy approaches for the individual forms of NCL prior to clinical translation.

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Delivery of Cas/gRNA RNP using the Viromer platform

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1: Lipocalyx

Viromer® transfection reagents are polymer-based nanoparticles featuring a viral mechanism of membrane fusion. As a synthetic copy of the Influenza virus machinery, they do form transfection complexes with various types of nucleic acids (siRNA, miRNA, plasmid DNA or shRNA, mRNA) that cells take up via the endocytosis pathway, a process that involves the formation of an acidic compartment. The low pH in late endosomes acts as a chemical switch that renders the Viromer® surface hydrophobic and facilitates membrane crossing. Once into the cytosol, the Viromer® releases its payload for cellular action. This ''Active Endosome Escape'' technology is safe and maxi-mizes transfection efficiency as it is using a natural uptake pathway. We have adopted the platform for delivery of the Cas/gRNA ribonucleoproteins (RNPs). Candidate selection started from a library of 6.000 Viromer compounds using RFP expressing HEK293 cells. Validation and optimization were performed in A549, HEK293 and HeLa cells. Using the Viromer CRISPR system, we demonstrated >80% of RNP delivery in these cells lines resulting in up to 50–90% of targeted editing events (NHEJ mutations) with no sign of toxicity. This, together with the capacity of DNA co-delivering opens great perspectives for HDR-oriented editing and in vivo application.

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Using nanobodies to target AAV vectors to specific cells

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A limiting factor for the use of AAV as vectors in gene therapy is the broad tropism of AAV serotypes, i.e. the parallel infection of several cell types. Here we show that nanobodies - the robust single binding domains of llama heavy chain antibodies - can function as ligands to target AAV vectors to specific cells. We used two strategies to target AAV vectors to cells expressing the ectoenzymes CD38 or CD296 or the P2X7 ion channel: Firstly, we generated bi-specific adaptors by genetically fusing an AAV1/2 dual-specific nanobody to a membrane protein-specific nanobody via a flexible peptide linker. These bi-specific adaptors strongly and specifically enhanced the transduction of cells expressing CD38, CD296, or P2X7 by both, AAV1 and AAV2RA. (We obtained the AAV-specific nanobodies from our nanobody-transgenic mice, the membrane protein-specific nanobodies from immunized llamas). Secondly, we genetically inserted a CD38-, CD296-, or P2X7-specific nanobody into a surface loop of VP1 of AAV2RA, using a plasmid that prevents expression of nanobody-containing VP2 or VP3. Conversely, we generated plasmids that permit expression of normal VP2 and VP3 of AAV2RA, AAV8 or AAV9, but prevent expression of VP1. By co-transducing HEK-cells with pairs of these plasmids, we generated AAV2RA, AAV8, and AAV9 serotypes exposing 2-5 nanobodies via VP1 of AAV2RA. These chimeric AAV vectors strongly and specifically transduced cells expressing the respective target membrane protein. These proof of principle studies provide powerful new nanobody-based approaches to optimize the target specificity of AAV vectors for gene therapy.

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