methodology for verifying functional impact of exon skipping strategies using an *in vitro* model.

P090

Effects of Ad5-DYSF transduction on skeletal muscles of dysferlin-deficient mice

O N Chernova[1] A A Titova[1] M O Mavlikeev[1] I A Yakovlev[1 2 3] V V Solovyeva[1] I G Starostina[1] A K Zeynalova[1] M S Kaligin[1] A A Rizvanov[1] A P Kiyasov[1] R V Deev[2 3]

1: Kazan (Volga region) Federal University, Kazan, 420008, Russia 2: Human Stem Cell Institute, Moscow, 129110, Russia 3: Ryazan State Medical University named after academician I.P.Pavlov, Ryazan, 390026, Russia

Mutations in DYSF gene lead to myopathy with progressive loss of striated muscles. One of the promising ways of their treatment is gene therapy. The aim of our investigation is evaluation of recombinant adenovirus Ad5-DYSF impact on skeletal muscles structure in dysferlin-deficient mice. Retro-orbital injection of 100ul 6.5×10^8 PFU of Ad5-DYSF was performed in 15-months old dysferlin-deficient Bla/J mouse. Paraffin sections of calf muscles obtained 30 days after injection were stained with H&E, Mallory's trichrome, immunohistochemically with antibodies against α-SMA, myogenin, Ki-67. The intact Bla/J mouse of the same age was used as control. Mean muscle fiber (MF) cross-sectional area in experimental gastrocnemius muscle was increased ($747.5 \pm 408.6\ \mu m^2$ vs $515.3 \pm 300.8\ \mu m^2$ in control, p=0,000002) reflecting hypertrophy caused by adenoviral transduction. Percentage of myogenin-positive nuclei in experimental muscle was increased (28.3±6.5% vs 15.8±5.7% in control, p=0.000031), together with decreased percentage of Ki-67-positive myonuclei (20.0± 5.2% vs 27.5±6.7%, p=0.013) expressing higher differentiation rate and more accomplished rhabdomyogenesis after Ad5-DYSF injection. Percentage of necrotic MF in experimental sample was slightly increased (14.4±4.0% vs 11.6±2.9%, p=0.04), as well as percentage of centrinucleated MF (21.2±7.0% vs 18.9±5.6%, n.s.). Number of blood vessels per MF was similar in both groups (0.23±0.03 vs 0.23±0.06 in control, n.s.). Percentage of Ki-67-positive interstitial cells was increased after Ad5-DYSF injection (35.1±17.2% vs 8.57±18.3%, p=0.0001), whereas connective tissue ratio was lower (18.1± 4.9% vs 25.7±7.1%, p=0.04). Our preliminary results show that Ad5-DYSF adenoviral treatment elevates rhabdomyogenic activity *in vivo* and may be used as a potential gene therapy of dysferlinopathy

P091

Intracoronary allogeneic cardiac progenitor cells improve myocardial salvage and enhance functional recovery compared to placebo in porcine experimental infarction

C Báez-Díaz[1] F M Sánchez-Margallo[1] R Blázquez[1] V Blanco-Blázquez[4] V Álvarez[4] J L Abad[2] J G Casado[1] A Bernad[3] V Crisóstomo[1]

1: Jesús Usón Minimally Invasive Surgery Centre and CIBERCV (CB16/11/00494) 2: Coretherapix 3: Spanish National Center for Biotechnology (CNB-CSIC) 4: Minimally Invasive Surgery Centre Jesus Uson

Purpose Myocardial Salvage Index (MSI) is a measure of treatment efficacy, and it could decrease sample size when used as cardioprotection-endpoint in clinical trials. Intracoronary (IC) allogeneic cardiac progenitor cell (CPC) infusion has been recently used for myocardial infarction (MI) therapy. Our aim was to evaluate the effects of IC porcine CPC (pCPC) injection on myocardial edema and cardiac function after experimental MI in swine. Methods Pigs received an IC injection of 50x10^6 pCPC (n=7) or vehicle (n=7) one week post-MI. MR was performed pre- and 10 weeks post-injection calculating: Area-at-risk (AAR), Ejection Fraction (EF), End Diastolic Volume (EDV), End Systolic Volume (ESV), final infarct size (FIS) and MSI (MSI=(AAR-FIS)/AAR). Results No differences were seen between groups in any MR-derived parameter before injection. IC infusion was successful in all cases. A trend towards EF recovery was seen in pCPC-group (50±5%) but not in vehicle-group (42±10%). Ventricular volumes were significantly smaller in pCPC-group (EDVi: 94±11mL/m^2 versus 119±25mL/m^2 and ESVi: 47±8mL/m2 versus 70±26mL/m^2 (p=0.018). There were no differences in FIS, despite a trend towards smaller scars in pCPC-group (6%±4% versus 8%±3%). However, MSI was significantly higher in pCPC-group (0.63±0.17 versus 0.35±0.20, p=0.01). Conclusion The IC injection of 50×10^6 pCPC one week post-MI is associated to improved cardiac function at 10 weeks. The absence of differences in EF and FIS could be attributed to the small sample size, since MSI, a parameter known to be able to decrease the sample size needed for clinical trials, was increased almost two-fold in treated animals.

P092

The combination of cell and gene therapy as new generation tool to boost MSCs immunosuppressive and tissue regeneration properties

A Dorronsoro[1] R Sánchez[1] M Gómez[1] V G Martínez[3] C Trigueros[2] A Vicente[3] P Sepúlveda[1]

1: Instituto de Investigación Sanitaria La Fe 2: VIVEbiotech 3: Universidad Complutense de Madrid

Cell therapy has emerged during the last decade as a promising candidate for the treatment of autoimmune diseases and tissue regeneration. Mesenchymal stem cells (MSCs) have been tested in multiple preclinical models obtaining excellent results in tissue regeneration and autoimmune diseases. However, clinical results obtained in the majority of the clinical trials using MSCs aren't as good as expected and just 2 studies reported significantly positive results. Our proposal is to use cutting-edge hypothesis fusing gen and cell therapy to boost the therapeutic properties of MSCs. Our results indicate that modulating the expression of genes involved in signaling pathways responsible of the regenerative and immunosuppressive capacity of these cells we can increase their therapeutic features. MSCs over-expressing HIF showed increased tissue regeneration but we have been able now to show that their immusuppressive capacity is increased as well. In the past, we demonstrated that NF-kB pathway is a key regulator of immunosuppression on MSCs. In this piece of work we have been able to modulate the activity of this pathway to significantly increase the immunomodulatory capacity of this cells. Using genetically modified MSCs may decrease the biosafety and overcome the potential benefits. Thus, we isolated and tested the exosomes derived from the improved MSCs in order to investigate if the exosomes have therapeutic properties as well. Acknowledgement: PI16/00107, RD16/0011/0004