and injected with lipase inhibitor, further verifying that this genetic approach markedly diminished hepatic lipid synthesis. In BAT, Fgf21 transfer induced a "super-brown" phenotypic change, which was accompanied by elevated expression of a panel of vital genes for adaptive thermogenesis, such as Ucp1, Pgc1-alpha, Cidea, Dio2 and Elovl3. Collectively, our findings unveil that the triglyceride-lowering effect of cold exposure can be fully recapitulated by liver-specific delivery of the Fgf21 using a hydrodynamics-based procedure, indicating that this new approach may provide an alternative and more acceptable option for hyperlipidemia therapy in humans.

178. Early Treatment for Growth Hormone Deficiency Based on Naked DNA Administration in Dwarf Mice Allows Efficient Catch-Up Growth

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Growth hormone deficiency (GHD) is the major disease of the pituitary gland and is characterized by significant changes in body composition, bone density, lipid metabolism amongst others. Current treatment consists of daily administration of recombinant human growth hormone. As an alternative, our group investigates a gene therapy strategy for GHD based on the administration of a plasmid encoding the human growth hormone gene (hGH) under the control of ubiquitin C promoter, followed by electrotransfer. In order to get the maximize phenotype correction and to compare treatment of pubertal relative to adult mice, a bioassay was performed using 40 or 80 days old lit/scid mice, respectively. The mice were injected with 50mg of plasmid DNA in each side of non-exposed tibialis cranialis muscle, followed by electrotransfer at high and low voltage pulses.

At day 30, the treated groups showed a significant body weight variation of 3.64g/mice/day for pubertal mice, 2.07g/mice/day for the adult mice, and -0.01g/mice/day for the control group.

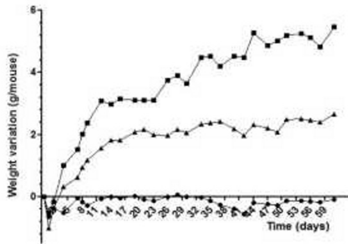

Fig. 1. Body weight variation in a 60-day bioassay of lit/scid mice with 40 (-■-) or 80 (-▲-) days of age, treated twice with 50 μg of hGH plasmid DNA injected into both tibialis cranialis muscles and of saline-treated lit/scid (-•-).

After 40 days, a second round of DNA administration was carried out, and as no significant body weight gain in the following days was observed, the assay was terminated at day 60. Remarkably, the parameters directly related to linear growth (table 1), like increases in nose-to-tail, tail and femur lengths, were 1.2 - 2.0 fold higher for pubertal mice as compared to the adult group, and treatment of pubertal mice resulted in a striking catch-up growth of 36 to 77%.

Table 1. Parameters directly related to longitudinal growth after 60 days of hGH-DNA administration in 40 days or 80 days old mice lit/scid mice

	n	Increase %	Catch-up (%)
Nose-to-tail length (cm)			
saline - 40 days	4	11.6	
hGH-DNA - 40 days	3	22.2	35.6
untreated scid - 40 days	4	9.0	
saline - 80 days	4	2.9	
hGH-DNA - 80 days	3	11.7	25.7
untreated scid - 80 days	5	11.2	
Tail lenght (cm)			
saline - 40 days	4	13.1	
hGH-DNA - 40 days	3	24.2	39.0
untreated scid - 40 days	4	5.8	
saline - 80 days	4	1.0	
hGH-DNA - 80 days	3	12.0	23.6
untreated scid - 80 days	5	11.3	
Femur lenght (mm)			
hGH-DNA - 40 days	3	18.7[1]	77.5
hGH-DNA - 80 days	4	16.1[1]	39.9

[1] Calculated with basis on saline-treated co-aged mice

Moreover mIGF-I, the main GH-dependent mediator of growth, was measured in plasma samples and the levels of mIGF-I in treated pubertal lit/scid mice were completely normalized 15 days post treatment and not significantly different to values for co-aged untreated non-dwarf mice, however the same did not occur to the adult lit/scid treated mice and their co-aged scid mice. These results are very promising and demonstrates the significance of early onset of gene therapy treatment in the lit/scid mouse model for phenotypic correction of GHD.

179. Correcting the Bleeding Phenotype in Hemophilia Ausing Lentivirally FVIII-Corrected Endothelial Cells Differentiated from Hemophilic Induced Pluripotent Stem Cell (iPSC)

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Background/Aim: Hemophilia A (HA) is a bleeding disorder caused by factor VIII (FVIII) gene mutations.Somatic cells can be reprogrammed to generate autologous, disease-free iPSCs, then differentiated into cell targetsrelevant for gene and cell therapy. Our aim is to develop a novel HA treatment strategy generating FVIII-corrected patient-specific iPSCs from peripheral blood cells anddifferentiating them into functional endothelial cells (ECs), secreting FVIII after transplantation.

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Methods:Mononuclear (MNC) and CD34+ cells were isolated from peripheral blood of healthy and hemophilic donors and reprogrammed with a Cre-excisable polycistronic LV carrying OCT4, SOX2 and KLF4. iPSCs were characterized by Alkaline Phosphatase (AP), immunofluorescence staining,telomeres length, RT-PCR for stem cell markers in iPSCs and three germ layers markers in embryoid bodies (EBs).The differentiation potential was assessed by adipogenic, osteogenic and chondrogenic differentiation. iPSCs were differentiated in ECs using hVEGF-supplemented EB medium. Endothelial markers expression was evaluated by FACS and RT-PCR. ECs,transduced with LV carrying GFP under the control of endothelial-specific promoters: Flk-1, Tie2 and VEC,were transplanted in NSG-HA mice. Cells engraftment was analyzed by GFP-staining of liver sections.

Results: Reprogrammed MNC and CD34+ cells gave rise to ESC-like-iPSCs coloniespositivesfor AP staining and stem cell markers. EBs differentiated in osteogenic, chondrogenic and adipose tissues, and expressed three germ layers markers. Increased iPSCs telomeres length suggested telomerase reactivation and normal karyotype. RT-PCR on FVIII-corrected HA-iPSCs showed hBDD-FVIII expression, confirming HA-MNC genetic correction by LV transduction.

iPSCs differentiated into ECs, acquiring endothelial-like morphology, expressing ECs markers and formed tubules when cultured in matrigel.

Flk-1/Tie2/VEC-GFP-LV transduced ECs were transplanted in NSG-HA mice and detected in liver sections up to 6 weeks after transplantation.

Conclusion: These results are instrumental to assess engraftment, proliferation and FVIII expression levels from differentiated, gene corrected and reprogramming factor-free iPSCs to confirm the suitability of this approach for HA gene-cell-therapy.

180. TARGT™ Shows Sustained Secretion of the Therapeutic Peptide GLP-2 Which Retains Functional Activity in SCID Mice and Rats

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Glucagon-like peptide-2 (GLP-2) is a naturally occurring 33 amino acid peptide, with $t_{1/2}$ of 6-7 min. This peptide is produced by L cells of small intestine and has several important functions, such as increasing intestinal absorption, stimulating intestinal growth, reducing bone breakdown and impacting gastric and intestinal motility. It is well known that individuals with short bowel syndrome benefit from exogenous GLP-2 to improve intestinal absorption.

Native peptides have been difficult to develop into drugs because of their short half-life, poor metabolic stability and rapid clearance. Manufactured peptides frequently require structural modification and, long-acting formulations. Moreover, the treatment with such peptides frequently uses high doses, which can lead to off-target effects such as nausea and vomiting in some patients. As an alternative, we developed a novel approach that allows our TARGT™ (Transduced Autologous Restorative Gene Therapy) technology to produce and secrete peptides such as GLP-2.

The TARGT™ system is an *ex-vivo* gene therapy, which we have now demonstrated is capable of providing autologous, continuous peptide therapies, at physiological ranges. Our group previously demonstrated such findings for certain proteins. In one set of experiments described herein, the TARGT™ system consists of several 2 x 30 mm biopsies of dermal tissue (Micro-Organ, MO), extracted under local anesthetic followed by fibroblast transduction with a Helper-Dependent Adenoviral Vector (HDAd) containing a novel GLP-2 gene expression cassette. After culture, and measurement of peptide production, one or more transduced MOs (TARGTs™) are re-implanted into the patient as required to achieve the desired dose. The system allows dose flexibility and the TARGTs™ may be added or removed according to *in-vivo* secretion levels.

Pre-clinical studies, using this novel technology, showed GLP-2 TARGT ($TARGT_{GLP-2}$) *in-vitro* secretion levels of tens of micrograms per day. Preliminary data from *in-vivo* SCID mice experiments confirms that TARGT-secreted human GLP-2 analog reaches the mouse blood stream and exhibits a sustained secretion profile similar to those observed and reported with other proteins using the TARGT™ technology (e.g. EPO, IFNα). In addition, *in-vivo* SCID mice and rat experiments suggest that TARGT™ secreted GLP-2 analog is active, and increases intestinal villous height and intestinal crypt cell proliferation rate.

Data obtained so far suggests that $TARGT_{GLP-2}$ may provide a favorable PK profile and physiologic levels of continuous GLP-2 for extended periods of time, avoiding supra-physiological peak serum concentration and achieving continuous coverage with less exposure compared to exogenous GLP-2 injections. The current results suggest that the TARGT™ platform is a promising novel therapy for short bowel syndrome and potentially other diseases of endogenous protein/peptide deficiency.

181. Real-Time Monitoring of Transcription Factor Activity Using In Vitro and In Vivo Models of Cholestasis

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Biliary obstruction results in cholestasis characterised by fibrosis, progressive cirrhosis and cholangiocyte hyperplasia leading to liver failure. The underlying molecular mechanisms remain unclear but likely involve deregulation of bi-potent progenitor cells. Transfection of cell lines with vectors containing serial transcription factor (TF) binding sequences upstream of a minimal promoter driving luciferase expression have been widely used to study TF activity in vitro. We have expanded on this technology, both in vitro and in vivo, to enable quantification of bioluminescence output in living cell cultures and animals. We have developed a library of lentiviral vectors expressing either firefly luciferase or a secreted NanoLuc luciferase conditionally activated by a range of TFs. Human HepaRG cells can be cultured as bi-potent progenitors capable of differentiating into cholangiocytes or hepatocytes and act as a valuable tool to understand cholangiocyte hyperplasia. We have evaluated TF activity in living HepaRG cultures subjected to pro-cholestatic agonists by measuring NanoLuc activity in conditioned medium. For in vivo experiments, high-titer VSV-G pseudotyped lentiviral preps containing TF activated reporter cassettes were administered by intravascular injection to P0 neonatal mice. This resulted in liver-restricted transduction and lifelong tolerance of the transgene. After establishing a bioimaging baseline, adult mice injected with either the NF-κB or the Smad2/3 reporter constructs, were subjected to partial bile duct ligation (pBDL) and serial bioimaging thereafter. Responses were observed in NF-kb ($p = 0.048$) and Smad2/3 ($p = 0.009$) reporter mice post-BDL. These tools have allowed us to evaluate and compare the temporal activity of candidate TFs during cholestatic insult in living cells and living mice. We envisage that this study could result in the elucidation of novel therapeutic targets.

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