51. LV.InsB9-23/Anti-CD3 mAb Inhibits Recurrence of Autoimmunity in NOD Mice After Islet Transplants

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Type 1 diabetes (T1D) is an autoimmune disease resulting in complete destruction of insulin-producing pancreatic beta-cells, which is mediated by auto-reactive T cells. In T1D in human and in the non-obese diabetic (NOD) mouse, the spontaneous murine model of T1D, induction of tolerance to beta-cell associated antigens represents a potential curative option. We previously showed that systemic administration of a single dose of integrase-competent (IC) or integrase-defective (ID) LV.ET.InsB9-23.142T (LV.InsB), enabling stable or transient expression of InsB9-23 in hepatocytes, arrests beta-cell destruction in NOD mice at advanced pre-diabetic stage. In these mice stable normoglycemia is maintained long term and InsB9-23-specific FoxP3$^+$ T regulatory cells (Tregs) are generated. Moreover, LV.InsB in combination with a suboptimal dose (1X 5µg) of anti-CD3 mAb reverts overt diabetes and preserves residual beta-cell mass. In the present study we tested the efficacy of the LV.InsB/anti-CD3 combination therapy to inhibit recurrence of autoimmunity and maintain insulin independence after pancreatic islets transplants. Pancreatic islets isolated from NOD-*scid* donor mice were transplanted under the kidney capsule of diabetic NOD mice with blood glucose levels >350mg/dL. Successfully transplanted mice (normoglycemic: blood glucose levels ~100 mg/dL) were treated with LV.InsB/anti-CD3; anti-CD3 mAb (1X 5µg) alone or left untreated as controls. LV.InsB/anti-CD3 combination therapy allowed stable normoglycemia up to 250 days whereas in mice receiving anti-CD3 mAb alone or untreated controls recurrence of diabetogenic responses cleared transplanted islets in 2 weeks. Autoreactive T cells were still present in the spleen of transplanted mice treated with LV.InsB/anti-CD3, but the frequency of FoxP3$^+$ Tregs within the CD4$^+$ T cells in renal (RLN) and pancreatic (PLN) lymph nodes significantly increased, as shown by phenotypic analysis. These data show that LV.InsB/anti-CD3 treatment induces active suppression of autoimmune responses in long-term normoglycemic transplanted NOD mice. Ag-specific FoxP3$^+$ Tregs accumulating in the PLN and RLN suppress effector T cells present in the target tissue. This mode of action is similar to what we previously described after LV.InsB treatment in a model of autoimmunity (Akbarpour M. et al. Science TM. 2015). Further studies are currently ongoing to evaluate the relative contribution of endogenous and exogenous beta-cell mass to insulin independence and to investigate the efficacy of the LV.InsB/anti-CD3 treatment after transplant of allogeneic pancreatic islets. The definition of novel gene therapy strategies to induce Ag-specific tolerance that at the same time control autoimmunity and transplant rejection would represent a major step toward the cure of type 1 diabetes.

52. Therapeutic Factor VIII Expression After AAV Delivery in Non-Human Primates

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Adeno-associated viral (AAV) vector delivery of factor VIII (FVIII) has been challenging due to its intrinsic properties that result in inefficient expression compared to similarly sized proteins. Early studies of AAV delivery in hemophilia A mice and dogs suggested that the therapeutic vector dose for FVIII will be higher than for factor IX. However, higher vector loads may induce stronger immune responses against capsid antigens, as evidenced in the clinical studies of AAV delivery for hemophilia B. The use of codon-optimization and novel FVIII variants with enhanced biological properties may provide strategies to increase FVIII expression or secretion to support clinical studies for hemophilia A. One published study has reported clinically relevant levels of hFVIII following AAV-hFVIII delivery in non-human primates (NHPs). This study utilized a hFVIII variant that included a 17 amino acid synthetic sequence within the 14 amino acid B-domain region that increased hFVIII expression compared to the parental B-domain deleted FVIII-SQ transgene (McIntosh, 2013). While this and other variants may increase expression after AAV delivery, the use of non-native FVIII sequences may also increase the risk of development of neutralizing antibodies to potential neo-antigens. In order to generate an AAV-hFVIII vector capable of expressing therapeutic levels of FVIII at a clinically relevant vector dose without introduction of any neoantigens, 28 hFVIII-SQ sequences were generated and introduced into our optimized expression cassette containing a modified transthyretin (TTRm) promoter. The constructs were initially screened by hydrodynamic delivery of plasmid DNA which identified 11 candidates that expressed FVIII 2-7 fold higher than our first generation codon optimized construct, CO3. AAV vectors (n=9) were generated using a novel AAV capsid, Spark100, with the best performing FVIII constructs. Hem A/CD4 KO mice were administered the vectors alongside CO3 (4x10e12vg/kg). At 8 weeks post vector administration, 2/9 expressed hFVIII similar to CO3, 5/9 were 4-8 fold higher than CO3 while 2/9 (SPK-8003 and SPK-8005) were >10 fold more potent than CO3. SPK-8005 was then evaluated in a dose escalation study in cynomologus macaques (n=3/group) treated with 3 doses: 2x10e12, 5x10e12 and 1x10e13 vg/kg and compared to vehicle controls (n=2). At 2 weeks post AAV administration, average hFVIII levels in the low, mid and high dose cohorts were 12.7 ± 2.1, 22.6 ± 0.8 and 54.1 ± 15.6 percent of normal, respectively. By 3-4 weeks, hFVIII expression started to decline in most of the animals concomitant with generation of antibodies against human FVIII. Of note, this is an expected and well-described observation that occurs in immune competent animal models due to differences between human and endogenous FVIII protein sequences. The 2 macaques that did not develop anti-hFVIII antibodies had sustained FVIII expression through the last time point evaluated. Finally, no vector-related toxicity events were observed. In summary, extensive codon-optimization identified novel AAV-hFVIII constructs capable of achieving therapeutic FVIII levels in macaques at clinically relevant doses. To our knowledge, the hFVIII levels observed in this study are the highest reported in a large animal model after treatment with an AAV vector expressing an unmodified FVIII-SQ protein. These safety and efficacy results in NHPs support the use of SPK-8005 hepatic gene transfer for the potential treatment of hemophilia A.

Gene Therapy for CNS Diseases

53. A Neuro-Specific Gene Therapy Approach to Treat Cognitive Impairment in Down Syndrome by RNA Interference

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Down syndrome (DS) is a genetic disorder caused by the presence of a third copy of chromosome 21. DS affects multiple organs, resulting in characteristic facial features, muscular hypotonia, heart defects, brain development impairment, and varying degrees of

intellectual disability. Trisomic mouse models of DS reproduce the main cognitive disabilities of the human syndrome. In particular, DS mice show structural and functional synaptic impairment as well as learning and memory deficits, largely determined by altered GABAergic transmission through chloride-permeable $GABA_A$ receptors ($GABA_AR$). In particular, we have recently found that intracellular chloride accumulation shifts $GABA_AR$-mediated signaling from inhibitory to excitatory in the adult brain of the Ts65Dn mouse model of DS. Accordingly, intracellular chloride accumulation was paralleled by increased expression of the chloride importer NKCC1 (Na-K-Cl cotransporter) in the brains of both trisomic mice and DS patients.

Our findings on NKCC1 as a pivotal molecular target for the rescue of cognitive deficits in DS opens the possibility of a gene therapy approach to treat the disease. Here, to normalize NKCC1 expression and rescue synaptic dysfunctions as well as cognitive deficits in Ts65Dn mice we have developed and characterized a knock-down approach to normalize NKCC1 activity. Reducing the expression of the chloride importer NKCC1 by RNA interference restored $GABA_AR$-mediated inhibition and also rescued the structural dendritic deficits found in trisomic neurons *in vitro*. Most importantly, focal administration of an AAV expressing a silencing RNA under the transcriptional control of a neuron-specific promoter in the hippocampus of Ts65Dn animals mediated NKCC1 knockdown *in vivo* and rescued behavioral performance on different learning and memory tests at levels undistinguishable from those of WT mice.

Our findings demonstrate that NKCC1 overexpression drives excitatory $GABA_AR$ signaling in trisomic cells, leading to structural neuronal abnormalities and behavioral impairments in DS mice. Moreover, our study identifies a new gene therapy target for treatments aimed at rescuing cognitive disabilities in individuals with DS.

54. Prevention of Sensory Ataxia in a Novel Mouse Model of Friedreich Ataxia Using Gene Therapy Approach

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Friedreich's ataxia (FRDA), the most common autosomal recessive ataxia, is characterized by a sensory and spinocerebellar ataxia, hypertrophic cardiomyopathy and increase incidence of diabetes. FRDA is caused by reduced levels of frataxin (FXN), an essential mitochondrial protein involved in the biosynthesis of iron-sulfur (Fe-S) clusters. Impaired mitochondrial oxidative phosphorylation, bioenergetics imbalance, deficit of Fe-S cluster enzymes and mitochondrial iron overload occur in individuals with FRDA. Proprioceptive neurons within the dorsal root ganglia (DRG) and cardiomyocytes are the most affected tissues in FRDA patients. To date there are not effective treatment for FRDA. We have previously established the primary proof-of-concept for developing gene therapy of FRDA cardiomyopathy and showed that adeno-associated virus (AAV) rh.10 vector expressing human FXN injected intravenously not only prevented the onset of the cardiac disease in a faithful FRDA cardiac mouse model, but also, when administered at the time of heart failure, rapidly and completely reversed the cardiac disease. To date, there were unfortunately no adequate neuronal mouse model to address the possibility of gene therapy for the neuronal aspects of FRDA. We therefore recently generated a novel mouse model that recapitulates faithfully the sensory ataxia associated to FRDA using the conditional approach to delete frataxin specifically in the proprioceptive neurons of the DRG. By behavioural analysis, the mice exhibit an ataxic phenotype beginning at 3 weeks of age, which is rapidly progressive. Electrophysiological studies reveal a significant decrease of sensory wave already at 4.5 weeks and almost a complete loss at 8 weeks of age. A significant loss of sensory neurons within dorsal root ganglia is observed at 17.5 weeks of age compare to age matched controls. Ultrastructural analysis of sciatic and saphenous nerves showed abnormalities at early time points. Using this mouse model, we have developed an AAV gene therapy approach based on an intravenous delivery of AAV9-CAG-hFXN-HA vector at an early symptomatic stage of the disease. Mice displayed a complete prevention of the ataxic phenotype and electrophysiological analysis showed maintenance of the sensory wave in the treated animals. Histological studies revealed a complete prevention of neuronal loss in the DRG as well as a normal ultrastructure of saphenous and sciatic nerve. As results are encouraging, we plan to evaluate the potential therapeutical effect at a more advanced stage of the disease.

55. Aquaporins and CSF Flux Are Critical Determinants of AAV Mediated CNS Gene Transfer

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The central nervous system (CNS) lacks conventional lymphatics for solute clearance. Convective currents of interstitial fluid (ISF) shunt macromolecules across the brain parenchyma into cerebrospinal fluid (CSF) compartments. Here the cargo is either reabsorbed into CNS via subarachnoid ducts or disseminated into the blood or peripheral lymph vessels. Recent studies have identified that flow of water via aquaporin (AQP) channels at the astroglial endfeet enables exchange of biomaterials between ISF and CSF. Conversely, loss of AQP expression due to aging or disease is correlated with ineffective clearance of neurodegenerative accumulations such as Amyloid β and tau. AAV mediated gene therapy of CNS disorders requires a deeper understanding of such factors and their effects on transduction efficiency and biodistribution/clearance. Here, we demonstrate that AQP mediated water transport dictates various aspects of AAV gene transfer in the CNS. We compared the CNS spread, transduction profile and systemic leakage of clinically relevant AAV vectors in wildtype (WT) or AQP knockout (AQP-/-) mice. Within minutes following intracerebroventricular (ICV) administration, AQP-/- mice exhibited highly restricted spread of fluorophore labeled AAVs when compared to wild type mice. Transgene expression was markedly increased from AAV administrations in AQP-/- mice. Further, systemic leakage and off-target transgene expression in peripheral tissues were markedly reduced for some AAV serotypes in AQP-/- mice when compared to WT mice. These results suggest that AQP-mediated water transport plays a critical role in determining the spread, transduction efficiency and systemic leakage of AAV vectors following CNS administration. We hypothesize that altered CSF flux under conditions pertinent to aging and CNS disease, can impact the residence time of AAV vectors and consequently gene transfer efficiency. Further results evaluating the CNS transport properties of AAV in mouse models of aging and disease will be presented.

56. Intracerebroventricular and Intravenous AAV Gene Therapy in Canine Globoid Cell Leukodystrophy

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Globoid cell leukodystrophy (GLD), or Krabbe disease, results from a deficiency in the hydrolytic enzyme galactosylceramidase