172. Correction of Profound Metabolic Impairments in MPS IIIB Mice by a Systemic rAAV9-hNAGLU Gene Delivery

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Mucopolysaccharidosis (MPS) IIIB is a lysosomal storage disease caused by deficiency in α-*N*-acetylglucosaminidase (NAGLU) leading to complex CNS and somatic pathology. This study was designed to characterize the metabolic impairments in MPS IIIB mice, and assess their responsiveness to effective gene therapy treatment. Using mass spectrometry targeting 361 metabolites, we detected significant decreases in 225 metabolites, and increases in 6, in serum from 7mo-old MPS IIIB mice, compared to wt mice. The metabolic disturbances in MPS IIIB mice emerge early, and involve virtually all major pathways of amino acid, peptide (58/102), carbohydrate (18/28), lipid (111/139), nucleotide (12/24), energy (2/9), vitamin and co-factors (11/16) and xenobiotics (11/28) metabolism. Notably, the reduced metabolites included 8 essential amino acids, Vitamins (C, E, B2, B6) and neurotransmitters (serotonin (5HT, glutamate, tryptophan, and N-acetyltyrosin). Importantly, the restoration of NAGLU activity with an IV injection of rAAV9-hNAGLU vector led to the efficient correction of these metabolic abnormalities, with 184 metabolites (79.7%) normalized, 6 (2.6%) partially corrected and 41 (17.7%) over-corrected. While the mechanisms are unclear, our data demonstrates that the lack of NAGLU activity triggers profound metabolic disturbances in MPS IIIB, leading to severely compromised functions in virtually all metabolic pathways. These metabolic impairments respond well to restoration of NAGLU activity using an effective gene therapy approach, supporting the biomarker potential of serum metabolomic profiles for MPS IIIB.

173. Insulin B9-23 LV-Driven Expression in Hepatocytes Combined With Suboptimal Dose of Anti-CD3 mAb Cures Type 1 Diabetes in NOD Mice

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Type 1 diabetes (T1D) is an autoimmune disease resulting in complete destruction of insulin-producing pancreatic β cells. In T1D in human and in the non-obese diabetic (NOD) mouse, the spontaneous murine model of T1D, auto-reactive T cells target islet-associated antigens. Induction of antigen (Ag)-specific tolerance could cure Type 1 Diabetes (T1D) but it has not been achieved yet. We previously showed that lentiviral vector (LV)-mediated gene expression in hepatocytes induces active tolerance toward the encoded-Ag. Systemic administration of a single dose of Integrase competent (IC) or integrase defective (ID) LV.ET.InsB9-23.142T, enabling stable and transient expression of InsB9-23 in hepatocytes, respectively, arrests β cell destruction in NOD mice at advanced pre-diabetic stage by generating InsB9-23-specific FoxP3+ T regulatory cells (Tregs). In the present study we tested the efficacy of hepatocytes-directed LV.ET.InsB9-23.142T gene transfer in protecting from disease progression at later stages and in reversing T1D.

Treatment with LV.ET.InsB9-23.142T in NOD mice with glucose levels ranging from 200mg/mL to 250mg/mL blocked T1D progression in only 27% of the mice. Co-expression of the late auto-Ags-derived epitopes GAD206-220 and IGRP195-214 in hepatocytes did not improve the efficacy of LV.ET.InsB9-23.142T treatment. LV.ET.InsB9-23.142T treatment in diabetic NOD mice with blood glucose levels ranging from 250mg/mL to 300mg/mL did not result in reversion to normoglycemic levels in any of the treated mice.

We next combined InsB9-23 gene transfer with anti-CD3 monoclonal antibody (mAb) treatment. Treatment with anti-CD3 mAb at optimal doses is able per se to reverse T1D in NOD mice. Therefore, we tested decreasing doses of anti-CD3 mAb in diabetic NOD mice with blood glucose levels ranging from 250mg/mL to 300mg/mL to identify the sub-optimal dose unable to revert T1D. We found that a single administration of anti-CD3 mAb at 5μg instead of 10μg results was not effective. This sub-optimal dose of anti-CD3 mAb (1X 5μg) was administered together with LV.ET.InsB9-23.142T to NOD mice with blood glucose levels ranging from 250mg/mL to 300mg/mL. Results showed T1D reversal in 75% of ICLV-treated and 40% of the IDLV-treated mice. These data indicate that the LV.ET.InsB9-23.142T treatment combined with sub-optimal anti-CD3 mAb treatment is able to reverse overt diabetes.

174. Liver Fibrosis in Aged OTC-KO Heterozygotes and Successful Correction by AAV8-Mediated Gene Therapy

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Ornithine transcarbamylase deficiency (OTCD) is an X-linked disorder of the urea cycle. Affected males are at risk of life threatening elevation of ammonia that can lead to irreversible cognitive impairment, coma and death. Female carriers show variable clinical manifestations depending on the level of X chromosome inactivation skewing. Histological analyses on liver samples from patients with urea cycle disorders found diffuse microvesicular steatosis, nuclear glycogen, and variable portal-to-portal bridging fibrosis. The OTC knockout (KO) model generated in our laboratory through the deletion of exons 2-3 closely mimics the severe neonatal onset form of OTCD in humans. Neonatal male OTC KO pups have elevated plasma ammonia levels due to the absence of OTC expression in the liver, and they inevitably die within 24 hours after birth. Heterozygous females breed normally, have normal plasma ammonia levels, reduced liver OTC enzyme activity, elevated urine orotic acid levels, and in some cases lower body weight compared to wild type (WT) littermates. Sirius red staining on liver samples from heterozygous mice of different ages (6, 12, and 18 months old) showed liver fibrosis in aged (18-month old) OTC-KO heterozygous female mice, similar to a liver sample from a 11-year-old OTCD patient. We then tested if gene therapy could prevent the liver fibrosis. Two-month old OTC-KO heterozygous received a single tail vein injection of a self-complementary AAV8 vector encoding a codon-optimized human OTC gene (hOTCco) at 1x10^10, 3x10^10, and 1x10^11 vector genome copies per mouse. One week following vector treatment, mice in all three vector dose groups had normal urine orotic acid levels which were maintained throughout the study (16 months). Liver samples were harvested from 18 month old treated mice for pathology analysis and compared to age-matched untreated heterozygous mice and WT littermates. All treated mice showed normal liver histology similar to WT, in contrast to the untreated heterozygous animals which had fibrosis throughout the liver. In conclusion, a single injection of AAV8sc-hOTCco vector can prevent liver fibrosis in OTC-KO heterozygous and has great potential for correction of liver fibrosis in OTCD patients.