Gb3 levels. Following the one-year observation period, subjects will be encouraged to participate in a long-term follow-up study for up to 4 years. Cohort 1 dosing was completed in 2 subjects and enrollment is ongoing for cohorts 2 and 3. An update will be provided on the design and progress of the study.

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Increased sulfatide disrupt mitochondrial function in Schwann and mesenchymal stromal cells in metachromatic leukodystrophy

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Metachromatic leukodystrophy (MLD) is characterized by deficient arylsulfatase A (ARSA) activity, resulting in intra-lysosomal sulfatide accumulation. Sulfatide buildup triggers systemic apoptosis, particularly in glia and oligodendrocytes in the central and Schwann cells (SCs) in the peripheral nervous systems, respectively. Clinical presentation of peripheral neuropathy is highly variable and disease pathology at a cellular level remains poorly understood. To delineate different cellular contributors to disease pathology, we characterized the mitochondrial morphology, cytokine secretion, and phagocytic activity in SCs and mesenchymal stromal cells (MSCs) isolated from $ARSA^{-/-}$ knock out (KO) mice treated with exogenous sulfatide (0–25 microgram/ml) for a period of 24 hours. We used MitoTracker Red CMRos to monitor live mitochondria through fluorescence imaging and intensity measurements. Imaging analysis tools MiNa and MicroP were used to quantify mitochondrial morphology. Proinflammatory cytokines (TNF-α and IL-1β) and cellular phagocytosis were assessed using enzyme-linked immunosorbent (ELISA) assays and imaging of fluorescent bead ingestion, respectively. Mitochondrial structures correspond with specific stages of the fission and fusion processes that cells undergo when trying to maintain homeostasis. We found that treated KO cells presented with persistent and increased ($P < 0.0001$) fragmented mitochondrial structures suggestive of prolonged mitochondrial fission within 6-hours following sulfatide exposure. Investigation of the immune response to sulfatide treatments revealed increased ($P < 0.05$) secretion of both TNF-α and IL-1β in KO cells at 12- and 24-hours. No differences were noted in the cellular phagocytic capacities following sulfatide treatment. These findings suggested that mitochondrial morphologies were impacted in MLD cells. Further characterization of in vivo mitochondrial function in MLD tissues may provide a clearer pathological picture.

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RNA-seq analysis in three Gaucher sib-pairs discordant for Parkinson disease

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Gaucher disease (GD) is a rare, autosomal-recessive lysosomal disorder caused by mutations in *GBA1*, the gene encoding for glucocerebrosidase. Mutations in *GBA1* are the most common genetic risk factor for developing the common neurodegenerative disorder, Parkinson disease (PD). To identify the secondary risk factors and biologically related pathways involved in this association, we analyzed three pairs of GD-affected siblings discordant for PD, and three age matched wild-type controls. Two of the discordant sib-pairs were of Ashkenazi Jewish ancestry and the third was half Ashkenazi-Jewish. RNAs were extracted from cell pellets of fibroblasts cultured at the same passage number. To identify significant differentially expressed genes (DEGs) that may explain the discordant PD phenotypes, we performed 3'-PolyA-tail RNA-seq on the extracted RNAs. Using the Partek Flow package, we analyzed RNA-seq reads in fastq format and identified three potential genes of interest using several thresholds: Total Read Count >10, FDR *p*-value <0.05, and Fold-Change >|2|. STAR and DESeq2 software were used with the Partek Flow pipeline for alignment and differential expression analysis. Qiagen's Ingenuity Pathway Analysis software was also employed to identify biologically significant pathways and upstream regulators that may shed light on possible mechanisms. The DEGs and variants identified by RNA-seq are currently being validated by RT-qPCR and in WES data, respectively. Ultimately, the final confirmed gene list will be examined using RNA from our larger cohorts of patients with GD and GD/PD to identify pathways which may be directly or indirectly related to the development of parkinsonism.

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Ex vivo hematopoietic stem cell gene therapy for mucopolysaccharidosis type I (Hurler syndrome)

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Transplantation of genetically-corrected autologous hematopoietic stem and progenitor cells (HSPC) represents a promising treatment approach for patients with lysosomal storage disorders. We conducted a phase I/II gene therapy (GT) study in children affected by Hurler syndrome. Eight patients were infused with autologous HSPC transduced with an alpha-L-iduronidase (IDUA)-encoding lentiviral vector following conditioning with busulfan and fludarabine. Seven of 8 patients had previous enzyme replacement therapy (ERT), which was discontinued several weeks before GT. Median transduction efficiency of the products was above 80%, with a median of 2.2 vector copies (VCN) per cell. All patients had rapid hematologic recovery, with median neutrophil engraftment on day +20, rapid resolution of neutropenic fever (median: 1.5 days) and short thrombocytopenia limited to grade 3 in most patients. GT did not induce IgG immune responses to IDUA, and the 5 patients that tested positive for antibodies while on ERT before GT, cleared them within 3 months. With a median follow up of 17 months (range: 9–26) as of October 2020, all patients are alive and well and show evidence for stable, polyclonal gene marking (median VCN in

granulocytes: 1.4). Notably, patients show extensive enzymatic reconstitution, with supraphysiologic median blood IDUA activity of 111 micromol/L/h (normal range: 3.8–35) accompanied by normalization of urinary glycosaminoglycan (GAG) levels. Importantly, IDUA activity was detectable in the cerebrospinal fluid (CSF) suggesting central enzyme expression, likely provided by local engraftment of microglia-like cells derived from transduced HSPC. Heparan sulfate levels in the CSF decreased up to 20-fold post-GT suggesting central metabolic correction. Patients with the longest follow-up demonstrated a stable cognitive score, improved findings on brain and spine MRI, resumed growth velocity and an amelioration of their skeletal phenotype. Our preliminary results show encouraging safety and efficacy data, highlighting the potential of genetic engineering of HSPC for the treatment of MPSI.

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Does extending enzyme replacement therapy after transplant provide neurocognitive benefit in Hurler syndrome?

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Neurocognitive decline is a hallmark feature of Hurler syndrome. Although hematopoietic cell transplantation (HCT) halts neurocognitive decline, most patients demonstrate below average cognition in the years following treatment. Enzyme replacement therapy (ERT) combined with HCT reduces transplant-related morbidity and mortality, and we have also reported a potential neurocognitive benefit, i.e., less dramatic neurocognitive decline after treatment than HCT alone. In the past 10 years, our institution has offered the option to continue ERT beyond the standard 8 weeks following transplant, with the rationale that organ function and somatic outcomes may be further improved. This study investigates whether any neurocognitive benefit is associated with continued ERT post HCT. We examined 9 children with Hurler syndrome who underwent HCT at our institution and continued on ERT for at least 1 year post-HCT (Extended group). We matched these children for age at HCT with 9 patients who received HCT + ERT for the standard period, i.e., 8 weeks following HCT (Standard group). Neurocognitive function, i.e., early IQ, was measured with accepted norm-referenced assessment tools where population IQ mean = 100 ± 15, average range is 85–115. Just prior to transplant, group level IQ means were as follows: Extended = 95, Standard = 89. In each of these groups, all but 1 patient measured in the average range. One year after HCT, Extended patients showed a mean loss of 8 IQ points, whereas Standard patients showed a mean loss of 14 points. Group level IQ means at 1-year post-HCT were as follows: Extended = 86, Standard = 75. Individual patient analysis revealed that 7 Standard patients fell below the average range at 1 year post-HCT, whereas only 3 Extended patients had this outcome, i.e., the majority of Extended patients remained in the average range intellectually. Findings suggest extended ERT may provide neurocognitive benefit and warrant longer-term investigation in a larger sample.

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HMI-202: A gene therapy development candidate for metachromatic leukodystrophy (MLD)

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MLD is an inherited autosomal recessive lysosomal disorder with a great unmet medical need. This fatal neurodegenerative disease occurs in three forms: late infantile, juvenile and adult. MLD is most commonly caused by mutations in the *ARSA* gene and results in deficiency in arylsulfatase-A (ARSA) enzyme. The disease is characterized by accumulation of supraphysiologic levels of sulfatides which lead to the destruction of myelin, a key protective layer of the nerve fibers that enhances propagation of action potentials. Herein, we report preclinical gene therapy data where a single I.V. dose of HMI-202 crosses the blood-nerve- and blood-brain-barriers (BNB and BBB) in juvenile non-human primates and in the *ARSA* KO murine model of MLD. In the HMI-202-treated *ARSA* KO mice, human ARSA (hARSA) expression was nearly identical to that of murine ARSA distribution in wild type controls, in both neuronal and glial cells. At one week post-dose, near normal human adult levels of hARSA activity are detected and levels are sustained at or above normal adult human brain levels through 52 weeks in the CNS of. A dose-response relationship in hARSA activity, transcript and vector genome copies in the CNS was observed in treated *ARSA* KO mice. In addition, we demonstrate HMI-202 modulation of key biochemical markers in the CNS, including sulfatide, MAL transcript, LAMP-1 and GFAP levels. In summary, a single I.V. dose of HMI-202 crosses the BNB and BBB in lower (mice) and higher (NHP) species. In addition, the ability to achieve hARSA enzymatic activity levels at or above normal human adult brain levels, rapid onset of expression, durability, broad biodistribution, and modulation of biomarkers in a murine disease model were demonstrated. Based on these preclinical data, IND-enabling studies of HMI-202 are ongoing to support the development of HMI-202 as a gene therapy for the treatment of MLD.

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Exploration of the efficacy of pabinafusp-alfa (JR-141) on neurocognitive development in Hunter syndrome (MPS II): 52-week data from clinical trials in Japan and Brazil

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Pabinafusp alpha (JR-141) is a novel iduronate-2-sulfatase fused with anti-human transferrin receptor antibody to enable enzyme delivery across the blood-brain-barrier to address neurodegeneration in severe MPS-II. It has been clinically evaluated in a total of 47 patients in Japan and Brazil, from which 52-week neurocognitive