372. Prevalence of Anti-AAV8 Neutralizing Antibodies and ARSB Cross-Reactive Immunologic Material in MPS VI Patients Candidates for a Gene Therapy Trial

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Recombinant vectors based on adeno-associated virus serotype 8 (AAV8) have been successfully used in the clinic and hold great promise for liver-directed gene therapy. Pre-existing immunity against AAV8 or the development of antibodies against the therapeutic transgene product might negatively affect the outcomes of gene therapy. In the prospect of an AAV8-mediated, liver-directed gene therapy clinical trial for Mucopolysaccharidosis VI (MPS VI), a lysosomal storage disorder due to arylsulfatase B (ARSB) deficiency, we investigated in a multiethnic cohort of MPS VI patients the prevalence of neutralizing antibodies (Nab) to AAV8 and the presence of ARSB cross-reactive immunologic material (CRIM), which will either affect the efficacy of gene transfer or the duration of phenotypic correction. Thirty-six MPS VI subjects included in the study harbored 45 (62.5%) missense, 13 (18%) nonsense, 9 (12.5%) frameshift (2 insertions and 7 deletions), and 5 (7%) splicing ARSB mutations. To the best of our knowledge, four mutations had not been previously described. These include: one missense (c.1178 A>G p.H393R) and three frameshift mutations [883-884duplTT (p.F295FfsX42), c.1036delG (p.E346SfsX11), c.1475delC (pP492LfsX80)] predicted to result in truncated proteins. The detection of ARSB protein in twenty-four patients out of 34 (71%) was predicted by the type of mutations. Pre-existing Nab to AAV8 were undetectable in 19/33 (58%) analyzed patients. Twelve out of 31 patients (39%) tested were both negative for Nab to AAV8 and CRIM-positive. In conclusion, this study allows estimating the number of MPS VI patients eligible for a gene therapy trial by intravenous injections of AAV8.

373. Expression of Human Iduronidase from Sleeping Beauty Engineered Human B Lymphocytes as a Cellular Therapy for Mucopolysaccharidosis Type I

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Mucopolysaccharidosis type I (MPS I) is an autosomal recessive lysosomal storage disorder cause by absence of the glycosidase alpha-L-idurondase (IDUA), resulting in the systemic accumulation of glycosaminoglycan storage materials heparan sulfate and dermatan sulfate. Affected individuals exhibit a range of manifestations including hepatosplenomegaly, skeletal dysplasias, and cardiopulmonary obstruction. The severe form of the disease (Hurler syndrome) is associated with progressive neurologic dysfunction and death by age 10. Current treatments for MPS I include enzyme replacement (ERT), and hematopoietic stem cell transplantation (HSCT). However, these treatments are expensive, insufficiently effective, and allogeneic HSCT is associated with considerable morbidity and mortality.

As an alternate approach to MPS I, Immusoft Corporation is developing as a cellular therapeutic primary B cells that have been genetically engineered for long-term expression and secretion of IDUA. At the core of the technology is the generation of genetically engineered autologous human B cells, which are isolated and expanded from patient blood. Mature B cells (specifically long-lived plasma cells) are the ideal cell type to produce protein since they live for long periods of time, occupy diverse tissues throughout the body and are capable of secreting very high levels of protein. In collaboration with Discovery Genomics, Inc., the University of Minnesota and the Fred Hutchinson Cancer Research Center, Immusoft has developed a treatment for Mucopolysaccharidosis type I (MPS I). In initial tests, Sleeping Beauty (SB) transposons expressing the human iduronidase gene were introduced into cultured B lymphoblastoid cells with and without a source of SB transposase, demonstrating long-term, high level expression of IDUA activity in vitro. SB transposons expressing both IDUA and a methotrexate-resistant human dihydrofolate reductase allowed for a >10-fold expansion of IDUA-expressing cells in the presence of methotrexate, as determined by intracellular staining for human IDUA and flow cytometry. Overall IDUA expression was also increased >10-fold in the methotrexate-selected cell population. IDUA encoding SB transposons were also nucleofected along with a source of SB transposase into primary human memory B cells, demonstrating expression of IDUA at levels more than 100 times greater than that observed in cultures of untreated primary B cells. These data support the development of a clinical trial to test SB-engineered B cells in the treatment of MPS I, with implications for improved treatment of other lysosomal storage disorders as well.