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Commentary

Brain-targeted *ex vivo* lentiviral gene therapy: Implications for MPS and beyond

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Mucopolysaccharidosis type II (MPSII) is a rare, x-linked, pediatric lysosomal storage disorder that is caused by mutations in the IDS gene. Pathogenic mutations result in deficiency in iduronate-2-sulphatase (IDS) enzyme activity and inability to break down glycosaminoglycans (GAGs), predominantly heparan sulfate (HS) and dermatan sulfate (DS). As GAGs accumulate throughout the body, multisystemic disease manifests as skeletal abnormalities, cardiac disease, hepatosplenomegaly, and in the majority of patients neurodegeneration. Enzyme replacement therapy (ERT) with a recombinant IDS enzyme, Elaprase™, is effective at reducing storage in somatic tissues; however, the large recombinant enzyme cannot cross the blood-brain barrier (BBB) and thus is not an effective treatment for neuronopathic MPSII. Herein, Ellison et al. detail long-term efficacy of *ex vivo* lentiviral-mediated gene therapy in a murine model of MPSII. The results showed sustained IDS enzyme activity, reduction in storage material in the brain and somatic tissues, attenuation of neuroinflammation, and improved cytokine and chemokine profiles. Interestingly, this gene therapy approach used an ApoEII tag to enhance targeting of the central nervous system (CNS); however, a secondary neuroprotective mechanism is likely at play. This study is particularly relevant and timely because in June 2023, this work translated to a phase I/II clinical study: Autologous $CD34^+$ Hematopoietic Stem Cells Transduced *Ex Vivo* With CD11B Lentiviral Vector Encoding Human IDS Tagged With ApoEII in Patients With Neuronopathic Mucopolysaccharidosis Type II (nMPS II, Hunters Syndrome) (NCT05665166). Furthermore, use of ApoEII targeting of viral vectors to enhance BBB penetration and/or reduce neuroinflammatory disease processes has potential implications in neurodegenerative diseases as a whole.

In the study, 6- to 8-week-old MPSII mice were conditioned with busulfan and treated systemically with hematopoietic stem cells transduced *ex vivo* with a lentiviral vector encoding therapeutic IDS fused to an ApoEII peptide (LV.IDS.ApoEII) to enhance CNS targeting from gene-modified cells in the periphery via receptor mediated transcytosis across brain endothelial cells (Figure 1) or IDS without the ApoEII peptide fusion (LV.IDS). Expression was driven by a myeloid-specific CD11b promoter. Chimerism and behavior analyses were performed 6 months post-treatment and previously reported by Gleitz et al.[1] In this long-term efficacy study, treated mice reached humane endpoint between 14 and 18 months of age. Supraphysiological levels of IDS activity were detected in most peripheral organs from the IDS and IDS.ApoEII treatment groups. In the brain, the investigators observed 14% and 4% of wild-type (WT) IDS activity levels in the IDS and IDS.ApoEII treatment groups, respectively, and subsequent reductions in brain HS storage of 4.3- and 2.8-fold, respectively. Although the higher level of enzyme activity in the brain without the brain-penetrating ApoEII tag was not the anticipated result, it was in line with the number of integrated vector copies (VCN), which was higher in the LV.IDS-treated animals. Although the two constructs perform similarly in terms of enzyme activity per VCN, it does leave a question regarding why the construct with the peptide tag was less efficient.

The addition of the BBB-targeting ApoEII tag resulted in greater reduction of astrogliosis and lysosomal swelling in specific brain regions, namely, the amygdala. Additionally, ApoEII appears to have had the greatest impact on microglia, demonstrating the most efficient reduction in activated microglia in all areas analyzed. Thus, despite 4 times lower enzyme activity and lower VCN, the addition of the ApoE tag resulted in greater correction of the neuroinflammatory profile associated with MPSII. However, the degree and changes were both brain region and cell type specific, with the greatest impact in the amygdala and microglia. Last, IDS and IDS.ApoEII were similarly effective at normalizing altered cytokine and chemokine levels observed in MPSII mice, suggesting that there were no toxic or immunogenic effects from the addition of the ApoEII tag.

In the initial study by Gleitz et al.,[1] 6 months post-treatment, the IDS-only cohort demonstrated no improvement in working memory, while the IDS.ApoEII cohort did. Given the superior brain IDS activity levels observed here at ~1 year post-treatment, it is plausible that behavioral outcomes were improved by LV.IDS at a later time point. Because of limited animal numbers, behavior was not assessed at this later time point and would have provided additional long-term translational data to correlate enzyme levels and behavior. Furthermore, in this study, mice were treated at a pre-symptomatic time point to offer the greatest potential for disease correction before the onset of irreversible neurocognitive and peripheral damage, leaving in question the impact on disease once symptoms are present. The authors have recently shown that MPSII mice transplanted at 4 months of age with *ex vivo* gene therapy have worse outcomes than those treated pre-symptomatically at 2 months of age.[2] Informed by these studies, the recently opened phase I/II clinical trial is recruiting only MPSII patients aged 4–12 months,

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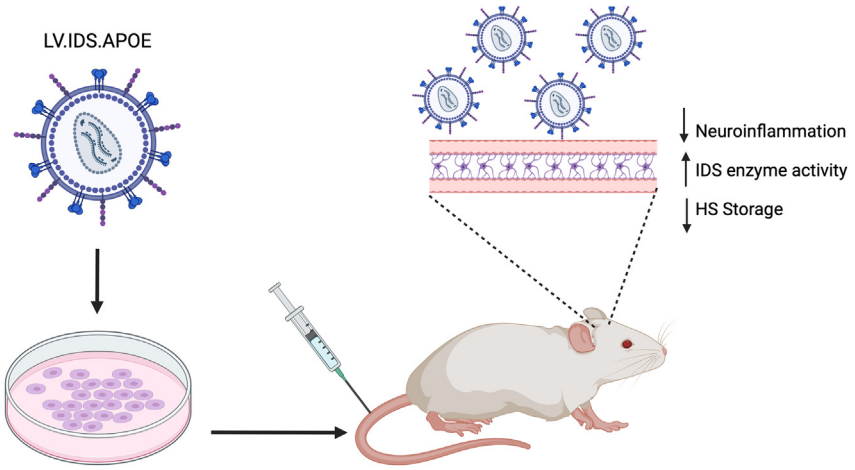

Figure 1. Brain-targeted *ex vivo* lentiviral-mediated gene therapy for the treatment of MPS

MPSII mice were conditioned with busulfan and treated with hematopoietic stem cells transduced *ex vivo* with a lentiviral vector encoding therapeutic iduronate-2-sulphatase (IDS) fused to an ApoEII peptide (LV.IDS.ApoEII) to enhance CNS targeting. At humane endpoint, IDS enzyme activity, heparan sulfate (HS) storage, and neuroinflammation were quantified. Created with BioRender.com.

which is before neuronopathic patients begin to fail cognitive milestones (NCT05665166).

First, this study has brought a treatment option for pre-symptomatic neuronopathic MPSII patients for which traditional ERT and hematopoietic stem cell transplantation (HSCT) do not treat the neurological disease manifestations. The results of this initial phase will likely determine if a broader cohort of MPSII patients will ultimately be eligible for this therapy. Second, the primary novelty of this study is the use of an ApoEII peptide to enhance targeting of the lentiviral vector to more efficiently traverse the BBB. However, as the addition of the ApoEII did not result in higher enzyme levels in the CNS, but did result in greater attenuation of neuroinflammatory properties, this suggests that enhanced brain penetration may not be the primary mechanism of action of ApoEII. In fact, ApoEII itself is used as a therapeutic transgene in an adeno-associated virus (AAV)-mediated gene therapy treatment for Alzheimer's disease (NCT03634007). It will be greatly informative for neurodegenerative diseases as a whole to reveal the intricate mechanism of ApoEII in the context of a pediatric neurodegenerative disease in MPSII patients.

A similar gene therapy approach has been used for mucopolysaccharidosis type I (MPSI) patients without the addition of the ApoEII peptide. In this clinical study, MPSI children received autologous hematopoietic stem and progenitor cells transduced *ex vivo* with an α-L-iduronidase (IDUA)-encoding lentiviral vector after myeloablative conditioning. The interim results were reported in the *New England Journal of Medicine* in 2021 and were overwhelmingly encouraging, demonstrating metabolic correction peripherally and in the CNS. Critically, patients showed stable cognitive performance, continued motor development, improved or stable findings on MRI of the brain and spine, reduced joint stiffness, and normal growth.[3] It is plausible that the brain-penetrating and/or neuroprotective properties of ApoEII could further enhance this effect. Regardless, this shows great potential for the use of *ex vivo* lentiviral gene therapy for the treatment of complex, multisystemic MPS diseases, and promise for the newly initiated MPSII trial based on the work herein.

Although clearly encouraging, *ex vivo* lentiviral mediated approaches are not the only gene therapy methods being evaluated for this class of diseases. For MPSI, MPSII, and mucopolysaccharidosis type IIIA (MPSIIIA), there are concurrent active AAV-mediated and *ex vivo* lentiviral mediated gene therapy approaches.[4] Long-term data from these two differing gene therapy approaches will be vital to determine the safest and most efficacious way to treat patients with this class of multifaceted pediatric lysosomal storage diseases.

DECLARATION OF INTERESTS

A.M.B. is a beneficiary of a licensing agreement with Axovant Gene Therapies (royalties) and Neurogene (royalties) and has received income from Neurogene (consulting and honorarium). A.M.B. is an inventor on multiple patents. None of these declarations is in conflict with the work herein.

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