MPS II is a rare X-linked lysosomal storage disorder (LSD) which occurs in ~1 in 100,000 to 170,000 males. This disease is caused by iduronate-2-sulfatase (IDS) gene mutations, resulting in loss of I2S enzyme and associated activity, leading to subsequent systemic (peripheral organs and central nervous system (CNS)) toxic accumulation of lysosomal glycosaminoglycans (GAGs). GAGs are large polysaccharides made of repeating disaccharide units responsible for providing structure and hydration to the cells and excess accumulation results in debilitating skeletal dysplasia, joint stiffness, hepatosplenomegaly, airway obstruction and, in severe cases, neurocognitive deficits. Together, these complications reduce the life expectancy of affected patients. Herein, we summarize our nonclinical gene therapy data where a single intravenous dose of investigational gene therapy HMI-203 delivering IDS in a MPS II murine model resulted in systemic tissue transduction, which was confirmed by detection of vector genomes, IDS transcript and I2S activity. Furthermore, in serum, significant levels of functional I2S were observed within a day of dosing, peaking by 1-week and stable out to 52 weeks post-dosing (end of study). We also demonstrated that the circulating enzyme can successfully be taken up via a cellular mannose-6-phosphate receptor pathway in cultured IDS-KO cells, further enhancing the gene therapy potential with that of cross-correction. The robust I2S expression significantly reduced GAGs (e.g., heparan sulfate) down to wildtype (WT) levels in the brain and peripheral organs, as well as in the cerebrospinal fluid and urine. Correspondingly, at 52 weeks, the integrity of the cellular lysosomes was similar to that seen in WT mice in the peripheral organs and CNS regions that were evaluated. In addition, amelioration of phenotypic symptoms associated with cranial and joint deformities in the MPS II mouse model was demonstrated. Together these nonclinical data support HMI-203 as a gene-therapy candidate for the treatment of MPS II.