Gene Therapy for Metachromatic Leukodystrophy: Lead Candidate Optimization

St. Martin T, Gall K, Newman J, Selby D, Zheng A, Przybylski D, Hanscom T, Prout J, Adjei S, Avila N, Lotterhand J, Rivas I, Wright T, Francone O, Seymour A and Gingras J

Homology Medicines, Inc., One Patriots Park, Bedford, MA 01730

Abstract

Metachromatic leukodystrophy (MLD) is an inherited autosomal recessive lysosomal storage disorder (LSD) with a significant unmet medical need. This fatal neurodegenerative LSD occurs in three forms: late infantile (prevalence of 1 in 40,000), juvenile and adult. The late infantile and juvenile forms represent the majority of the MLD patients where mortality at 5 years is estimated at 75% and 30%, respectively. MLD is most commonly caused by mutations in the *ARSA* gene and patients suffering from the disease are deficient in arylsulfatase-A (ARSA) enzyme activity. The disease is characterized by accumulation of supraphysiologic levels of lipids (sulfatides) in the nervous system and peripheral organs, leading to toxicity and cell death. In the brain, excess sulfatides initially lead to the destruction of myelin, an insulating lipid layer that forms a protective sheath around the nerve fiber that enhances propagation of action potentials.

Herein, we report the outcome of the optimization of an initial gene therapy construct (HMI-202) resulting in the nomination of a one-time, *in vivo* gene therapy development candidate, HMI-204. The design optimization focused on achieving ARSA expression in all disease-relevant tissues, in addition to overall manufacturing improvements. HMI-204 is a single-stranded codon-optimized (co) *ARSA* sequence driven by a ubiquitous promoter (AAVHSCco*ARSA*). Following a single intravenous administration, HMI-204 resulted in broad and targeted systemic biodistribution and robust expression in the central nervous system, consistent with our previously reported crossing of the blood-brain barrier in the *Arsa* knockout (KO) murine model of MLD. In the brain of HMI-204-treated adult *Arsa* KO mice, ARSA cellular expression patterns were nearly identical to that of murine Arsa distribution in wildtype age-matched littermates. In HMI-204-treated adult *Arsa* KO mice, sustained levels of ARSA activity reaching normal adult ARSA brain activity levels were achieved out to 12 weeks post-dose (end of study).

Results

First-generation construct: HMI-202

ARSA Activity

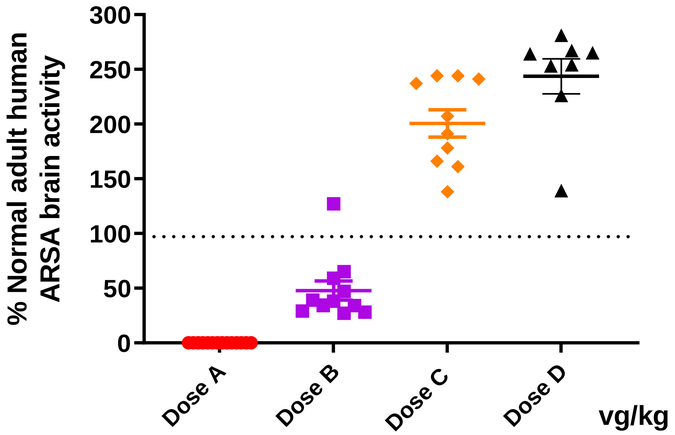


Figure 1: At study termination, levels of ARSA activity were assessed in the brain of mice that were on study. Doses C and D achieve >100% WT ARSA activity. Efficacy in the motor assay appears to require >50% of active ARSA activity (whether compared to WT mouse or normal human brain tissue).

Rotarod

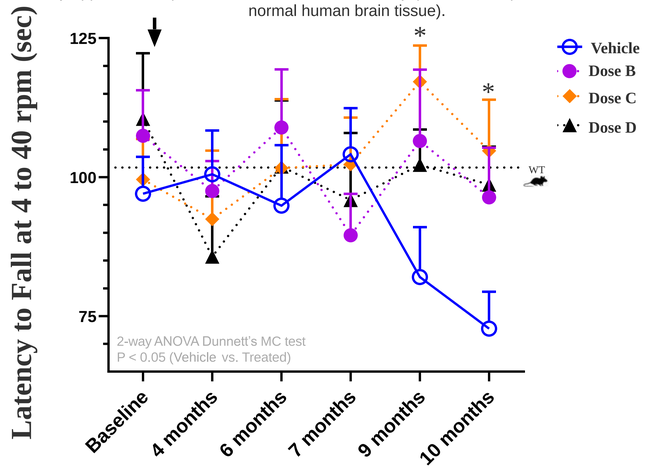
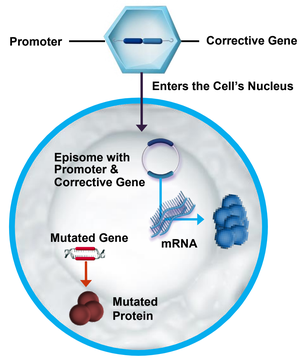


Figure 3: *Arsa* KO mice develop a motor deficit evident by 9 months of age. Dosing of HMI-202 prior to onset of deficit (arrow) leads to a statistically significant improvement in motor outcome at doses C and D (with dose B trending similarly).

Single I.V. Administration



I.V.=intravenous injection

Optimization

Conclusions

- ✓ HMI-204 maintained high levels of ARSA activity in CNS predicted to lead to efficacy *in vivo*
- ✓ Optimized tissue expression profile by lowering levels of expression in heart
- ✓ Improved manufacturing productivity and packaging

Second-generation construct: HMI-204

ARSA Activity

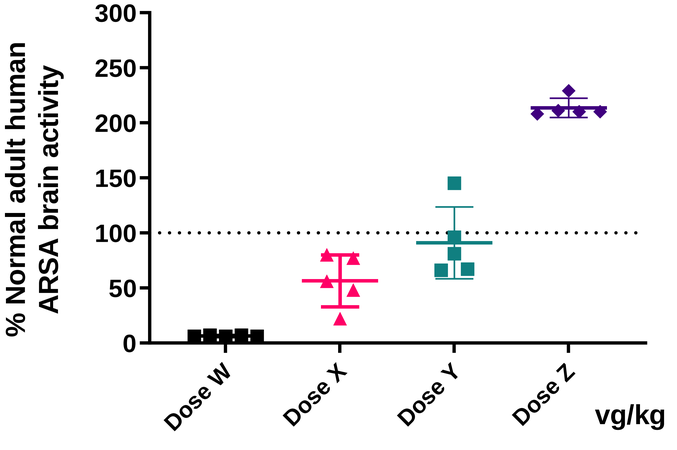


Figure 2: ARSA activity assessed in the brain of *Arsa* KO mice 12 weeks post-dosing. HMI-204 achieves levels of ARSA activity (Dose Y and Z) predicted to lead to a direct motor benefit in the rotarod assay, as demonstrated with HMI-202.

Anti-ARSA Biodistribution

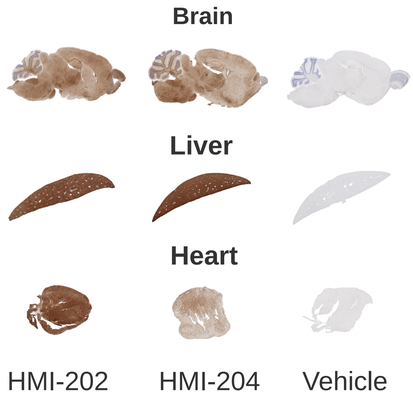


Figure 4: In *Arsa* KO mice, HMI-204 maintained a robust and broad distribution of ARSA across the entire axis of the brain and liver while lowering its expression in heart tissue, as compared with the anti-ARSA biodistribution achieved with HMI-202.

Productivity

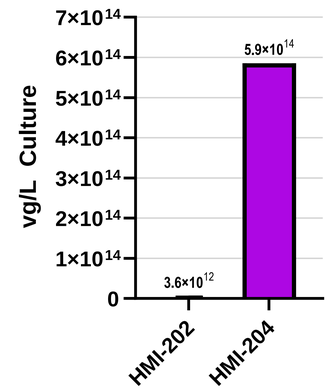


Figure 5: Outcome of HMI-204 packaging productivity achieved leading to an ~120% improvement in vector genome yields compared with historical HMI-202 data.