# Gene Therapy Candidate for Metachromatic Leukodystrophy (MLD): Optimization of HMI-202 Leading to HMI-204 Nomination

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## Abstract

Metachromatic leukodystrophy (MLD) is an inherited autosomal recessive lysosomal storage disorder (LSD) with a great unmet medical need. This fatal neurodegenerative LSD occurs in three forms: late infantile (prevalence of 1 in 40,000), juvenile, and adult. The first two 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 sulfatides to supraphysiological toxic levels in the peripheral organs and nervous system. In the brain, excess sulfatides lead to the destruction of myelin, a key protective sheath that forms a layer around the nerve fibers that enhances propagation of action potentials.

Herein, we report the outcome of the optimization and nomination of a development candidate, HMI-204, for the treatment of MLD. The optimization addressed both biology and manufacturability. The goal was to maintain an intravenous (I.V.) delivery route for HMI-204 to target this disease systemically, while also providing the expression of high levels of ARSA activity in the brain of *Arsa* knockout (KO) mice, predicted to lead to direct motor deficit improvements as demonstrated with the previous lead construct. The team also sought to lower, but not eliminate, ARSA expression in the heart tissue.

Following a single I.V. dose of HMI-204, anti-ARSA expression patterns in the brain of adult *Arsa* KO mice remained nearly identical to that of anti-murine Arsa distribution in wild type age-matched littermates, confirming successful crossing of the blood-brain barrier (BBB). A dose-response in ARSA brain activity was achieved in adult and neonate *Arsa* KO mice, reaching normal human levels of expression as measured in normal post-mortem human brain tissue samples. This range covered the expression levels predicted to lead to motor deficit prevention in the MLD mouse model (50-100% of normal human levels) across multiple doses. In the heart of adult *Arsa* KO mice, the biodistribution of anti-ARSA was significantly reduced, while that in the liver remained similar, when compared to the previous lead construct. In neonatal mice, a dose-response in ARSA activity was achieved in heart and liver tissues, as well as in serum, leading to a durable systemic expression for the entire study duration (12 weeks). Lastly, the manufacturing productivity profile of HMI-204 (vg/L) was substantially improved as compared to the previous lead construct.

In summary, a single I.V. dose of HMI-204 achieved a broad and sustained systemic biodistribution, including the central nervous system, while lowering expression in heart tissues. Levels of ARSA activity detected in each organ tested reached normal human levels for the corresponding organ, at one or multiple doses. Lastly, the optimization improved both the biological and manufacturability profile of HMI-204 and these preclinical data continue to support the potential of HMI-204 as an effective gene therapy for the treatment of MLD.

## Results (for details on the Methods, please see St. Martin et al., 2023)

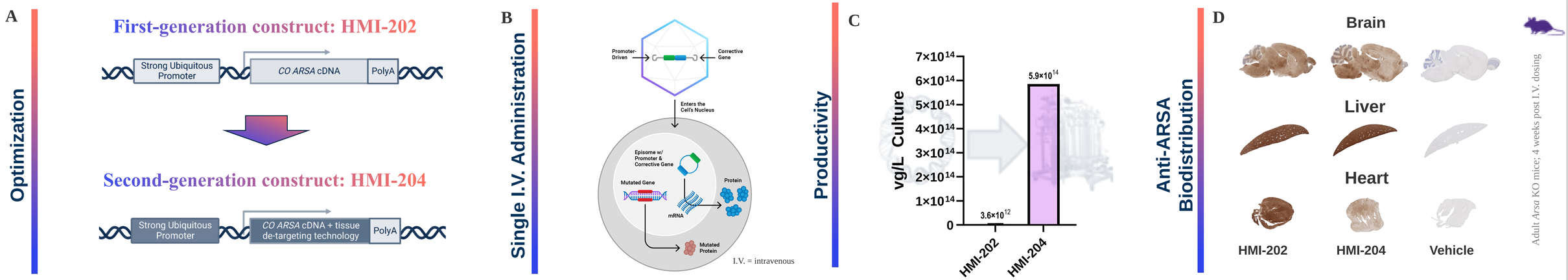


**Figure 1**: **A**) HMI-204 is the result of an optimization process centered around HMI-202 and **B**) is intended as a single I.V. administration gene therapy. **C**) Outcome of HMI-204 packaging productivity led to an ~120% improvement in vector genome (vg) per liter yield compared with historical HMI-202 data. **D**) Four (4) weeks post dosing, HMI-204 (dose C) successfully crossed the BBB and 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 (at the same dose) in adult *Arsa* KO mice. Thus, the team has successfully achieved its goal of improving the productivity (vector genome/ liter (L) yield in culture), and the biological profile with tissue de-targeting technology while maintaining the levels of ARSA activity in the CNS predicted to prevent the development of the motor deficit in *Arsa* KO mice (St Martin et al., 2023).

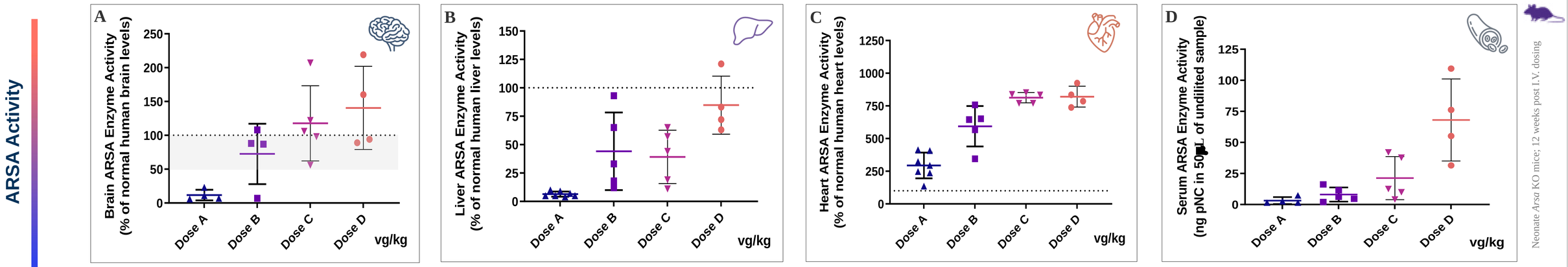


**Figure 2**: Twelve (12) weeks following a single .I.V.-administration of HMI-204 in neonate *Arsa* KO mice, we demonstrated a dose-response in ARSA activity levels and achieved normal human ARSA activity levels (and greater; see dotted lines) across corresponding murine organs, including brain (**A**), liver (**B**), heart (**C**), as well as in serum (**D**). Furthermore, in brain tissue (**A**), levels of ARSA activity achieved are predicted to lead to a direct motor benefit in the rotarod assay (see St. Martin et al., 2023 (*Journal of Neuroscience;* (>50% -100% at doses B, C and D overlapping the gray zone))). In liver sample (**B**), following active tissue growth over the course of the first 4 weeks of life (see Nomura, 1976), the residuals levels of ARSA activity reached near normal range at the highest dose (Dose D) and remained above pseudo-deficient ARSA levels at all doses (~5-20% ARSA activity; Patil and Maegawa, 2013; Doherty et a;, 2019). In heart tissue (C), the de-targeting approach utilized for HMI-204 lowered the total ARSA detected in heart tissue (Figure 1D). Resulting supraphysiological ARSA activity levels remained within the safety range (15-fold above normal range, see Capotondo et al., 2007). In serum (**D**), the detected levels of ARSA activity supports the prospect of cross-correction (albeit not demonstrated), in addition to that of broad and robust systemic transduction and biodistribution as demonstrated by the transduction levels achieved in the brain and peripheral organs..

## Conclusions

**Optimization Leading to HMI-204 Resulted in**

- ✓ **Improvements in manufacturing productivity and packaging**
- ✓ **Improvement in biological profile via decrease of ARSA protein expression in heart tissue**
- ✓ **Successfully crossing of the blood brain barrier**
- ✓ **ARSA activity levels anticipated to prevent the motor deficit in *Arsa* KO mice**
- ✓ **Normal to supraphysiological ARSA activity levels in key tissues evaluated**
- ✓ **A broad and sustained (12-week study duration) systemic biodistribution**
- ✓ **Expression of near-normal ARSA activity levels following active liver growth**
- ✓ **Detection of active ARSA secreted in the circulation (serum)**

**These data support the potential of HMI-204 as an effective gene therapy for the treatment of MLD**

**References**: Nomura, 1976 (*Br J Cancer*); Capotondo et al., (*Human Genet Ther*); Patil and Maegawa, 2013 (*Drug Des Devel Ther*); Doherty et al., 2019 (*Mol Gent Metab Rep*) & St Martin et al., 2023 (*J of Neurosci*);