Preclinical Studies with HMI-104, an AAVHSC Vectorized C5 Monoclonal Antibody, for the Treatment of Paroxysmal Nocturnal Hemoglobinuria

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Abstract # 386

AAVHSC GTx-mAb Therapy Targeting Complement Mediated Disorders

- Adeno-associated virus (AAV) gene therapy has the potential to offer a long-term resolution for diseases that rely on chronic dosing of therapies such as monoclonal antibody (mAb)-based drugs.
- GTx-mAb platform is an extension of our gene therapy approach that aims to provide systemic and sustained levels of a therapeutic mAb with one-time intravenous (I.V.) dose.
- Previously,[1] we showed that GTx-mAb platform constructs achieved dose-dependent and sustained expression of a functional C5 monoclonal antibody (C5mAb) in NOD SCID mice, and humanized liver FRG® mice,[2] supporting the use of a vectorized approach for diseases relying on chronic anti-C5 dosing, such as Paroxysmal Nocturnal Hemoglobinuria (PNH).

PNH is a Rare, Acquired, Life-Threatening Blood Disease Treatable with Complement Inhibitors

- PNH is caused by acquired *PIGA* mutations in hematopoietic stem cells, leading to loss of surface binding of the complement inhibitors CD55 and CD59.[3]
- Blood cells derived from mutant PNH clones expand and are vulnerable to complement-induced hemolysis, leading to major complications such as anemia, thrombosis and bone marrow failure.
- Life expectancy in untreated PNH patients is 10-15 years after diagnosis.[4, 5]
- Treatment of PNH relies on chronic intravenous (I.V.) dosing of anti-C5 mAbs (eculizumab or ravulizumab every 2 or 8 weeks, respectively), or twice-a-week subcutaneous dosing of an anti-C3 inhibitor (pegcetacoplan) (Figure 1A).

Nonclinical Studies with HMI-104

- HMI-104 is an AAVHSC vector and our developmental GTx-mAb candidate designed to elicit hepatic expression of a C5mAb for the treatment of PNH and alleviate the dependency on chronic dosing of anti-C5 therapeutics (Figure 1A).
- HMI-104 is delivered via a single (I.V.) injection and the expression of C5mAb can inhibit C5-mediated lysis of red blood cells (Figure 1B).
- Here, we present the results from two IND-enabling studies with HMI-104:
  - A 16-week dose range finding (DRF) study in NOD SCID mice which lack murine C5 (Figure 2)
  - A 4-week DRF study in humanized liver FRG® KO mice which express human C5 (Figure 3)

Results

16-Week DRF Study of HMI-104 in NOD SCID Male Mice

Objective

To determine the relationship between dose levels, liver vector genomes (vgs), mRNA levels, C5mAb serum concentrations, and *ex vivo* hemolysis over time.

Results (Figure 2)

- ✓ Dose-dependent increase in serum C5mAb levels, with concentrations rising steadily through 5 weeks post dose, and plateauing through 16 weeks post dose
- ✓ Linear range for steady state concentrations as a function of dose for Doses B-D
- ✓ Functional C5mAb determined in an *ex vivo* hemolysis assay using serum from HMI-104-treated NOD SCID mice
- ✓ Dose-dependent increase in liver vgs, with stable levels achieved by 4 weeks
- ✓ Dose-dependent increase in mRNA levels in liver mirroring changes in vgs
- ✓ Pharmacologically relevant dose range for HMI-104 in NOD SCID mice established as >A to <E, given that Dose A is considered to be minimally efficacious, and Dose E yielded same C5mAb levels as Dose D

4-Week DRF Study in the Humanized Liver FRG® KO Male Mice

Objective

To evaluate HMI-104 transduction (vgs) and mRNA levels in human hepatocytes *in vivo* and assess C5mAb levels in the presence of human C5.

Results (Figure 3)

- ✓ HMI-104 successfully transduced human hepatocytes *in vivo* and expressed sustained serum C5mAb levels in the presence of human C5
- ✓ C5mAb expressed in the presence of human C5 was functionally active at all doses and timepoints tested, as determined by complete inhibition of *ex vivo* hemolysis using serum from HMI-104 treated FRG® KO mice
- ✓ Vgs and transcript levels in purified human hepatocytes were dose dependent
- ✓ Antibody levels achieved in FRG® KO mice were greater than or equal to those obtained at steady state in the NOD SCID mouse model

References: (1) Sharma, Y, et al. "Transducing the Liver as an Antibody Factory Using AAVHSCs." Molecular Therapy. Vol. 29. No. 4. 50 Hampshire St, Floor 5, Cambridge, MA 02139 USA: Cell Press, 2021. (2) FRG® KO Model details (https://www.yecuris.com/frg-ko-mice/). (3) Hill, A, et al. "Paroxysmal nocturnal haemoglobinuria." Nature reviews Disease primers 3.1 (2017): 1-14. (4) Gérard, S, et al. The Lancet 348.9027 (1996): 573-577. (5) Kelly, R. J., et al. Blood, The Journal of the American Society of Hematology 117.25 (2011): 6786-6792.

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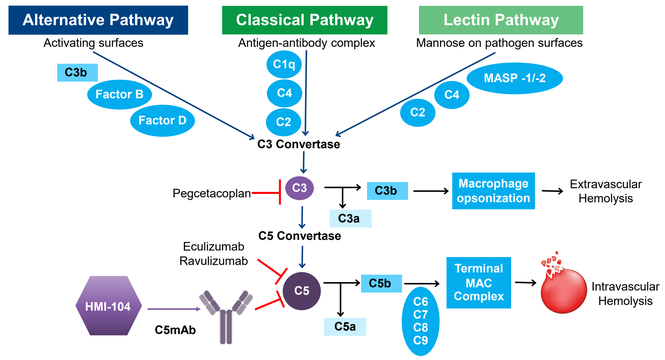
HMI-104 is a Single Dose GTx-mAb that Expresses a Vectorized Monoclonal Antibody Against C5 for the Treatment of PNH

A. Targeting C5 Node, Common to Three Complement Pathways



B. HMI-104: Systemic Delivery Results in Continuous and Sustained mAb Levels

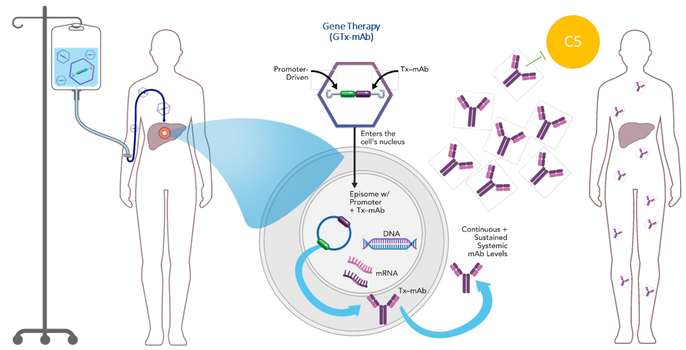
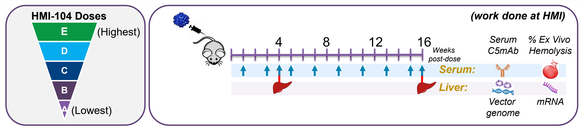


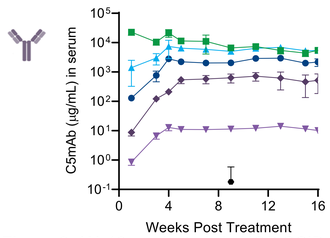
Figure 1: (A) Complement cascade highlighting approved C5 and C3 inhibitors and HMI-104 GTx-mAb approach; **(B)** HMI-104 is developed for one-time I.V. dosing, using the liver as an antibody factory to produce sustained expression of a C5mAb.

Treatment of NOD SCID with HMI-104 Results in Sustained, Dose-Dependent Expression of a Functional C5mAb, and Durable Liver VG and mRNA Levels

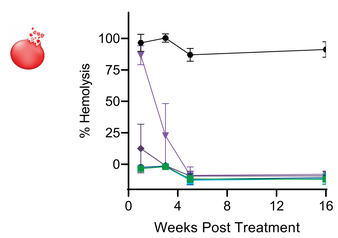
A. 16-Week DRF Study in NOD SCID Mice



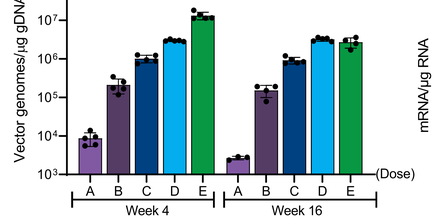
B. Dose-Dependent Expression of C5mAb



C. C5mAb is Active in *Ex Vivo* Hemolysis Assay



D. Liver Vg are Durable and Dose Dependent in A-D Dose Groups



E. mRNA Changes Mirror Changes in Vg

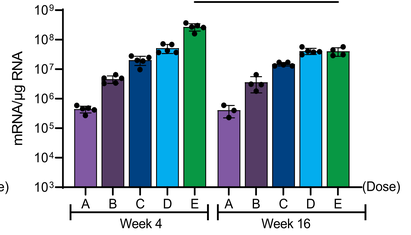
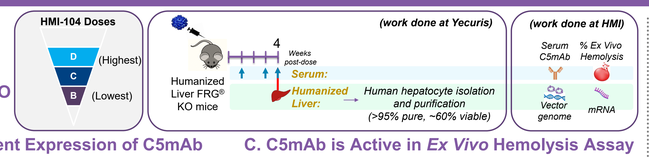


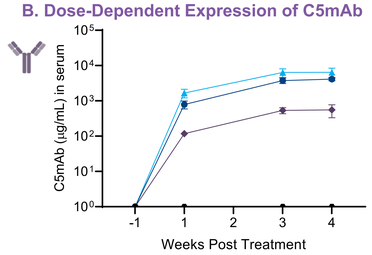
Figure 2: **(A)** 16-week study in NOD SCID male mice treated with HMI-104; **(B)** Time course of C5mAb levels in serum of NOD SCID mice following administration of HMI-104 at 5 doses (A-E) or vehicle control; **(C)** Inhibition of *ex vivo* hemolysis with serum from HMI-104-treated NOD SCID mice as a function of dose and time; **(D)** Dose-dependent changes in vgs and **(E)** mRNA in livers of HMI-104 treated mice. *(Data represented as mean ± S.D.)*

Treatment of Humanized Liver FRG® KO Mice with HMI-104 Results in Dose-Dependent Expression of a Functional C5mAb, with VG and mRNA in Human Hepatocytes

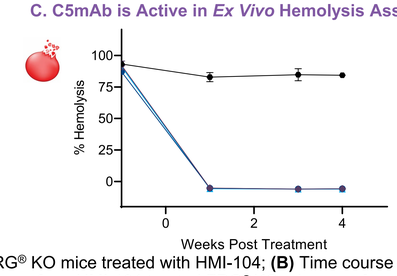
A. 4- Week DRF Study in Humanized Liver FRG® KO Mice



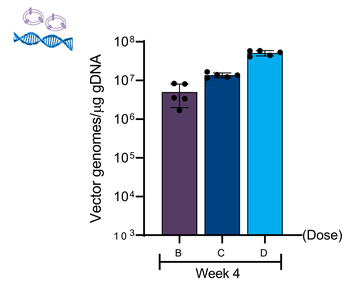
B. Dose-Dependent Expression of C5mAb



C. C5mAb is Active in *Ex Vivo* Hemolysis Assay



D. Dose-Dependent Changes in Vg in Purified Human Hepatocytes from FRG® KO Mice Treated with HMI-104



E. Dose-Dependent Changes in mRNA in Purified Human Hepatocytes from FRG® KO Mice Treated with HMI-104

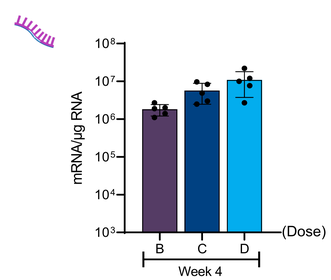


Figure 3: **(A)** 4-week study in humanized liver FRG® KO mice treated with HMI-104; **(B)** Time course of C5mAb in serum of FRG® KO mice following administration of HMI-104 at 3 Doses (B-D) or vehicle control; **(C)** Inhibition of *ex vivo* hemolysis with serum from HMI-104-treated FRG® KO mice as a function of dose and time (humanized liver FRG® KO mice express human and mouse C5, so assay adapted to inhibit murine C5; **(D)** Dose-dependent changes in vgs and **(E)** mRNA in human hepatocytes of HMI-104 Treated Mice. *(Data represented as mean ± S.D.)*

Conclusions

In conclusion, we present nonclinical data with HMI-104 demonstrating sustained expression of functional C5mAb levels in NOD SCID and humanized liver FRG® KO mice. These results support the development of HMI-104, which is currently in IND-enabling studies, for the treatment of PNH and complement-mediated disorders. Given the severity of PNH and the unmet need associated with available therapies, HMI-104 aims to provide, with a single I.V. treatment, sustained serum C5mAb sufficient to inhibit C5 complement-mediated lysis and reduce breakthrough and residual intravascular hemolysis associated with insufficient C5 antibody levels in PNH patients.