Development of an AAV Gene Therapy Vector FT-004 for Hemophilia B

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Introduction

Hemophilia B (Hemo B) is an inherited X-linked recessive bleeding disorder caused by mutations in *F9* gene leading to coagulation factor IX (FIX) deficiency or dysfunction. The current standard of care for severe Hemo B is prophylactic replacement of hFIX via frequent intravenous injections for life. Systemic delivery of adeno-associated viral (AAV) vectors engineered for liver transduction and hFIX expression is a promising method to treat patients with Hemo B. However, several early programs have failed likely due to low activity of wildtype FIX or loss of transgene expression.

To improve delivery efficiency and maintain FIX expression, Frontera has developed a series of AAV cassettes in either scAAV or ssAAV form containing a codon-optimized human FIX Padua variant driven by different liver-specific promoters. The CpG motifs in FIX Padua transgene sequence are eliminated to reduce potential immunogenic effect. We tested the 6 AAV vectors *in vitro* using Huh-7 cell line and in an F9-deficient hemophilia B mouse model. PA097 was selected as clinical candidate named FT-004 based on higher hFIX expression and activity to produce a significantly faster clotting *in vivo*. FT-004 was further evaluated the dose-dependent efficacy and safety in preclinical studies to support the clinical development for treat patients with Hemo B.

Result

Cassette optimization and screening

Construct ID	Design
PA092 (scAAV)	LP1-mSV40i-hF9-Padua-co1-SV40pA
PA093 (scAAV)	LP1-mSV40i-hF9-Padua-co2-SV40pA
PA094	LP1-mSV40i-hF9-Padua-co1-SV40pA
PA095	LP1-mSV40i-hF9-Padua-co2-SV40pA
PA096	LP2-hF9-Padua (intron1A)-co3-bGHpA
PA097	LP2-hF9-Padua (intron1A)-co2-bGHpA

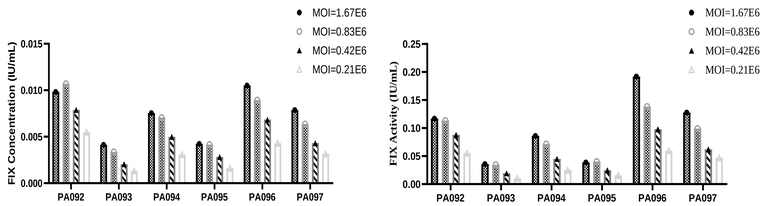

Fig 1. *In vitro* Screening of 6 rAAV5 Cassettes at Expression and Activity of FIX. A dose-dependent expression and activity of FIX were produced by 6 cassettes at 2 days after transduction in Huh-7 cells using ELISA and Chromogenic assay.

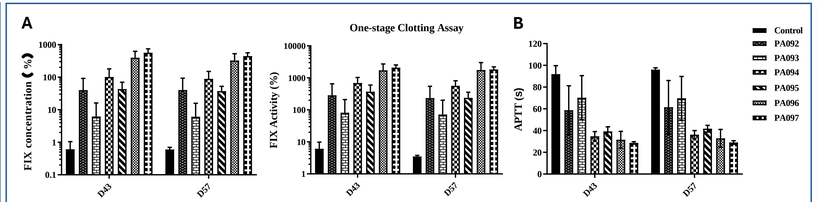

Fig 2. *In vivo* Evaluation of 6 rAAV5 Cassettes at Expression, Activity and Potency of FIX. 6 vectors were delivered to F9-deficient mice at 5×10^{11} vg/mouse via intravenously injection. At D43 and D57, the expression of FIX and FIX activity (A) and coagulation time (B) were measured using ELISA and one-stage clotting assay. The results showed a higher FIX activity and a significantly shorter APTT time produced by PA096 and PA097, and PA097 was selected as clinical candidate named as FT-004.

In vivo efficacy of FT-004 in F9-deficient mice

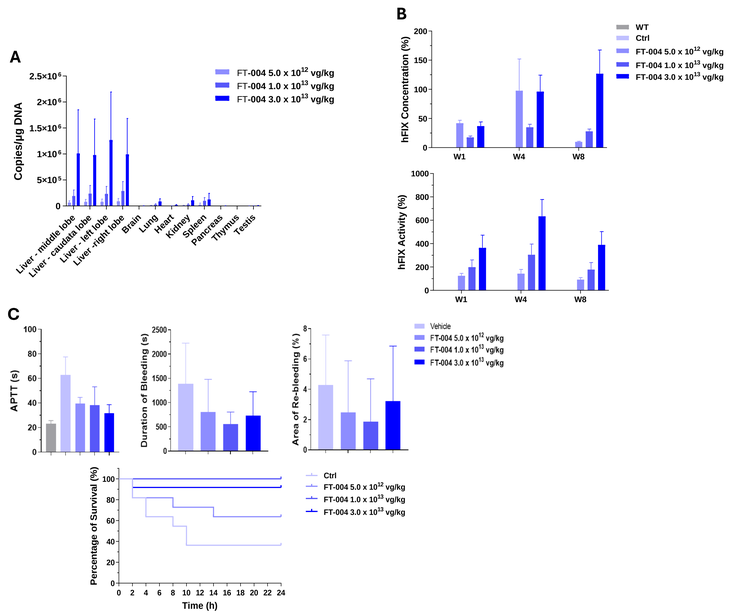

Fig 3. *In vivo* Efficacy Evaluation of FT-004 in F9-deficient Mice. A single dose of FT-004 at 5.0×10^{12}, 1.0×10^{13} and 3.0×10^{13} vg/kg via intravenously injection, FT-004 mainly distributed in liver (A), and expressed highly active hFIX in a dose-dependent manner (B), resulting in the shorter APTT and bleeding duration, decrease in re-bleeding area, and increase in survival rate (C). The minimum effective dose (MED) was determined to be 5.0×10^{12} vg/kg.

Comparison of hFIX expression and potency by FT-004 at different production scales

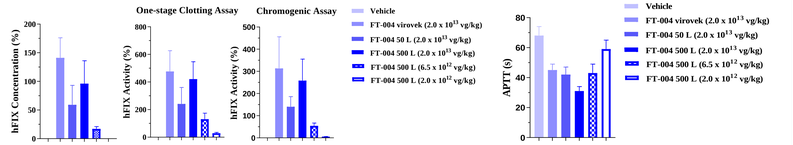

Fig 4. *In vivo* Comparison of Different Production Scales of FT-004 at hFIX Expression and Activity. 4 weeks after IV administration at 2×10^{13} vg/kg, different production scales of FT-004 (virovek-0.2 L, 50 L and 500 L) expressed highly active hFIX to improve the coagulation function (shorter ATPP) in F9-deficient mice, and no significant difference was noted. Also, FT-004 at different doses from 500 L scale showed a dose-dependent hFIX expression level and potency in F9-deficient mice.

Summary of FT-004 tolerability, biodistribution and hFIX expression in a 13-week GLP-compliant NHP study

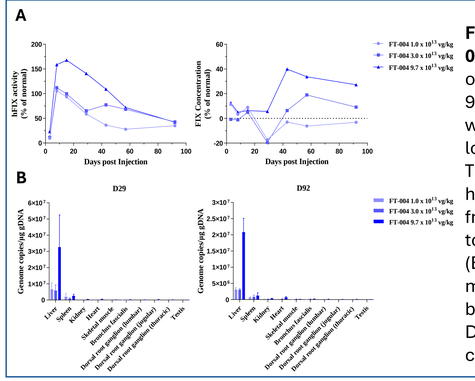

Fig 5. Safety and Biodistribution of FT-004 in NHPs. A single IV administration of FT-004 at 1.0×10^{13}, 3.0×10^{13} and 9.7×10^{13} vg/kg were well tolerated, where no test article related systemic or local abnormalities were observed. (A) The high baseline-corrected activity of hFIX was detected at 1W and declined from 4W post-dose, which may be due to the production of anti-hFIX antibody. (B) Genome copies of FT-004 were mainly distributed in the liver followed by the spleen, kidney, heart and etc. on D29 and D92, and minimal vector copies were detected in testis.

Conclusion

- Codon-optimized FIX Padua variant packaged into rAAV5 capsid (FT-004) provided robust expression of highly potent hFIX in vitro and in vivo.
- Intravenous administration of FT-004 specifically targeted to liver and expressed active hFIX leading to a significant improvement of coagulation function in F9-deficient mice during entire study period.
- FT-004 was well tolerated by NHPs, and NOAEL identified as 9.7×10^{13} vg/kg.
- FT-004, Frontera's clinical candidate, is currently advancing to Ph I clinical study.

Acknowledgement

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