Human Dose Prediction of a Novel Factor IX Variant Gene Therapy Candidate (AMT-180) Mediating Clotting Independently of Factor VIII

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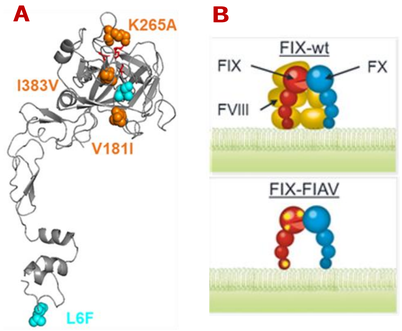
BACKGROUND

Hemophilia A (HemA) is an X-linked recessive disorder characterized by absent or deficient clotting factor VIII (FVIII). Expression of FVIII constructs following AAV delivery in HemA patients may wane over time, perhaps due to liver stress or other factors [1]. In addition, up to 30% of patients are currently ineligible for FVIII-based gene therapy due to current or past inhibitors. Management of patients with inhibitors is particularly challenging and relies on the use of bypass agents, such as recombinant FVIIa, prothrombin complex or bispecific antibodies that bridge FIX(a) and FX(a). However, this may be associated with increased thrombosis risk. Hence, there is a need for a non-FVIII, non-thrombogenic and durable gene therapy to treat HemA.

The novel AAV5 gene therapy clinical candidate AMT-180 encodes a transgene for a variant of FIX, FIX-FIAV, which possesses 4 amino acid substitutions *vs* wild-type FIX (Figure 1A) under the control of a proprietary primate-specific liver promoter. Upon activation of FIX-FIAV to activated FIX-FIAV (FIX-FIAVa) hemostasis is induced through FVIII-independent activation of FX (Figure 1B, lower) [2]. AMT-180 is therefore a strong candidate to treat inhibitor and non-inhibitor HemA patients.

Figure 1. Normal and FVIII-independent clotting in the presence of FIX-FIAV



A: FIX-FIAV consists of 4-amino acid additions in WT FIX **B.** the mechanism of action of WT (upper) and FIX-FIAV (lower).

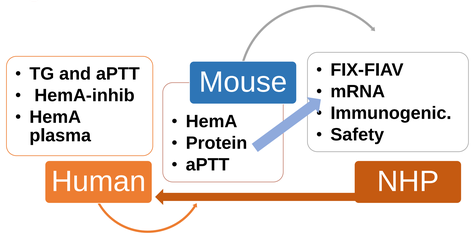
OBJECTIVES

- Translational interplay between human, mouse and non-human primate (NHP) was assessed to provide a thorough understanding of dose-translation for first-in-human (FIH) dosing.

METHODS

- Experiments were performed in different species [3]:
  - Human: Thrombin Generation and activated partial thromboplastin time assays (TGA and aPTT, respectively) were carried out.
  - HemA mice were injected intravenously (i.v.) with AAV5-FIX-FIAV, under the control of a promoter optimized for murine expression. Blood samples were taken (5 and 8 weeks) and aPTT and protein levels used to determine the percentage of FVIII-independent activity / 100% FIX-FIAV protein expression.
  - Pilot and GLP-toxicological experiments were performed in the NHP (cynomolgus macaque).

Figure 2. AMT-180: Dose Translation



Human data were used as confirmatory, while mouse aPTT and FIX-FIAV data were used to determine FVIII-independent activity / 100% FIX-FIAV protein. These data were used to estimate FVIII-independent levels in the NHP. NHP immunogenic responses were assessed to help interpret data and decide whether data from specific subjects be included.

RESULTS

FIX-FIAV gene therapy showed excellent tolerability and pharmacodynamic (PD) properties.

- Human Plasma
  - rFIX-FIAV induced TG and aPTT effects in the presence of FVIII-inhibitors in NPP and in HemA plasma, characteristic of FVIII-independent clotting/hemostasis.

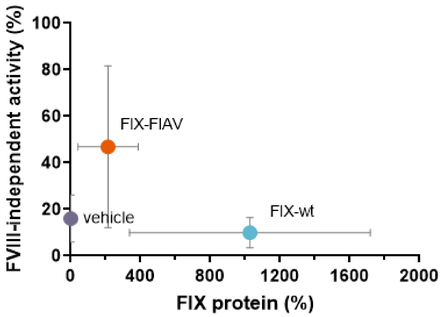
Table 1. rFIX-FIAV-induced Thrombin Generation and aPTT in Human Plasma

Matrix	TGA (%)	aPTT (%)
Depleted NPP	29	32±6
Hem A Plasma	18	*nd*

%, values are *vs* 100% FVIII-control. Studies were in depleted FVIII-normal pooled human plasma (NPP) or HemA plasma. All rFIX-FIAV studies were performed at 5 µg/mL FIX-FIAV, equivalent to 100%.

- Mouse
  - Injection of AAV5-FIX-FIAV in HemA mice was well tolerated and did not increase coagulation activation markers (TAT and D-dimer).
  - PD data showed FVIII-independent clotting, as expected [2]. Up to 24% FVIII-independent activity was seen in HemA mice.

Figure 3. AAV5.FIX-FIAV: FVIII-independent clotting in the HemA mouse

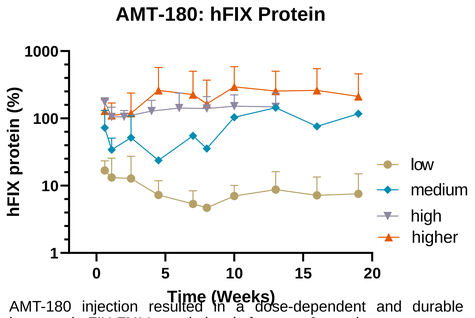


Blood samples were taken (5 and 8 weeks; 5-week data shown). aPTT was used to determine the percentage of FVIII-independent activity / 100% FIX-FIAV protein expression, i.e. 24 and 13.5% at week 5 and 8, respectively.

NHP

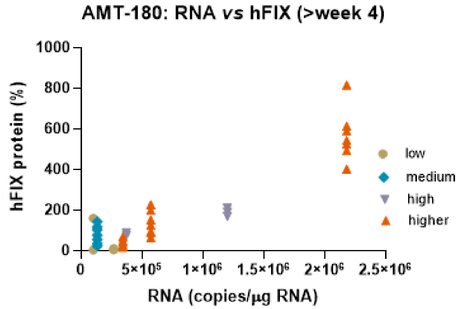
- AMT-180 in the NHP was well tolerated, with no hepatotoxicity nor any coagulation activation.
- Cross-species antibodies (IgG) were seen in some subjects after ~2 weeks, which is common. These subjects were excluded from analyses after week 4. Where possible, day 8 data are shown.
- There was a clear relationship-between FIX-FIAV mRNA and plasma FIX-FIAV protein.
- Dose *vs* FIX-FIAV protein comparison showed a clear dose-protein relationship.

Figure 4. AMT-180: Dose-dependent Increase of FIX-FIAV in the NHP



AMT-180 injection resulted in a dose-dependent and durable increase in FIX-FIAV protein levels for up to 6 months.

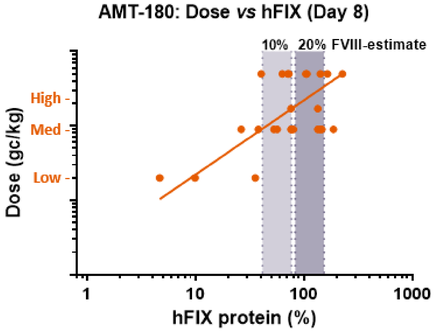
Figure 5. Dose-dependent correlation between mRNA and plasma hFIX



Terminal hepatic FIX-FIAV mRNA (liver transduced) and circulating protein correlated well with protein measurements.

- Finally, dose *vs* FIX-FIAV protein-relationships were assessed to completely understand the interrelationship between FIX-FIAV and theoretical FVIII-independent activity.

Figure 6. NHP AMT-180-induced hFIX and FVIII-independent activity relationship



AMT-180 injection resulted in a dose-dependent increase in FIX-FIAV protein levels, as shown on day 8. The bars indicate the estimated FVIII-independent activity based on Hem A mouse data.

CONCLUSIONS

Despite translational challenges and immunogenic responses in some subjects, the current preliminary data demonstrated i. RNA *vs* protein correlation and ii. dose-dependent protein responses. Primate studies used an optimized promoter, enabling a much higher protein/RNA ratio, which is critical in obtaining clinically-relevant FVIII-indirect actions. These data support meaningful hemostasis in Hemophilia A patients with and without inhibitors[4] following administration with AMT-180.

REFERENCES

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