Predictable Protein Expression with Enhanced Factor IX Activity Following Administration of a Modified AAV5-hFIX Vector to Nonhuman Primates

Ying Poi Liu, Jacek Lubelski, Erich Ehlert, Sander Gielen, Paula Montenegro-Miranda, Martin de Haan, Bart Nijmeijer, Harald Petry

uniQure N.V., Amsterdam, The Netherlands

BACKGROUND

Gene therapy for hemophilia B is advancing rapidly and offers the possibility to achieve sustained amelioration of the bleeding phenotype with a single treatment in patients with severe disease. The efficacy and safety of AMT-060, an investigational gene therapy construct comprising an adeno-associated virus (AAV) serotype 5 capsid containing a codon-optimized wildtype FIX gene under control of a liver-specific promoter, were extensively studied during a phase I/II clinical trial. Patients who received $2x10^{13}$ gc/kg AMT-060 showed sustained and durable factor IX (FIX) activity of 3-13% and substantial reductions in spontaneous bleeding without evidence of T-cell mediated hepatotoxicity. The Padua variant has been previously identified as a hyperfunctional variant with 8-9-fold increased specific activity compared to wildtype FIX (Simioni *et al.* 2009).

OBJECTIVES

- To evaluate the efficacy and safety of introducing the Padua, gain-of-function substitution, in the AMT-060 cassette in nonhuman primates (NHPs).

METHODS

- AMT-060 is an AAV5 vector with a gene cassette containing a liver specific (LP1) and a codon-optimized wildtype FIX gene.
- The novel vector, AMT-061, is based on AMT-060, created by incorporation of the Padua substitution, R338L, by a 2-nucleotide change into the codon optimized FIX gene.
- Male cynomolgus macaques (n=3 per group) received intravenous administrations of AMT-060 (5e12 gc/kg) or AMT-061 (the Padua variant) in a range of doses (5e11 to 9e13 gc/kg) or the vehicle control.
- Blood was collected throughout the study and the following measurements were made in plasma:
  - Human FIX (hFIX) protein expression (using a human-specific ELISA)
  - FIX activity by activated partial thromboplastin (APTT) assay
    - hFIX activity was estimated by correcting for each monkey's endogenous baseline activity
  - Thrombotic markers included D-dimer and thrombin-anti-thrombin (TAT) complex
  - Markers of liver function including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)
- Vector DNA levels were determined as the Area-Under-Curve (AUC) of the plasma curves over time.
- Toxicological parameters (clinical observations, biochemistry) were assessed at various time points after treatment and full (histo-) pathological examination was performed at the end of the study.

RESULTS

At equal doses, as expected, circulating vector DNA plasma levels, liver distribution, liver cell transduction and hFIX protein expression were consistent for both AMT-060 and AMT-061 (**Table 1**).

Table 1. Characteristics of AMT-061 vs AMT-060

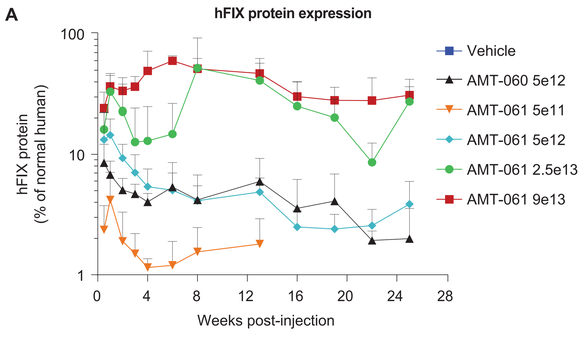
AMT	Dose gc/kg	Plasma AUC gc/ml	Liver vector DNA gc/µg	Liver FIX RNA gc/ µg	Liver cell transduction (%)	hFIX protein (%) wk 4-13	FIX clotting activity (%) wk 4-13*	Ratio FIX activity 061/060*
060	$5.0x10^{12}$	$3.22x10^{10}$	$1.0x10^{6}$	$7.3x10^{4}$	17.1	4.89	9.1	6.5
061	$5.0x10^{12}$	$3.25x10^{10}$	$1.7x10^{6}$	$7.3x10^{4}$	16.7	4.85	58.9	

**FIX activity was corrected for monkey endogenous baseline activity; gc, genome copies.*

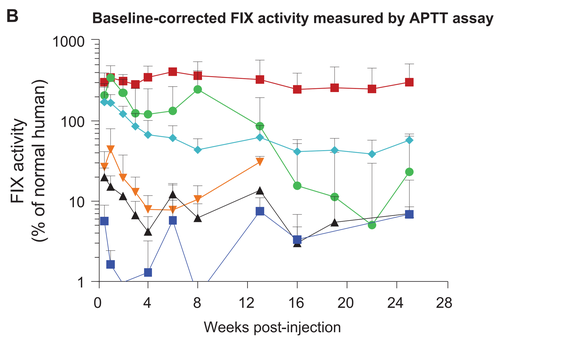
EFFICACY TESTING OF AMT-061 IN CYNOMOLGUS MACAQUES

- AMT-061 resulted in dose-dependent increases in hFIX protein expression and activity (**Figure 1a and 1b**).
- hFIX protein expression correlated well with FIX activity (**Figure 1c**).
- AMT-061 and AMT-060 showed similar hFIX protein expression, while FIX activity was ~6.5-fold higher in animals receiving AMT-061 (**Figure 2a and b**).

Figure 1. Compared to AMT-060, AMT-061 shows similar hFIX protein expression with enhanced FIX activity.



hFIX protein expression in NHPs after administration of AMT-061 and AMT-060. Lines represent average of 3 animals per group +/- standard deviations.



Baseline corrected FIX activity in the plasma of NHPs that received AMT-061 and AMT-060. Lines represent average of 3 animals per group +/- standard deviations.

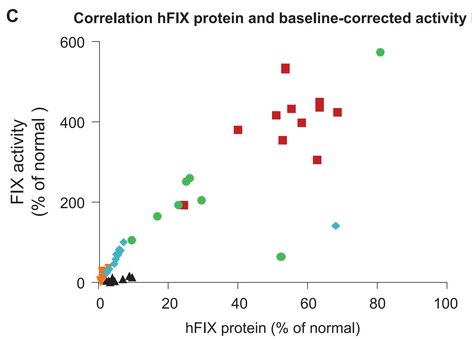
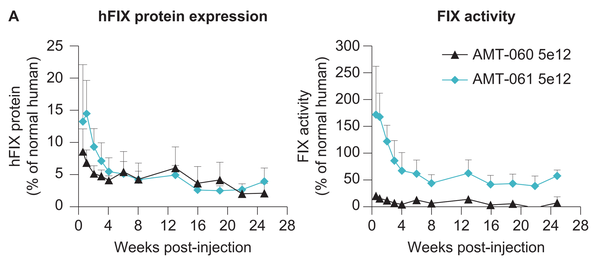
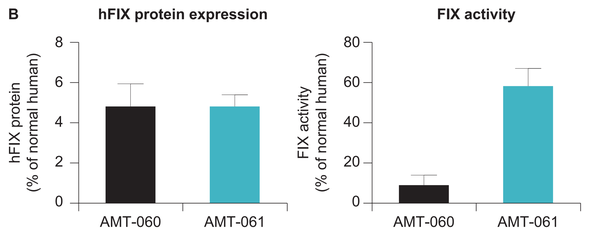


Figure 2. AMT-061 shows similar protein expression, but 6.5-fold increased FIX activity after baseline correction



hFIX protein expression and FIX activity in plasma of the NHPs that received AMT-060 and AMT-061 at 5e12 gc/kg. The lines represent average levels of 3 animals.

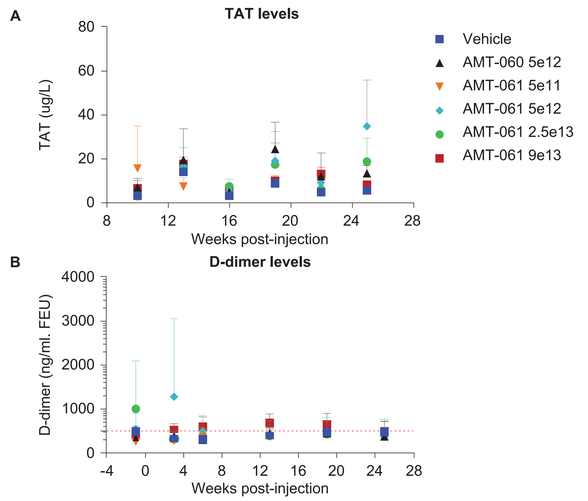


Average of hFIX protein and FIX activity levels in plasma of the NHPs that received AMT-060 and AMT-061 at 5e12 gc/kg from Week 4 to Week 13 (+/- standard deviation).

SAFETY TESTING OF AMT-061 IN CYNOMOLGUS MACAQUES

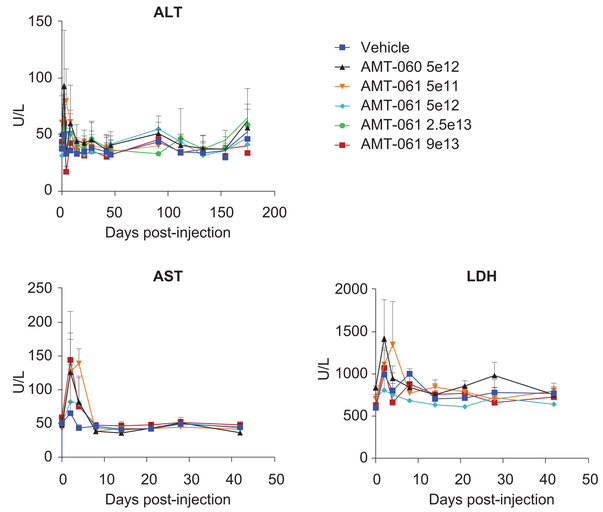
- No increase in thrombogenic risk was associated with AMT-061 at equivalent doses to AMT-060 (**Figure 3**).
- At day 2 post-treatment, minor elevations in ALT, AST and LDH were observed in almost all groups, which returned to pre-dose values at day 4 and stayed similar during the observation period (**Figure 4**).

Figure 3. Administration of AMT-061 is not associated with an increased risk of thrombosis in NHPs



TAT, Thrombin anti-thrombin. TAT levels were measured at different time points and the average per group is indicated. D-dimers were measured at different time points and the average per group is shown. The red dashed line indicates the 500 ng/ml (FEU) that is the upper limit in the normal adult population.

Figure 4. Liver enzyme profile of NHP plasmas post-treatment



ALT, Alanine amino-transferase; AST, aspartate amino-transferase; LDH, lactate dehydrogenase. Assessment of liver enzymes (ALT, AST, and LDH) was performed before treatment and at several different time points after treatment.

SUMMARY AND CONCLUSIONS

- Administration of AMT-061 via peripheral vein infusion resulted in a dose-dependent expression of FIX protein in NHPs.
- Compared to AMT-060, AMT-061 mediated similar hFIX protein expression, but 6-7-fold higher baseline-corrected FIX activity levels confirming the higher potency of the FIX-Padua variant.
- At a dose of 5e12 gc/kg, AMT-060 and AMT-061 showed similar circulating vector DNA plasma levels, liver distribution, liver cell transduction and transgene expression.
- Expression of hFIX-Padua was not associated with abnormal activation of coagulation in NHPs.
- The in-life, hematology and clinical chemistry parameters were unaffected by treatment with AMT-060 and AMT-061.
- Safety assessments revealed no adverse findings and the highest dose was the No Observed Adverse Effect Level (NOAEL).
- No toxicological findings or target organ defects were detected after either AMT-060 or AMT-061 administration in the toxicity study.
- Results from this study demonstrate that the safety of AMT-061 is comparable to AMT-060 in NHPs and confirm that AMT-061 displays the expected increase in clotting activity.

REFERENCES

Simioni P, Tormene D, Tognin G, Gavasso S, Bulato C, Iacobelli NP, Finn JD, Spiezia L, Radu C, Arruda VR. X-linked thrombophilia with a mutant factor IX (factor IX Padua). N Engl J Med. 2009 Oct 22;361(17):1671-5. doi: 10.1056/NEJMoa0904377.

DISCLOSURES

YP Liu, J Lubelski, E Ehlert, S Gielen, P Miranda and B Nijmeijer are employees of uniQure Biopharma B.V. M de Haan is working as a consultant at uniQure Biopharma B.V.

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