Clearance of Vector DNA Following Systemic Administration of AAV5-hFIX or AAV5-hFIX Padua in Patients with Severe or Moderate-Severe Hemophilia B

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INTRODUCTION

- Current adeno-associated viral (AAV) vector-based gene therapy strategies for hemophilia rely on systemic administration of the vector.
- Durable expression of the transgene has been reported, yet information on vector clearance is still limited.
- **Vector clearance:** Measurement of the persistence of the vector in the bodily matrices (eg: "shedding").
- We examined the presence of vector DNA in participants from a Phase 1/2 study of an AAV5-hFIX wildtype construct (AMT-060; NCT02396342) and a Phase 2b study utilizing the enhanced version, AAV5-hFIX Padua (etranacogene dezaparvovec [AMT-061]; NCT03489291).[1,2]

METHODS

- Adult males with severe or moderately-severe hemophilia B received a single intravenous infusion of AMT-060 (Phase 1/2) at 5×10^{12} genome copies (gc)/kg (low dose) or 2×10^{13} gc/kg (high dose), or etranacogene dezaparvovec (Phase 2b) at 2×10^{13} gc/kg in two ongoing trials.[1,2]
- Samples were collected at all visits. Vector clearance was confirmed by finding of vector DNA either zero or below the limits of detection (LOD) for three consecutive measurements.
  - AMT-060 trial: weekly visits (weeks 0-12), every 2 weeks (weeks 13-26), quarterly week 27 to year 3 and twice yearly to year 5.
  - Etranacogene dezaparvovec trial: weekly visits (weeks 0-12), every two weeks (weeks 13-26), monthly (weeks 27-52) and twice yearly to year 5.
- Assessments in both trials included efficacy and safety outcomes as well as vector clearance in whole blood and semen.
  - In the AMT-060 trial, vector clearance was also measured in nasal secretions, feces, urine, and saliva.
- Vector clearance was analyzed using a validated, real time, qPCR for vector DNA in bodily fluids.
  - Results were presented as copies per mL (blood, semen, saliva, urine), per mg (feces) and per swab (nasal secretions).
  - Theoretical LOD were <400 copies per mL (semen and saliva), <571 copies/mL or copies per swab (whole blood, urine, nasal swab) and circa 1 copy per mg (feces).

RESULTS

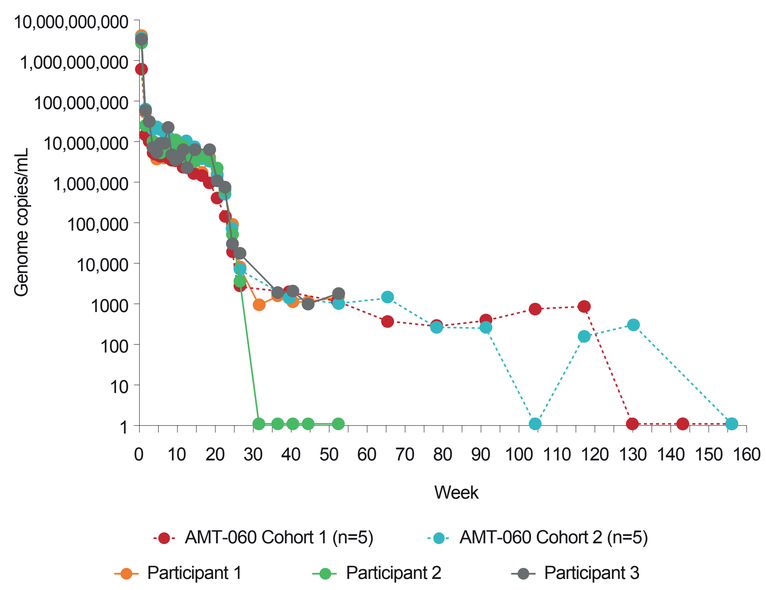
- AMT-060 resulted in sustained improvement in FIX activity for up to 4 years (mean FIX activity was 5.1% [low dose group at 4 years] and 7.5% [high dose group at 3.5 years]) and treatment with etranacogene dezaparvovec resulted in mean FIX activity of 41% at 52 weeks.
- Both AAV5-hFIX and AAV5-hFIX Padua were safe and well tolerated; no unexpected treatment-related adverse events (TRAE) have been observed with longer-term follow up.
- Table 1 describes the time in weeks to the first and last of three consecutive measures of vector DNA of either zero or <LOD for all bodily fluids for AMT-060 and etranacogene dezaparvovec.
  - AMT-060 at the higher dose was cleared from semen, feces, urine, nasal secretions and saliva in all participants by week 78 (range 7–78 weeks).
  - In blood, the lower dose of AMT-060 was cleared in all participants by 3 years (range 1.0–3.0 years).
  - The higher AMT-060 dose was cleared from the blood in all participants by 3.7 years (range 1.8–3.7 years).
- With etranacogene dezaparvovec, vector DNA was <LOD in blood in 2 participants by weeks 31–48 and in semen by week 26 in 2 of 3 participants (**Table 1**).
  - Three consecutive <LOD values achieved by week 40 by participant 2 (blood) and by week 52 in participant 1 (semen).
  - Participants 1 and 3 achieved <lower limit of quantification (LLOQ) values in blood by weeks 31-36, however, <LLOQ measurements were not considered to be negative.
  - Participant 3 only had semen samples up to week 12.

Table 1. Time to clearance of vector DNA from bodily fluids

Bodily fluid/ secretion	Range in weeks until first and last of three consecutive measurements of vector DNA either zero or below LOD					
	AMT-060 (5×10^{12} gc/kg, n=5)		AMT-060 (2×10^{13} gc/kg, n=5)		Etranacogene dezaparvovec (2×10^{13} gc/kg, n=3)	
	First	Last	First	Last	First	Last
Blood	27-130	52-158	69-159	93-192	Participant 1: 48	NA
					Participant 2: 31	40
					Participant 3: NA	NA
Feces	6-16	14-20	16-40	20-64	Not tested	
Nasal secretions	5-18	7-22	7-26	9-64	Not tested	
Saliva	6-20	8-24	9-26	11-78	Not tested	
Semen	9-52 (n=4)*	14-90 (n=4)*	12-40	17-64	Participant 1: 26	52
					Participant 2: 26	2nd consecutive LOD week 52
					Participant 3#: Not available	Not available
Urine	3-11	5-14	8-22	10-26	Not tested	

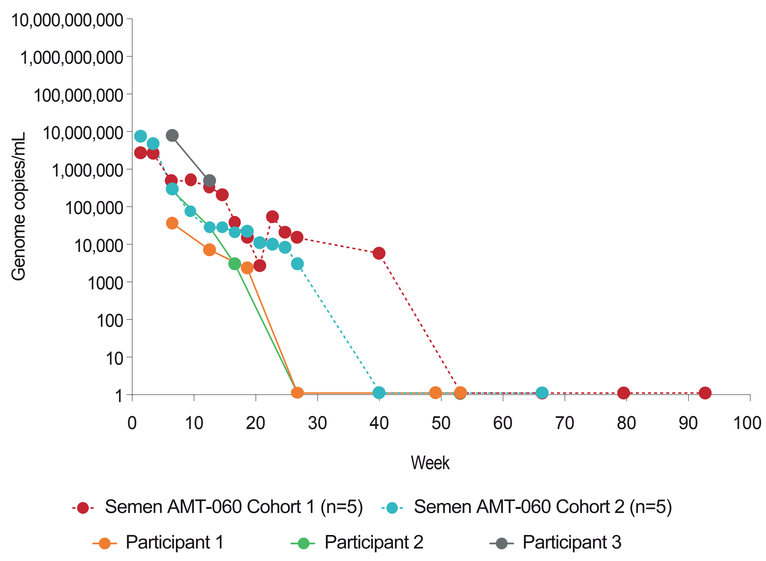
**Participant 4 unable to provide sample; #No data after week 12, samples were positive up to that timepoint. LOD, limit of detection; NA, not applicable.*

Figure 1. Comparison of vector clearance from blood with AMT-060 and etranacogene dezaparvovec



Values at 1 gc/mL were below the theoretical limit of detection (<571 copies/mL for blood).

Figure 2. Comparison of vector clearance from semen with AMT-060 and etranacogene dezaparvovec



Values at 1 gc/mL were below the theoretical limit of detection (<400 copies/mL for semen). Etranacogene dezaparvovec shedding data in participant 3 were only available up to week 12.

CONCLUSION

- Post-AMT-060 treatment, vector DNA in the high dose group was cleared by 18 months in all bodily fluids except blood.
  - AMT-060 was cleared from the blood in 100% of participants in the low dose group at 3 years and in all participants in the high dose group by 3.7 years.
- Etranacogene dezaparvovec vector DNA was cleared from the blood in 1 participant by week 40, and was low but detectable (<LLOQ) in the other 2.
- Etranacogene dezaparvovec vector DNA was cleared in the semen of 1 participant by week 52 and had tested <LOD on 2 consecutive tests in a second participant.
- The presence of vector DNA in bodily fluids assessed was not associated with any adverse safety or efficacy findings.

REFERENCES

1. Miesbach W, et al. Blood 2018;131:1022-1031; 2. Von Drygalski A, et al. Blood Advances 2019; 3:3241-3247.

DISCLOSURES

All authors are full time employees of uniQure biopharma B.V.

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