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

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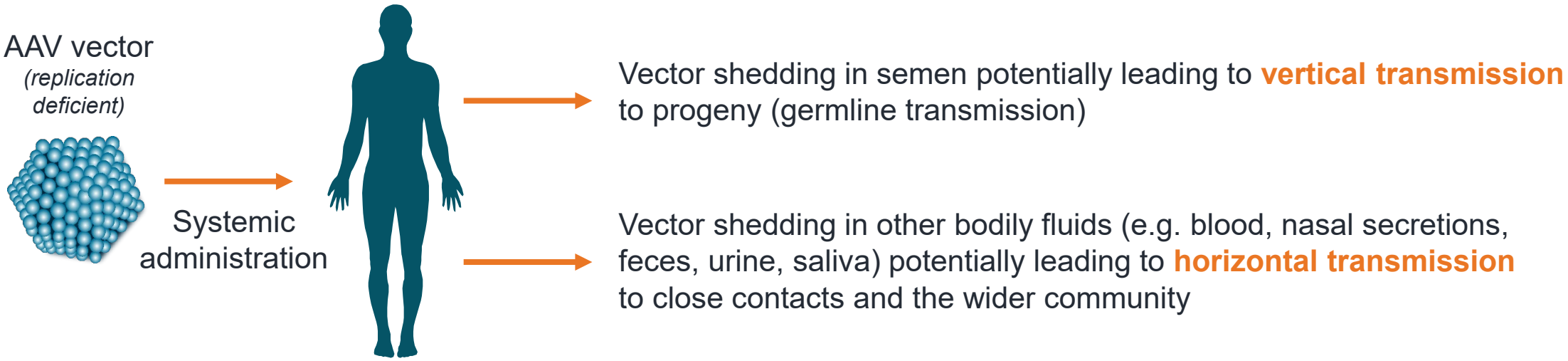
Disclosures

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

Why examine vector shedding?

- **Shedding** is a term typically used to describe the release of infectious virus into the environment [1]



- It is a **regulatory requirement** to assess vector shedding data during development of gene therapy products[2]

AAV, adeno-associated virus

1. 1. Pipe S, et al. Mol Ther Meth Clin Dev. 2019;15:170-178; 2. https://www.fda.gov/media/89036/download

Transmission risk from shedding of AAV vectors is considered low

- First clinical trial of AAV gene therapy for hemophilia B (delivered via the hepatic artery) was placed on hold after vector DNA was detected in semen[1]
  - Raised concerns about the risk of inadvertent germline transmission
- A study in nonhuman primates showed infectious vector was present exclusively in serum for 48-72 h after receiving IM injections of AAV vector[2]
  - Vector DNA was detectable by PCR in all excreta
- No evidence of vertical transmission following IV administration of AAV5-hFIX to male mice[3]
- Risk of vertical transmission is considered negligible;[4] however, barrier contraception is recommended as a precaution in humans treated with gene therapy until **clearance** of vector DNA in semen is confirmed

AAV, adeno-associated virus; NHP, non-human primates; IM, intramuscular; IV, intravenous; hFIX, human Factor IX

1. Manno CS, et al; Nat Med 2006;12:342-7, 2. Favre D, *et al. Mol Ther.* 2001;4(6):559-566, 3. Sponck L, et al. Poster presented at ISTH 2019, 4. https://osp.od.nih.gov/wp-content/uploads/2013/12/Dec01minutes.pdf

Vector shedding versus vector clearance

- Current **vector shedding assays** use qPCR to measure vector DNA in bodily fluids post-treatment
  - They do not distinguish between different forms of vector DNA (free, encapsidated, episomal, or integrated)[1,2]
  - Detection of vector DNA in bodily fluids does not necessarily imply an infectious risk, nor diminished efficacy[1]

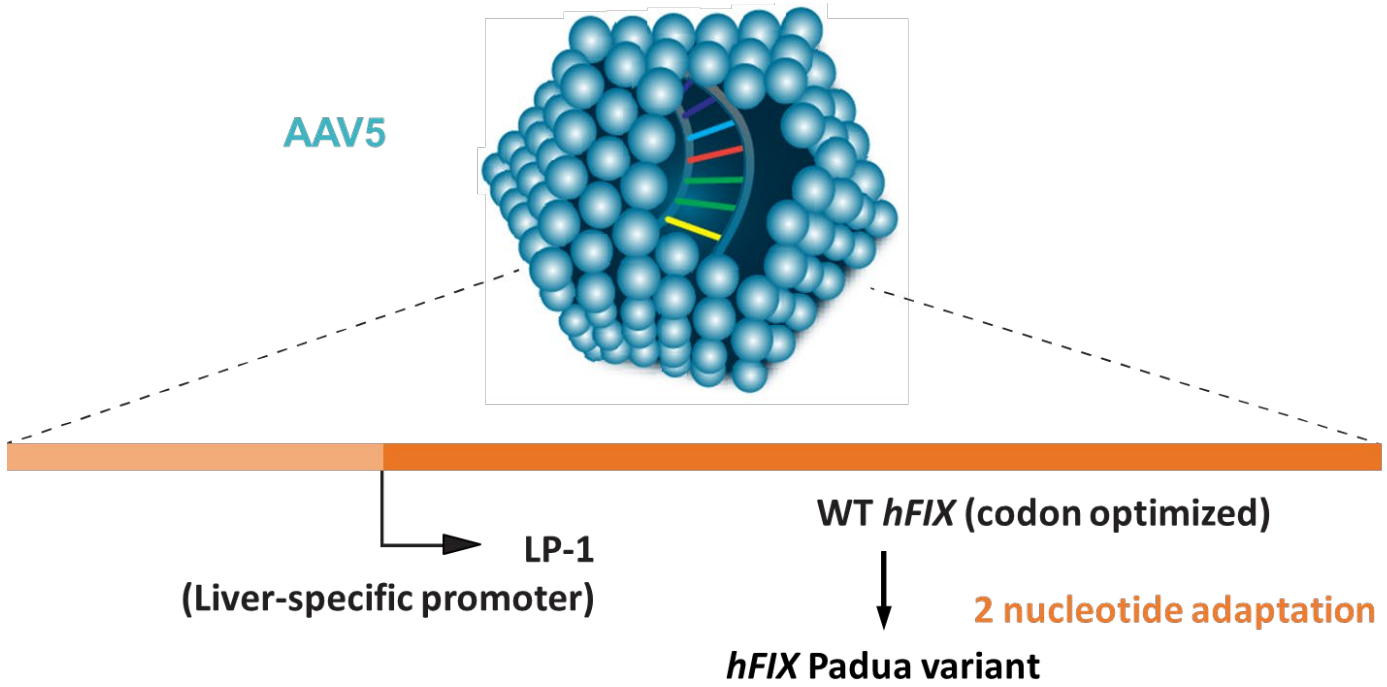
- **Vector clearance** reflects the rate at which vector is removed from the body following administration of gene therapy[1]

IV, intravenous; hFIX, human Factor IX; qPCR, quantitative polymerase chain reaction

1. Pipe S, et al. Mol Ther Meth Clin Dev. 2019;15:170-178; 2. Gil-Farina, et al. Mol Ther. 2016;24:1100-1105

Investigational AAV Gene Therapy for Severe or Moderate-Severe Hemophilia B

- **AMT-060** comprises an AAV5 vector containing WT hFIX protein and a liver-specific promotor[1,2]
- **Etranacogene dezaparvovec** is a modified version of AMT-060
  - Encodes hFIX Padua protein
  - 6-7-fold greater FIX activity vs. WT hFIX[3-4]
- Both are administered intravenously
- Durable transgene expression has been demonstrated;[1-3] information on **vector clearance** is limited



AAV, adeno-associated virus; WT, wildtype; hFIX, human FIX gene

1. Miesbach et al. Blood 2018;131:1022-31; 2. Leebeek et al. Res Pract Thromb Haemost. 2019;3(S1):8; 3. Von Drygalski A, et al. Blood Adv. 2019;3:3241-3247; 4. Cantore A, et al. Blood 2012;120:4517-20

AAV5-hFIX gene therapy is being evaluated in two ongoing clinical studies

Phase 1/2 study[1-3] AMT-060 (AAV5-hFIX)

Patients

10 adults with moderate-severe or severe hemophilia B (FIX activity ≤2%)

Treatment

A single IV dose of AMT-060:
Cohort 1: $5x10^{12}$ gc/kg
Cohort 2: $2x10^{13}$ gc/kg

Phase 2b study[4-5] Etranacogene dezaparvovec (AAV5-hFIX Padua)

Patients

3 adults with severe or moderate-severe hemophilia B (FIX activity ≤2%)

Treatment

A single IV dose of etranacogene dezaparvovec ($2x10^{13}$ gc/kg)

Key outcomes

FIX activity, bleeds, FIX replacement usage, and safety (**including vector clearance**) monitored for up to 5 years

hFIX, human Factor IX; IV, intravenous

1.NCT02396342; 2. Miesbach et al. Blood 2018;131:1022-31; 3. Leebeek et al. Res Pract Thromb Haemost. 2019;3(S1):8;
4. NCT03489291; 5. Von Drygalski A, et al. Blood Adv. 2019;3(21):3241-3247

AAV5-hFIX gene therapy is well tolerated and leads to sustained increases in FIX activity, reduced bleeds and reduced FIX use

Phase 1/2 study[1-3] AMT-060

Dose-dependent increases in FIX activity

Cohort 1: Mean FIX activity 5.1% at 4 years
Cohort 2: Mean FIX activity 7.5% at 3.5 years

Sustained reductions in FIX use and bleeds

FIX use reduced by 90-100%
Bleeds reduced by 77-100%
8/9 patients discontinued prophylaxis

Generally safe and well-tolerated

No development of inhibitors
Mild asymptomatic elevations in liver enzymes in 3/10 patients; resolved without loss of FIX activity or capsid-specific T-cell activation

Phase 2b study[4-5] Etranacogene dezaparvovec

Increased FIX activity

Mean FIX activity 41% at 52 weeks

Sustained reductions in FIX use and bleeds

97.6-100% reduction in FIX use
Bleeds reduced by 100%

Generally safe and well-tolerated

2 AEs possibly related to treatment
No loss of FIX activity; no FIX inhibitor development
No clinically significant elevations in liver enzymes
No requirement for immunosuppression

hFIX, human Factor IX; AE, adverse event

1.NCT02396342; 2. Miesbach et al. Blood 2018;131:1022-31; 3. Leebeek et al. Res Pract Thromb Haemost. 2019;3(S1):8;
4. NCT03489291; 5. Von Drygalski A, et al. Blood Adv. 2019;3(21):3241-3247

Vector clearance following AAV5-hFIX gene therapy is being evaluated in two ongoing clinical studies

Phase 1/2 study[1-3] AMT-060 (AAV5-hFIX)

Sample collection

Samples were collected weekly (weeks 0-12), every 2 weeks (weeks 13-26), quarterly week 27 to year 3, and twice yearly to year 5

Vector clearance was determined in **whole blood, semen, nasal secretions, feces, urine, and saliva**

Trial Status

In long-term follow-up; data available for 3.5-4 years

Phase 2b study[4-5] Etranacogene dezaparvovec (AAV5-hFIX Padua)

Sample collection

Samples were collected weekly (weeks 0-12), every two weeks (weeks 13-26), monthly (weeks 27-52), and twice yearly to year 5.

Vector clearance was determined in **whole blood** and **semen**

Trial Status

In follow-up; data available for 1 year

Vector clearance:

Analyzed using qPCR to measure the presence of vector DNA in bodily fluids

Confirmed when 3 consecutive measurements of vector DNA resulted in values <LOD* or zero

*Theoretical LOD were <400 copies/mL (semen and saliva), <571 copies/mL or copies/swab (whole blood, urine, nasal swab) and circa 1 copy/mg (feces). FIX, factor IX; qPCR, quantitative real time polymerase chain reaction; LOD, limit of detection

1. NCT02396342; 2. Miesbach et al. Blood 2018;131:1022-31; 3. Leebeek et al. Res Pract Thromb Haemost. 2019;3(S1):8;
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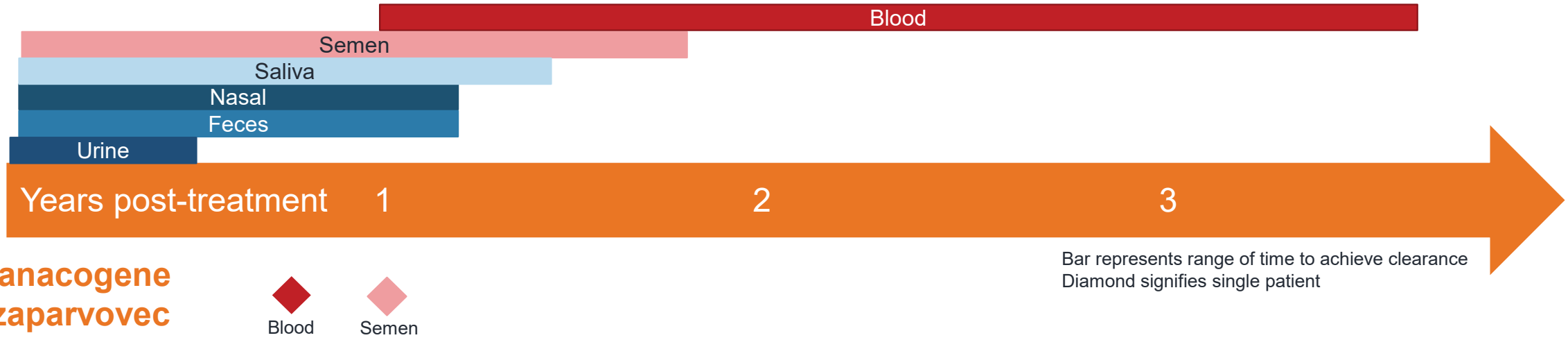
Time to clearance of vector DNA from bodily fluids

AMT-060



Etranacogene dezaparvovec

Vector clearance:
3 consecutive measurements of vector DNA resulted in values <LOD* or zero

LOD, limit of detection; LLOQ, lower limit of quantification
Theoretical LOD: <400 copies /mL (semen and saliva), <571 copies/mL or copies/swab (whole blood, urine, nasal swab), ~ 1 copy /mg (feces)

AMT-060 study: Time to clearance of vector DNA from bodily fluids

	Weeks (range) until the first and last of 3 consecutive measurements of vector DNA were zero or <LOD			
Bodily fluid/secretion	**AMT-060 ($5x10^{12}$ gc/kg); n=5**		**AMT-060 ($2x10^{13}$ gc/kg); n = 5**	
	First	Last	First	Last
Blood	27-130	52-158	69-159	93-192
Feces	6-16	14-20	16-40	20-64
Nasal secretions	5-18	7-22	7-26	9-64
Saliva	6-20	8-24	9-26	11-78
Semen	9-52 (n=4)*	14-90 (n=4)*	12-40	17-64
Urine	3-11	5-14	8-22	10-26

*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.
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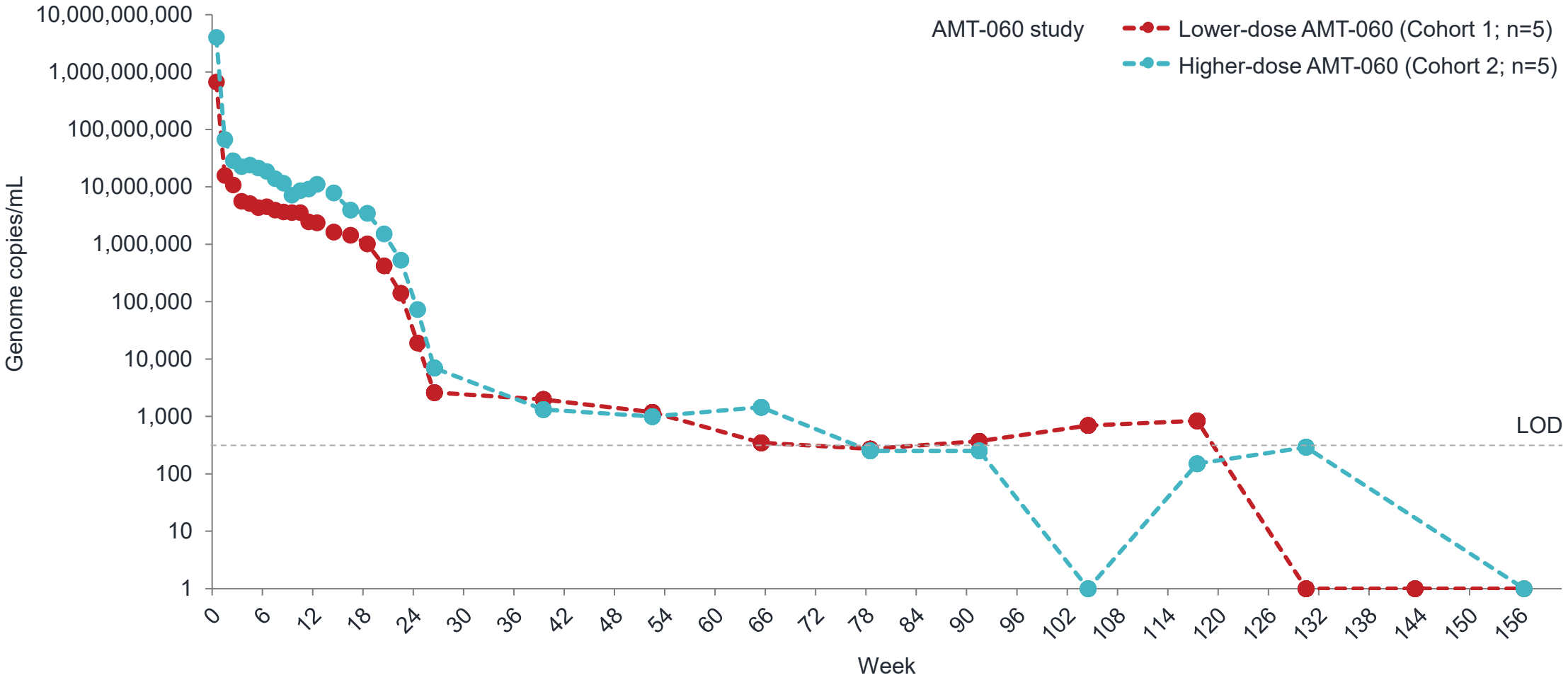
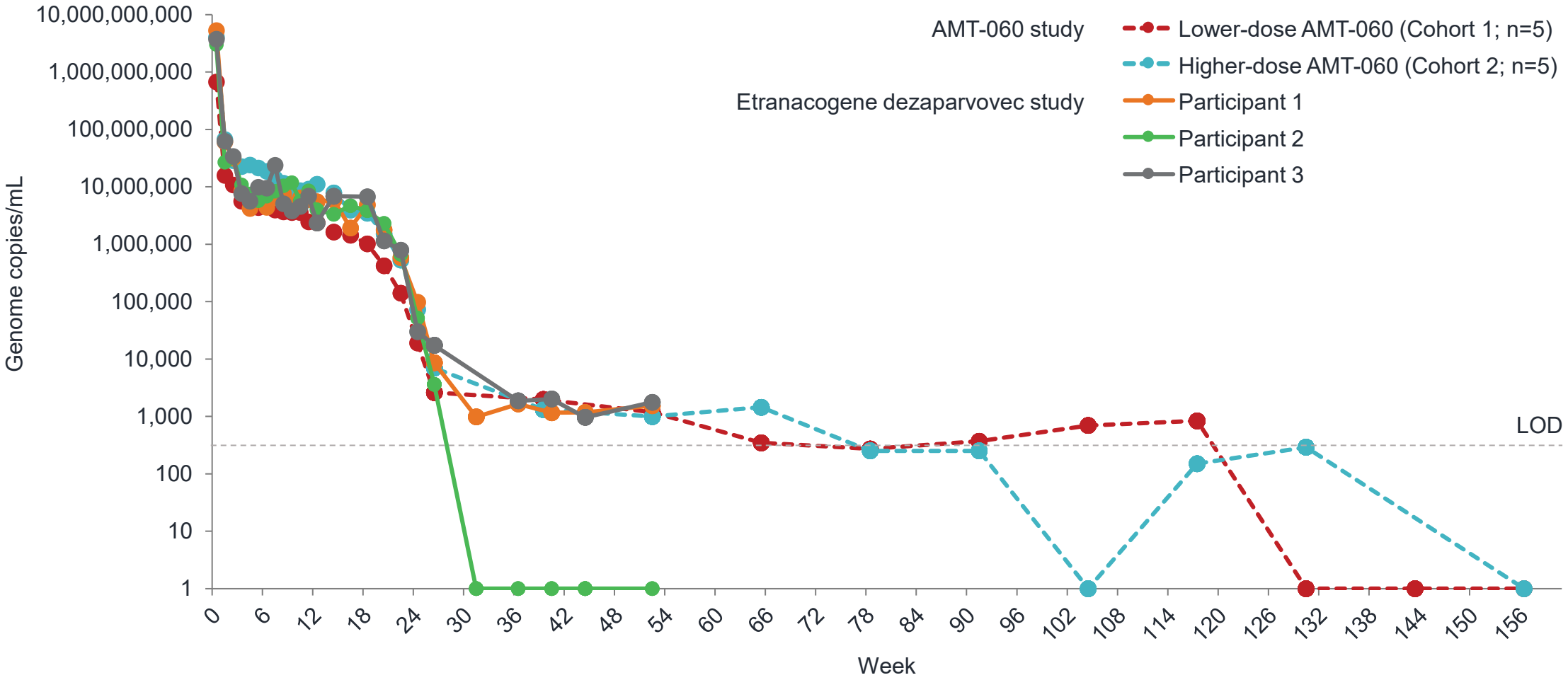
Vector clearance from blood after treatment with AMT-060 and etranacogene dezaparvovec



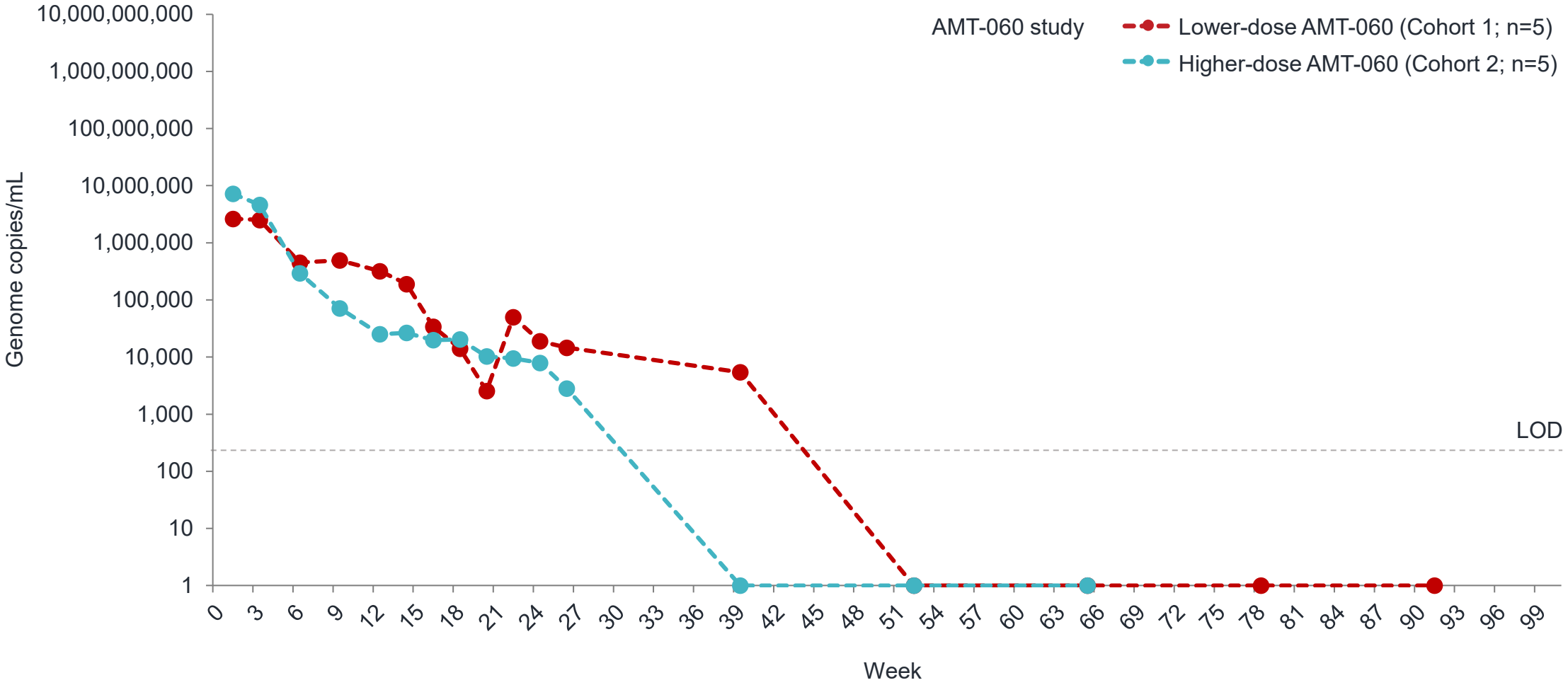
Values at 1 gc/mL were below the theoretical LOD (<571 copies/mL for blood); Vector clearance was reached when vector DNA was either zero or <LOD for three consecutive measurements. LOD, limit of detection; ED, etranacogene dezaparvovec

Vector clearance from blood after treatment with AMT-060 and etranacogene dezaparvovec



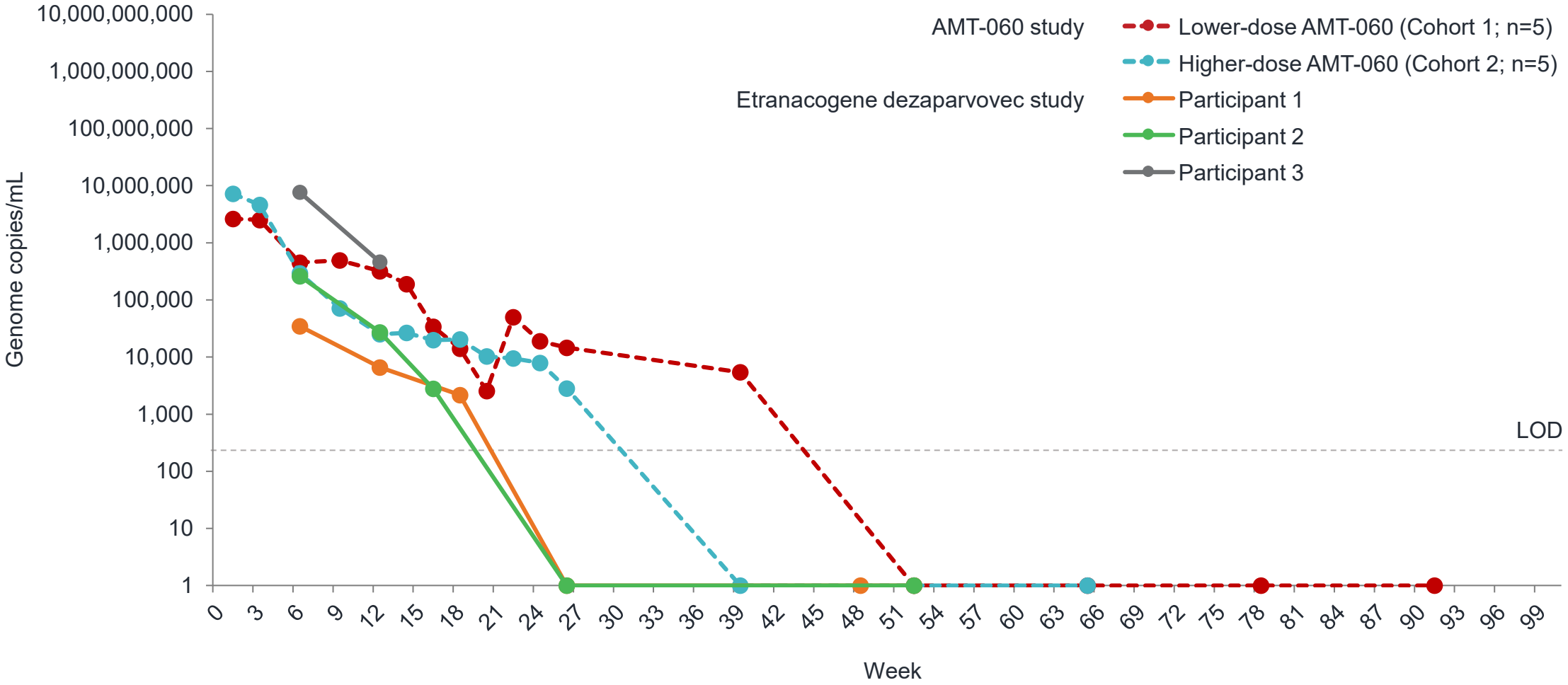
Values at 1 gc/mL were below the theoretical LOD (<571 copies/mL for blood); Vector clearance was reached when vector DNA was either zero or <LOD for three consecutive measurements. LOD, limit of detection; ED, etranacogene dezaparvovec

Vector clearance from semen after treatment with AMT-060 and etranacogene dezaparvovec



Values at 1 gc/mL were below the theoretical LOD (<400 copies/mL for semen). Vector clearance was reached when vector DNA was either zero or <LOD for three consecutive measurements. Etranacogene dezaparvovec shedding data in participant 3 were only available up to week 12. LOD, limit of detection; ED, etranacogene dezaparvovec

Vector clearance from semen after treatment with AMT-060 and etranacogene dezaparvovec



Values at 1 gc/mL were below the theoretical LOD (<400 copies/mL for semen). Vector clearance was reached when vector DNA was either zero or <LOD for three consecutive measurements. Etranacogene dezaparvovec shedding data in participant 3 were only available up to week 12. LOD, limit of detection; ED, etranacogene dezaparvovec

Summary & conclusions

- 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 appears to be following **similar trajectories** for clearance
  - 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.

- Information on clearance can help manage patient expectations regarding **duration of lifestyle restrictions**

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