Blood Biodistribution and Vector Shedding of Valoctocogene Roxaparvovec in People with Severe Hemophilia A: Results from the Phase 3 GENEr8-1 Trial

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Introduction

- Valoctocogene roxaparvovec uses a recombinant adeno-associated virus (AAV) gene therapy vector to deliver a B-domain–deleted FVIII coding sequence to hepatocytes and enable steady, endogenous factor VIII (FVIII) expression in people with severe hemophilia A[1–4]
- Although AAV vectors are replication incompetent and therefore pose minimal risk for horizontal transmission or environmental release, comprehensive assessment of vector shedding in secreta and excreta is a necessary safety evaluation
- Here, we characterized vector DNA biodistribution and shedding over 2 years following valoctocogene roxaparvovec administration in the global phase 3 GENEr8-1 trial

Methods

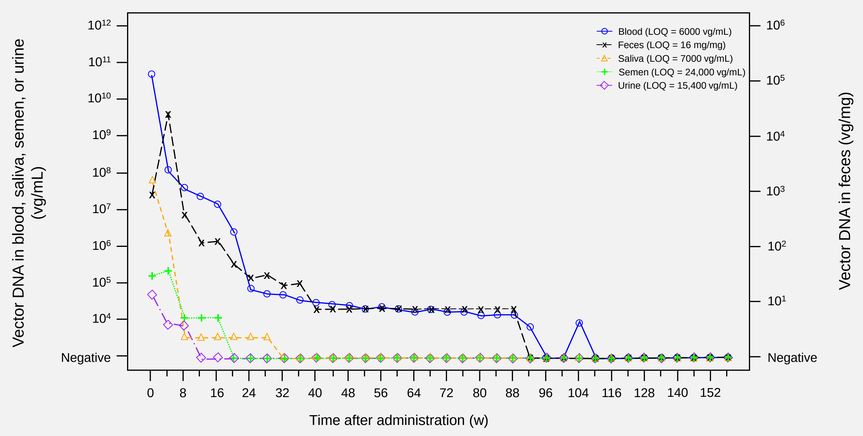
- GENEr8-1 was a multicenter, open-label, single-arm phase 3 trial assessing the efficacy and safety of a single 6x10^{13} vg/kg infusion of valoctocogene roxaparvovec[3,4]
- Participants were adult men (≥18 years) with severe hemophilia A (FVIII ≤1 IU/dL) who were negative for anti-AAV5 antibodies, had no history of inhibitors, and were previously receiving prophylactic FVIII replacement
- Blood, saliva, urine, stool, and semen were collected for evaluation of biodistribution and vector shedding at baseline and on days 1, 4, 8, 15, 22, and 29, then on weeks 6, 8, 12, 16, 20, 24, 26, 32, 36, 40, 44, 48, and 52 until at least 3 consecutive negative samples were obtained
  - Semen testing continued through week 12 even if three consecutive negative samples were previously collected
  - Participants who did not have 3 consecutive negative samples by week 52 continued testing every 4 weeks during year 2
- Vector DNA was detected and quantified with a validated quantitative PCR (qPCR) assay. The limit of quantitation (LOQ) for blood, stool, saliva, semen, and urine was 6000 vg/mL, 16 vg/mL, 7000 vg/mL, 24,000 vg/mL, and 15,400 vg/mL, respectively
  - Clearance was defined as time to below the limit of quantitation (BLQ) or negative qPCR sample confirmed by 2 additional consecutive samples
  - This definition is more clinically meaningful, given the high sensitivity of the qPCR method and the higher threshold of BLQ[5]
- Encapsidated vector DNA potentially capable of cell transduction was detected and quantified with an immunoprecipitation-coupled qPCR (iqPCR) assay for samples of plasma and semen.[6] The LOQ was 2.08x10^5 vg/mL for both plasma and semen
  - Clearance was defined as time to first negative iqPCR sample confirmed by 2 additional consecutive samples
- The contiguity of vector genomes was measured in whole blood and peripheral blood mononuclear cells (PBMCs) using a drop-phase droplet-digital (dd)PCR assay. Vector genome inverted terminal repeat (ITR) fusions were measured in whole blood and PBMCs using ddPCR and primers directed outward from the 5' and 3' ends of the linear vector genome
- All participants who received a dose of valoctocogene roxaparvovec were included in the analysis. Missing data were not imputed

Results

Vector DNA kinetics after valoctocogene roxaparvovec dosing

- Overall, 134 participants received a single 6x10^{13} vg/kg dose of valoctocogene roxaparvovec in GENEr8-1
- Median peak vector DNA levels were observed 1–8 days after dosing and were highest in blood, followed by saliva, semen, stool, and urine; concentrations then declined steadily (**Figure 1**)

Figure 1. Median vector DNA biodistribution and shedding profiles in blood, saliva, semen, stool, and urine following 6x10^{13} vg/kg valoctocogene roxaparvovec infusion



Data were binned within 4-week intervals. LOQ, limit of quantitation; w, week.

- As of this data cut, clearance (defined as three consecutive BLQ or negative qPCR samples) of blood, saliva, semen, stool, and urine was achieved by 7 (5.2%), 134 (100%), 132 (99.2%), 113 (84.3%), and 134 (100%) participants, respectively (**Table 1**)

Table 1. Vector DNA biodistribution and shedding following 6x10^{13} vg/kg valoctocogene roxaparvovec infusion

		N (%) of detectable participants	Time to first detectable sample[a] (wk)	Peak concentration[b] median (vg/mL)	Time to peak concentration (wk)	Time to first BLQ/negative sample confirmed by 2 consecutive samples[c] (wk)
Blood	n	134 (100)	134	134	134	7
	Median		**0.14**	**4.7x10^{10}**	**0.14**	**101**
	Min		0.14	1.6x10^8	0.14	32
	Max		1.0	2.0x10^{11}	1.0	130
Saliva	n	134 (100)	134	134	134	134
	Median		**0.14**	**6.6x10^7**	**0.14**	**6.6**
	Min		0.14	1.3x10^6	0.14	3.1
	Max		1.1	4.3x10^9	2.3	69
Semen	n	133[e] (100)	133	133	133	132
	Median		**0.14**	**1.8x10^6**	**1.0**	**6.1**
	Min		0.14	1.2x10^4	0.14	0.57
	Max		2.1	1.0x10^{10}	12	36
Stool	n	134 (100)	134	134	134	113
	Median		**0.14**	**2.7x10^5**	**1.1**	**44**
	Min		0.14	2.1x10^2	0.14	12
	Max		6.3	5.7x10^6	6.3	88
Urine	n	134 (100)	134	134	134	134
	Median		**0.14**	**8.9x10^4**	**0.21**	**2.3**
	Min		0.14	7.7x10^3	0.14	0.29
	Max		0.86	3.7x10^7	2.3	8.1

[a]Defined as time to first positive shedding sample; [b]Units for stool reported as vg/mg; [c]Confirmed by 2 consecutive negative or BLQ samples; [d]Reported as time of first negative sample confirmed by 2 additional consecutive negative samples; [e]One participant did not have available semen shedding assessments.
BLQ, below the limit of quantitation; max, maximum; min, minimum; wk, weeks.

Encapsidated vector DNA in plasma and semen

- As qPCR is unable to distinguish between non-encapsidated vector DNA and potentially transduction-competent vector DNA contained within the AAV5 capsid, iqPCR of plasma and semen was used to assess encapsidated vector DNA
- Encapsidated vector DNA was cleared more rapidly than total vector DNA (**Table 2**)
  - Complete clearance of both plasma and semen was achieved by all participants with samples in ≤12 weeks

Table 2. Encapsidated vector DNA biodistribution and shedding following 6x10^{13} vg/kg valoctocogene roxaparvovec infusion

		N (%) of detectable participants	Peak concentration (vg/mL)	Time to peak concentration (wk)	Time to last detectable sample (wk)	N (%) with 3 consecutive negative samples	Time to first negative sample confirmed by 2 consecutive samples (wk)
Plasma (n = 134)	**Median**	130 (97.0)	**BLQ**	**1.1**	**2.2**	134 (100)	**3.3**
	Min		BLQ	0.86	0.86		1.3
	Max		1.1x10^7	4.1	10.0		10.1
Semen (n = 133)[a]	**Median**	131 (98.5)	**9.3x10^5**	**0.57**	**1.9**	131[b] (98.5)	**3.0**
	Min		BLQ	0.14	0.14		0.4
	Max		3.8x10^8	4.0	8.1		12

[a]One participant did not provide semen shedding assessments; [b]Two participants had insufficient sample quantity for vector DNA assessment by iqPCR.
BLQ, below the limit of quantification; iqPCR, immunoprecipitation-coupled quantitative PCR; max, maximum; min, minimum; wk, week.

Contraception is recommended for men 6 months following treatment with valoctocogene roxaparvovec

- This recommendation is based primarily on time to clearance in semen and plasma of potentially transduction-competent encapsidated vector DNA per iqPCR with a 3-month washout period, as well as the time to clearance in semen of all other residual vector DNA
- Maximum time to clearance of encapsidated vector DNA was ≤12 weeks in both plasma and semen; maximum time to clearance of all other residual vector DNA in semen was 36 weeks. Amounts of vector DNA less than the BLQ likely pose a negligible risk
- Most participants (131/133) achieved clearance of residual vector DNA in semen by 6 months; the 2 participants who did not clear before 6 months had vector DNA concentrations close to the LOQ at weeks 25.9 and 31.8, which were not considered meaningfully different compared to those of the other 131 evaluable participants

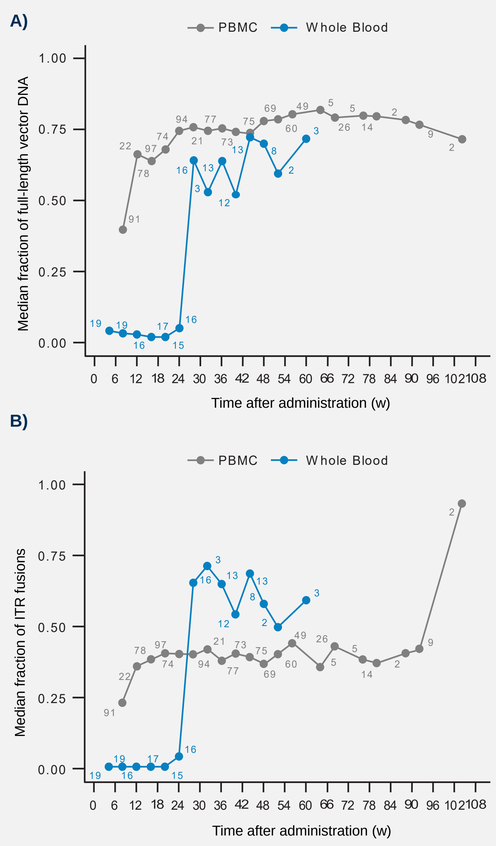
Conclusions

- Vector DNA and vector capsids were steadily cleared from the blood and shedding matrices of people with severe hemophilia A treated with valoctocogene roxaparvovec
- The transition of vector genomes into stable, circular episomes in PBMCs likely contributes to the duration of detection in blood by qPCR
- The replication-incompetent nature of valoctocogene roxaparvovec and the rapid clearance of encapsidated vector make the risk of transmission to untreated individuals extremely low
- The presence of vector DNA in assessed bodily fluids was not associated with any adverse safety or efficacy findings

Blood biodistribution and full-length vector genomes

- In blood, vector genomes transitioned from initial noncontiguous forms into full-length forms over time; by week 52, the majority of vector DNA in whole blood was full-length (**Figure 2A**)
  - The fraction of ITR fusions steadily increased over time, indicating formulation of stable, circularized episomes (**Figure 2B**)

Figure 2. Median fraction of A) full-length vector genomes and B) ITR-fusions in blood matrices following 6x10^{13} vg/kg valoctocogene roxaparvovec infusion



The average of each participant's fractions within each interval was calculated and the median fraction across participants for each interval is presented. The number next to each point represents the n-value for the median calculation. Bins with n = 1 were excluded from median calculations.
ITR, inverted terminal repeat; PBMC, peripheral blood mononuclear cells; w, week.

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Disclosures

All authors are employees and stockholders of BioMarin Pharmaceutical Inc.

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