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Human liver biopsy analysis reveals lower RNA transcription may contribute to a decline in FVIII levels following AAV5-hFVIII-SQ gene therapy

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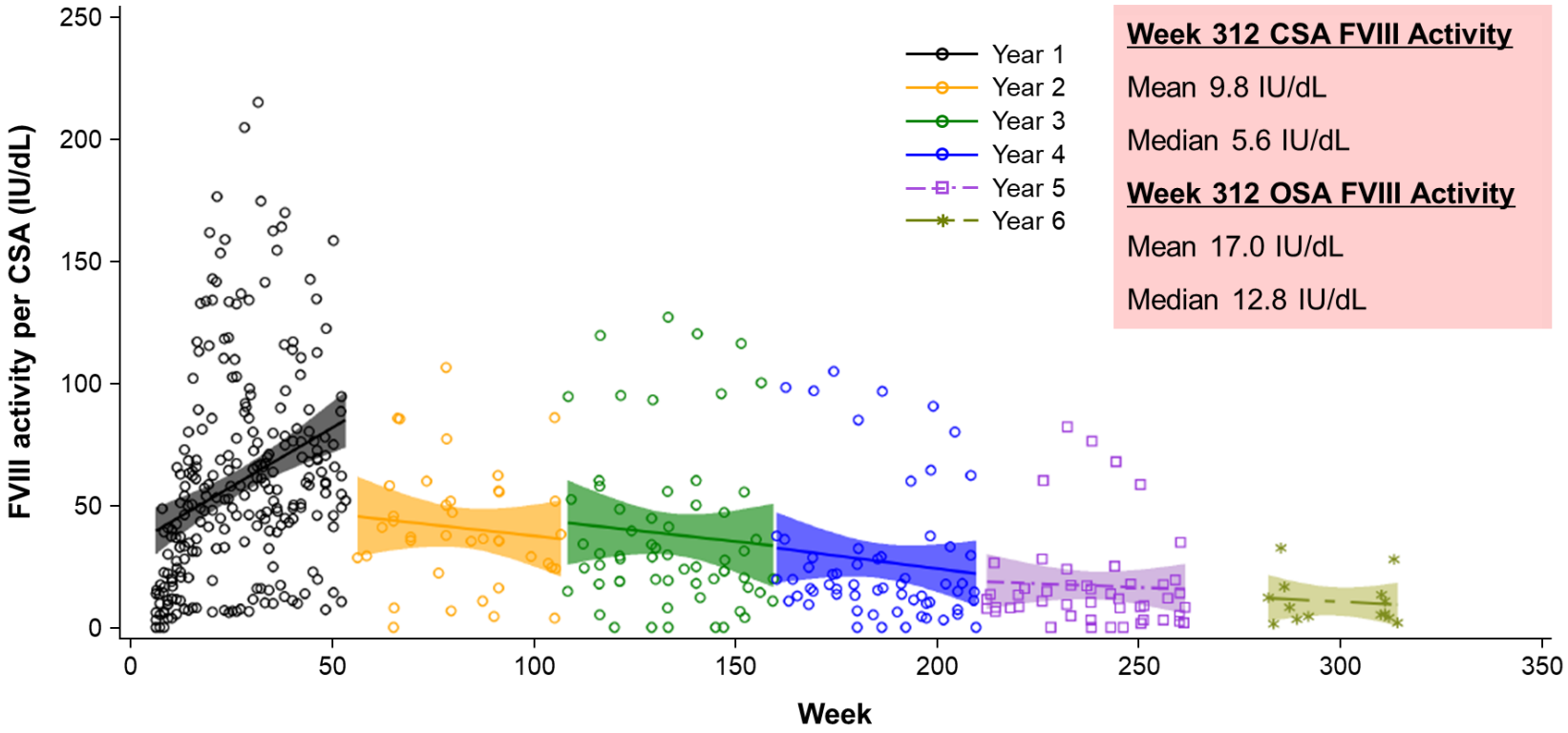
Disclosures

- Ashrafali M Ismail is a BioMarin Inc. employee and stockholder

FVIII activity declines over time following AAV5 gene therapy



- Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is a gene therapy for hemophilia A that uses an AAV5 vector to deliver a B-domain-deleted hFVIII coding sequence controlled by a liver-selective promoter
- A single infusion has provided hemostatic efficacy for up to 3 years in a phase 3 study[1] and up to 6 years in a phase 2 study,[2] but FVIII activity declines over time

- **Understanding the mechanisms behind the decline in FVIII activity is needed to identify intervening strategies that could maximize the durability of response**

AAV5, adeno-associated virus serotype 5; CSA, chromogenic substrate assay; FVIII, factor VIII; GT, gene therapy; hFVIII, human FVIII; OSA, one-stage assay.

1. Madan B, et al. *Journal of Thrombosis and Haemostasis.* In preparation. 2. Symington E, et al. *Haemophilia.* Under review.

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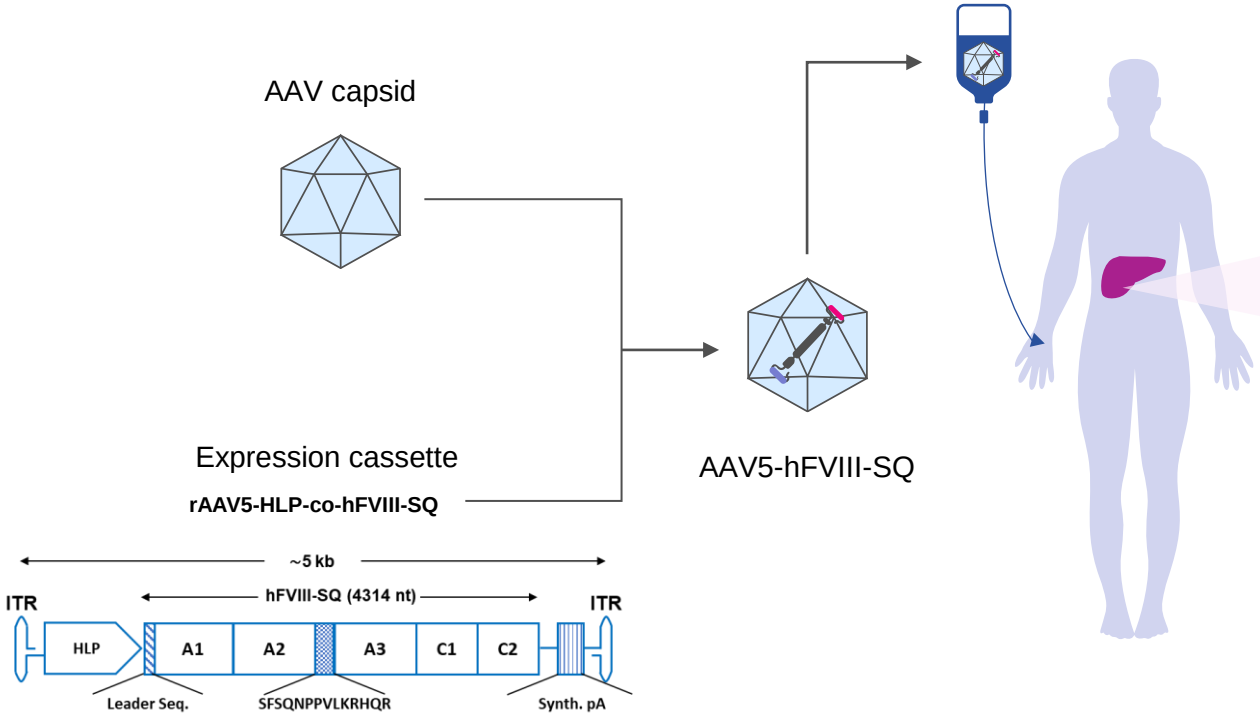
Aims

- To assess rAAV5-HLP-co-hFVIII-SQ vector **transduction efficiency**
- To characterize and quantify episomal forms of **vector DNA**
- To quantify **transgene expression** (hFVIII-SQ RNA) and correlate with FVIII activity
- To examine liver **histopathology**

FVIII, factor VIII; hFVIII-SQ, B-domain-deleted human FVIII; HLP, hybrid liver-selective promoter; rAAV5, recombinant adeno-associated virus serotype 5; rAAV5-HLP-co-hFVIII-SQ, valoctocogene roxaparvovec expression cassette.

Sub-study design

- Optional sub-study of the phase 2 trial and the phase 3, single-arm, open-label GENEr8-1/3 trials assessing $6x10^{13}$ vg/kg valoctocogene roxaparvovec in adult males with severe hemophilia A (FVIII ≤1 IU/dL)
- **Biopsy exclusion criteria** were any condition, detected via liver ultrasound, precluding safe liver biopsy



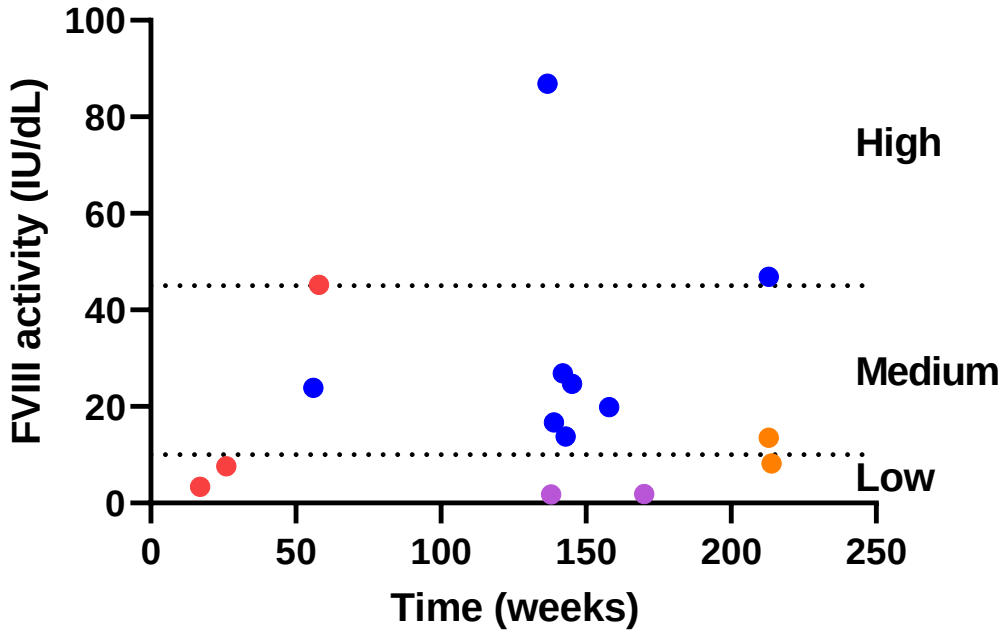
Sub-study subjects (n = 15)

- Liver biopsies were collected from 12 participants, 1.1 to 4.1 years post-dosing
- SoC liver biopsies in response to transient transaminitis were collected from 3 additional participants 0.3 to 1.1 years post-dosing

NCT02576795, phase 2 trial; NCT03370913/NCT04323098, phase 3 GENEr8-1/3 trials.
AAV, adeno-associated virus; AAV5, AAV serotype 5; AAV5-hFVIII-SQ, valoctocogene roxaparvovec; hFVIII-SQ, B-domain-deleted human factor VIII; HLP, hybrid liver-selective promoter; ITR, inverted terminal repeat; pA, polyadenylation sequence; rAAV5, recombinant AAV5 vector; rAAV5-HLP-co-hFVIII-SQ, valoctocogene roxaparvovec expression cassette; Seq., sequence; SoC, standard-of-care; Synth., synthetic.

Collection of liver biopsy samples

- Biopsy collection ranged from early post-gene transfer to beyond 4 years
- Participants' FVIII activity level varied
- Two participants originally had high FVIII activity, but it had since declined to <3 IU/dL
- Three biopsy samples collected at the time of transaminitis (SoC)



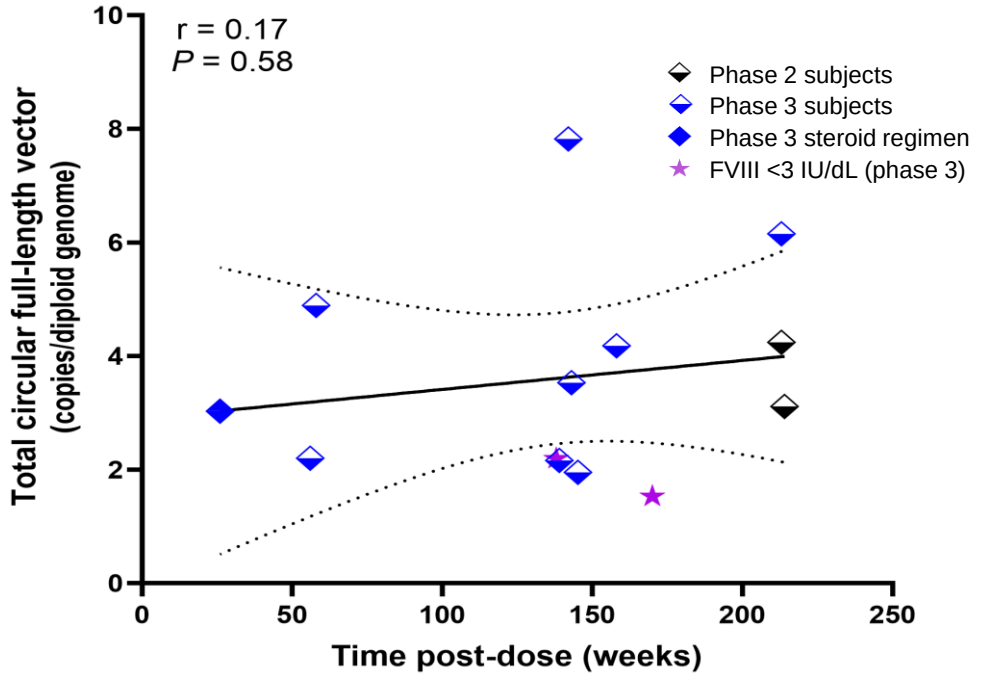
Red = SoC samples
Blue = phase 3 studies
Orange = phase 2 study
Purple = levels declined to <3 IU/dL

FVIII activity levels
>45 (high): 3 biopsies (1 SoC)
10–35 (medium): 7 biopsies
<10 (low): 5 biopsies (2 SoC)

One participant was using CS at the time of biopsy (SoC). CS treatment consisted of a single intravenous infusion in conjunction with receipt of a 19-week prophylactic CS regimen starting on the day of valoctocogene roxaparvovec infusion. FVIII activity measured using chromogenic substrate assay.
CS, corticosteroid; FVIII, factor VIII; SoC, standard-of-care.

Circular full-length episomes persist through 4 years of post-gene therapy follow-up

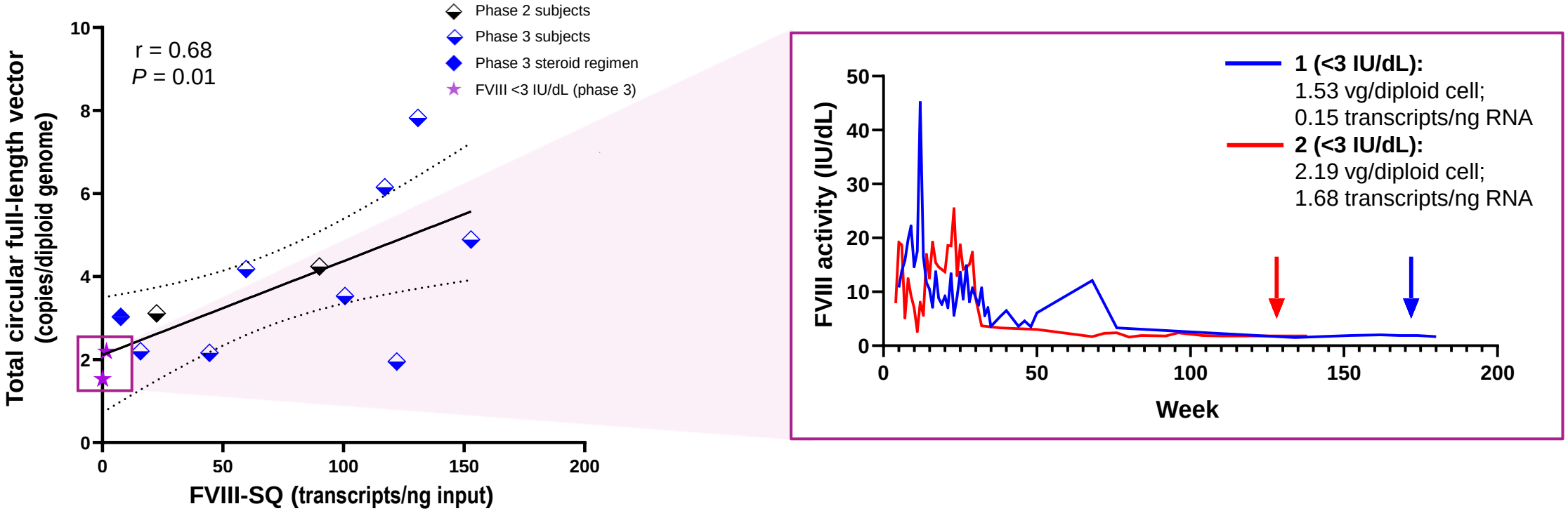
- Circular full-length episomes did not decrease over time
  - Mean ± SD circular full-length episomes in the study participants were 3.60 ± 1.99 (range, 1.53–7.82) vg/diploid cell
- Circular full-length episomes correlated with FVIII activity (r = 0.61, *P* = 0.026)



Two study participants did not have liver biopsy tissue collected for molecular analysis; n = 13.
FVIII, factor VIII; SD, standard deviation; vg, vector genome.

FVIII-SQ RNA expression influences FVIII response

- Mean ± SD hFVIII-SQ RNA transcript levels in participants with FVIII >3 IU/dL were 78.5 ± 50.9 (range, 7.6–152.9; n = 11) transcripts/ng RNA
- Two participants' FVIII activity declined to <3 IU/dL:
  - Poor RNA transcript levels (0.15 and 1.68 transcripts/ng RNA)
  - Level of transduction and full-length vector genomes were similar to those with FVIII >3 IU/dL (1.95–2.2 vg/diploid cell)

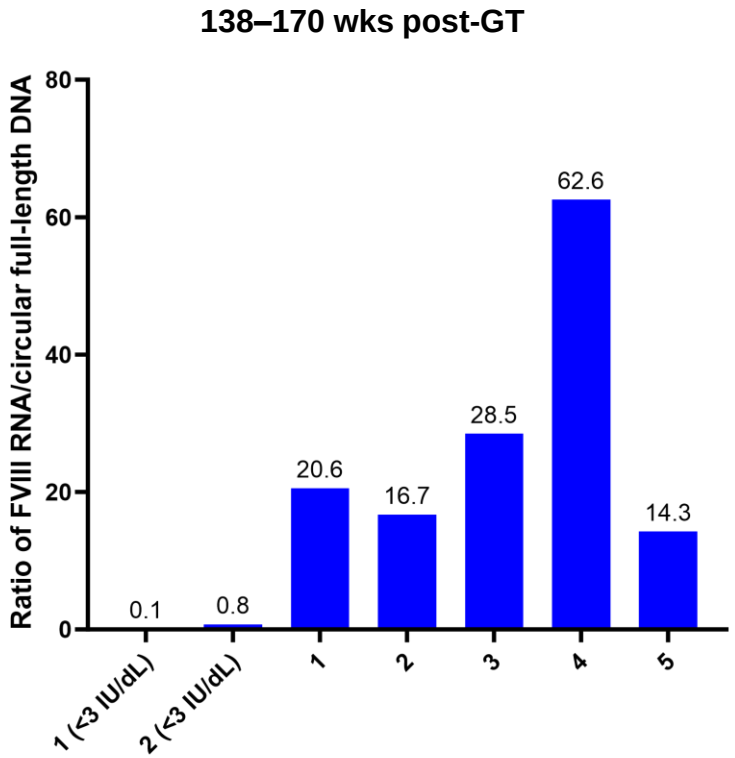


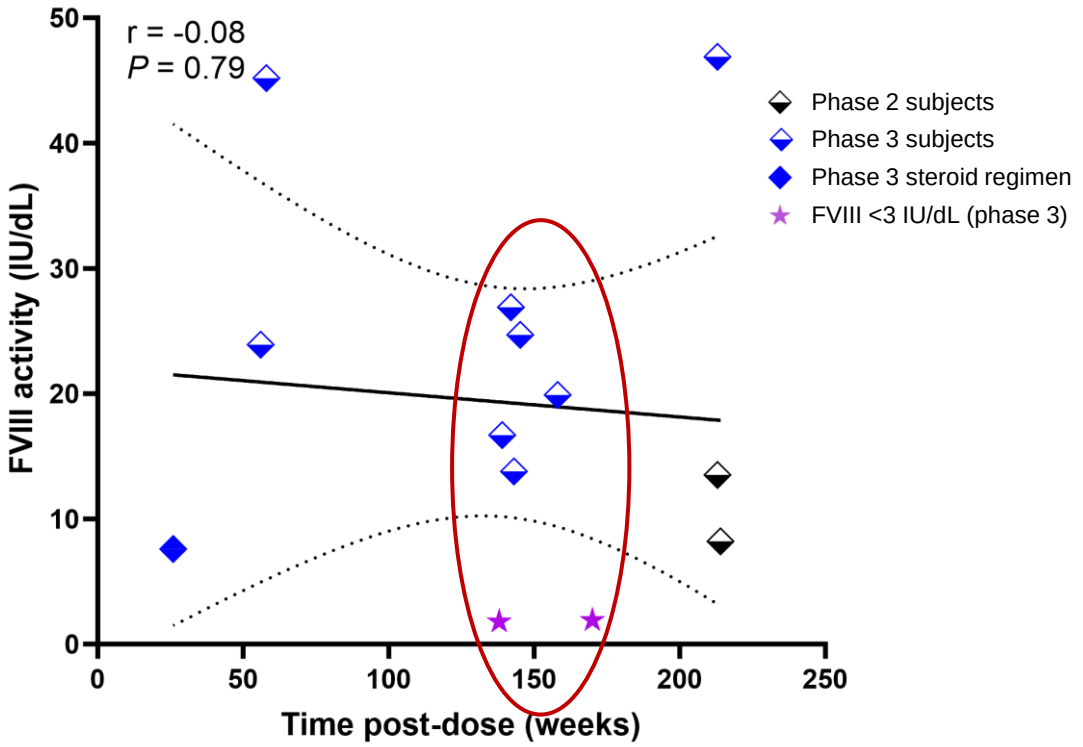
Two study participants did not have liver biopsy tissue collected for molecular analysis; n = 13.

FVIII, factor VIII; hFVIII-SQ, B-domain-deleted human FVIII; SD, standard deviation; vg, vector genome.

Transcriptional efficiency is significantly lower in those with FVIII <3 IU/dL, suggesting transgene silencing may mediate the decline of FVIII expression following AAV5-hFVIII-SQ treatment

- Participants with FVIII that declined to <3 IU/dL (n = 2) had a significantly lower RNA/DNA ratio than participants with FVIII >3 IU/dL (n = 5) at a similar time duration (Welch's t-test, *P* = 0.034)





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AAV5, adeno-associated virus serotype 5; AAV5-hFVIII-SQ, valoctocogene roxaparvovec; FVIII, factor VIII; GT, gene therapy; hFVIII-SQ, B-domain-deleted human FVIII.

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Conclusions

- Follow-up liver biopsy analysis of AAV5-hFVIII-SQ gene therapy suggests efficient hepatocyte transduction occurred
- Full-length circular episome levels were persistent and did not decrease over time
- FVIII-SQ RNA expression impacts FVIII activity and may contribute to decline in FVIII expression and activity over time
- Ongoing work will assess additional factors contributing to expression variability, safety, and mechanisms of action mediating transaminitis

AAV, adeno-associated virus; AAV5, AAV serotype 5; AAV5-hVIII-SQ, valoctocogene roxaparvovec; FVIII, factor VIII; hFVIII-SQ, B-domain-deleted human FVIII.

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