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Human liver biopsy analysis shows decline in FVIII levels following AAV5-hFVIII-SQ gene therapy may be due to low RNA transcription levels despite persistence of full-length episomal vector genomes

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Disclosures

- Bridget Yates is an employee and stockholder of BioMarin Pharmaceutical Inc.

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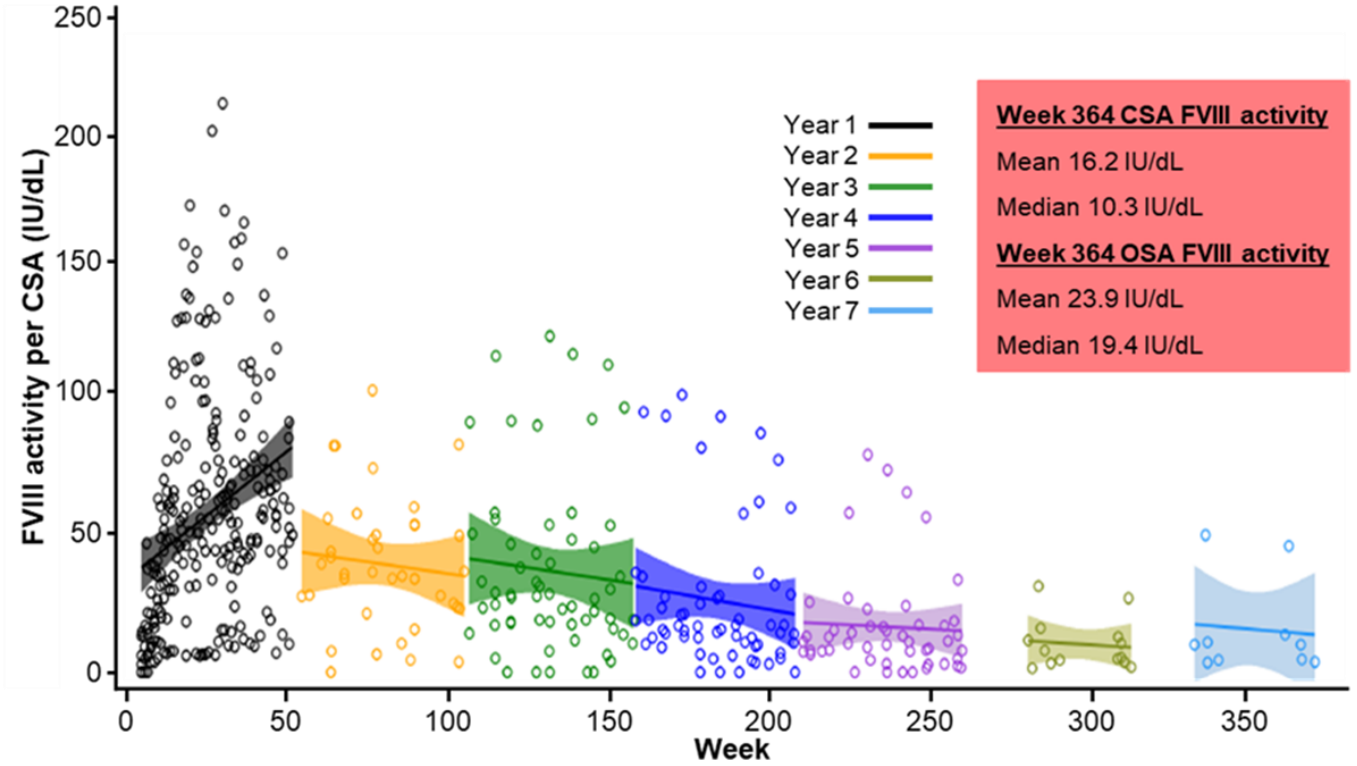
FVIII activity declines over time following AAV-mediated gene therapy



- 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 >3 years in a phase 3 study[1] and >7 years in a phase 1/2 study,[2] but FVIII activity declines over time

Understanding the mechanisms behind the decline in FVIII activity and ALT elevation is necessary to identify intervening strategies that could maximize the durability of response

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

1. Madan B, et al. *J Thromb Haemost.* Under review. 2. Symington E, et al. *Haemophilia*. 2024. 30(S1):96.

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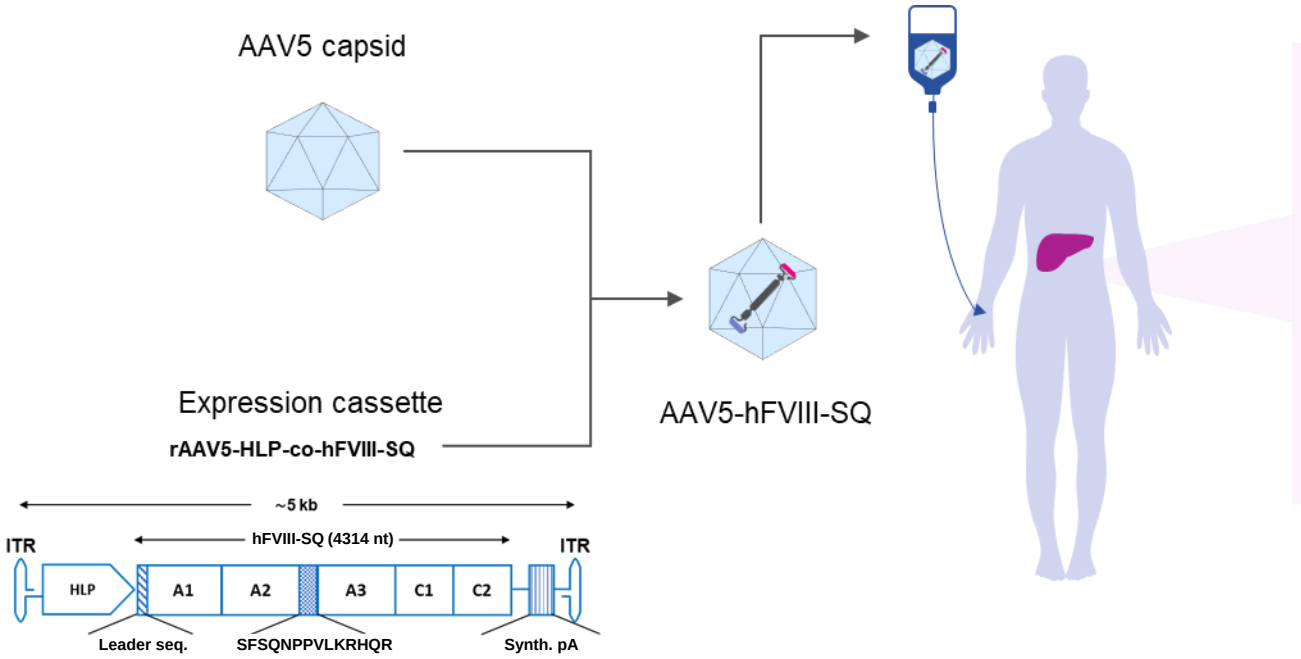
Study design

- Optional sub-study of the phase 1/2, phase 3 GENEr8-1, and phase 3b GENEr8-3 trials, assessing the efficacy and safety of valoctocogene roxaparvovec in adult males with severe hemophilia A (FVIII ≤1 IU/dL)
  - Participants were administered $6x10^{13}$ vg/kg
  - Phase 3b trial incorporated the use of prophylactic corticosteroids*
- SoC liver biopsies were received from both phase 3 and 3b trials following ALT elevation
  - Biopsy considered if ALT is >2x baseline without improvement within 14 days



Study participants (n = 16)

- Liver biopsies were collected from 12 sub-study participants, 2.1 to 4.1 years post-dosing
- SoC liver biopsies in response to transient transaminitis were collected from 4 additional participants 0.1 to 1.1 years post-dosing
- **Biopsy exclusion criteria** were any condition, detected via liver ultrasound, precluding safe liver biopsy

*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. NCT02576795, phase 2 trial, NCT03370913/NCT04323098, phase 3 GENEr8-1/3 trials.

AAV5, adeno-associated virus serotype 5; AAV5-hFVIII-SQ, valoctocogene roxaparvovec; ALT, alanine aminotransferase; CS, corticosteroid; FVIII, factor VIII; hFVIII-SQ, B-domain-deleted human factor VIII, SQ variant; HLP, hybrid liver-selective promoter; ITR, inverted terminal repeat; nt, nucleotide; pA, polyadenylation sequence; rAAV5, recombinant AAV5 vector; rAAV5-HLP-co-hFVIII-SQ, valoctocogene roxaparvovec expression cassette; seq., sequence; SoC, standard of care; Synth., synthetic; vg, vector genome.

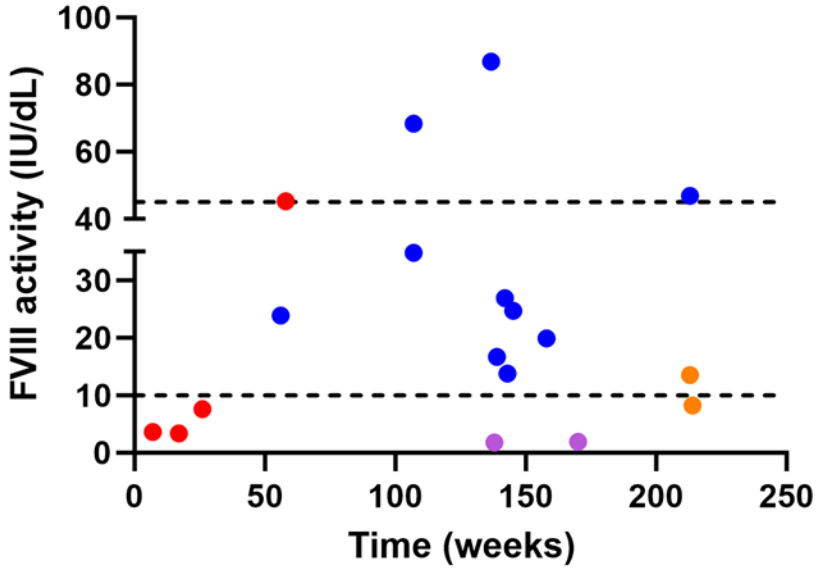
Aims

- To examine liver histopathology
- 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

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

Liver biopsy samples were collected from a wide variety of participants

- Participants' FVIII activity level varied from <3 to 87 IU/dL
- Two participants originally had high FVIII activity, but it had since declined to <3 IU/dL
- Four biopsy samples collected at the time of transaminitis (SoC)



Red = SoC samples
Blue = phase 3 studies
Orange = phase 1/2 study
Purple = levels declined to <3 IU/dL

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

Goal: to investigate mechanisms underlying durability and variability of response

One participant was using CS treatment 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 was measured using chromogenic substrate assay.

CS, corticosteroid; FVIII, factor VIII; SoC, standard of care.

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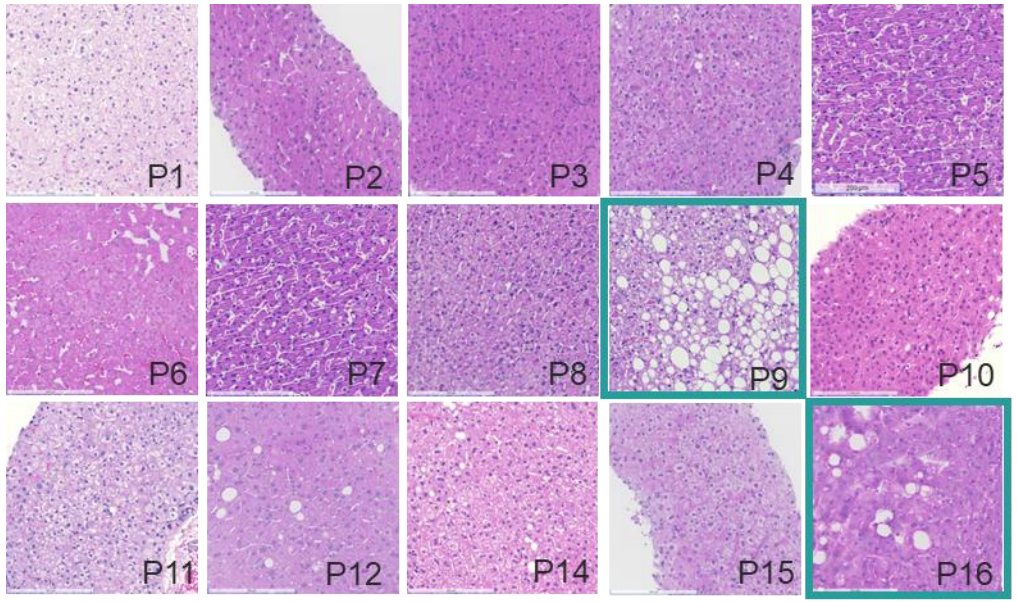
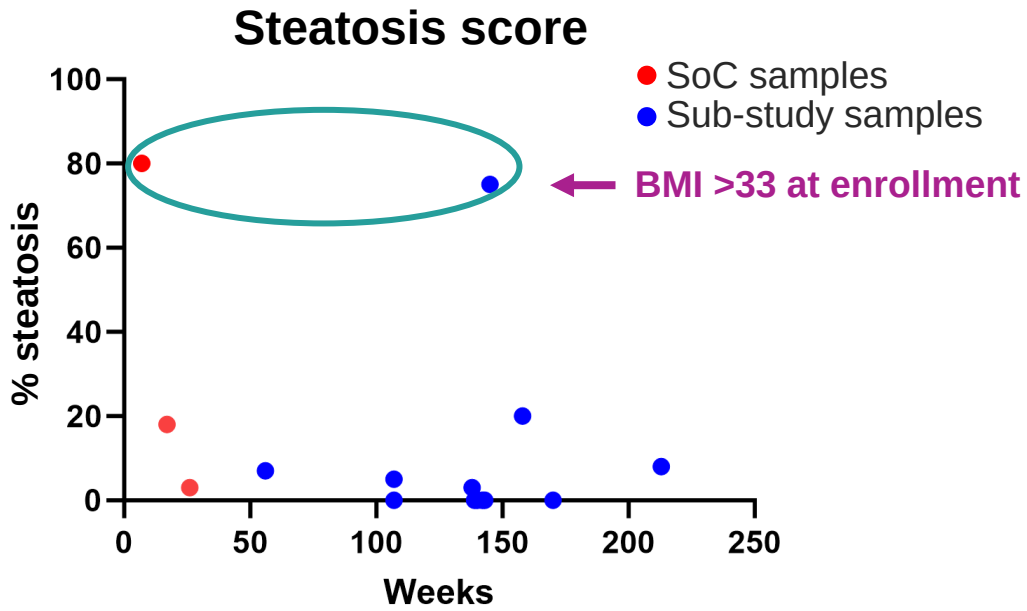
Histopathology revealed no evidence of abnormal architecture or dysplasia

Histopathology sections stained with hematoxylin and eosin





- Varying levels of steatosis were detected
  - 11/13 showed mild steatosis consistent with high prevalence in normal male populations from developed countries
  - 2/13 with severe steatosis had BMI >33 at enrollment
- No clinically relevant inflammation was observed
- Ground glass hepatocytes observed in 1 participant with prior HepB/C infection and possibly as result of herbal supplementation
  - Ground glass hepatocytes negative for HBs Ag, FVIII, or GRP78

BMI, body mass index; FVIII, factor VIII; GRP78, glucose-regulated protein 78; HBs Ag, hepatitis B surface antigen; HepB/C, hepatitis B/C; Px, participant number; SoC, standard of care.

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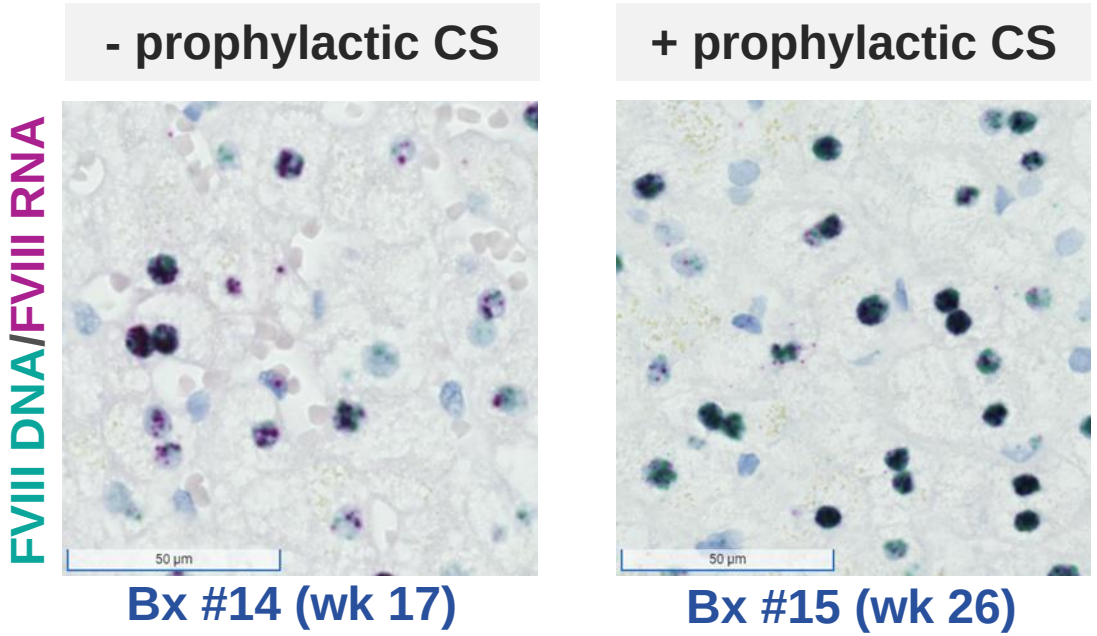
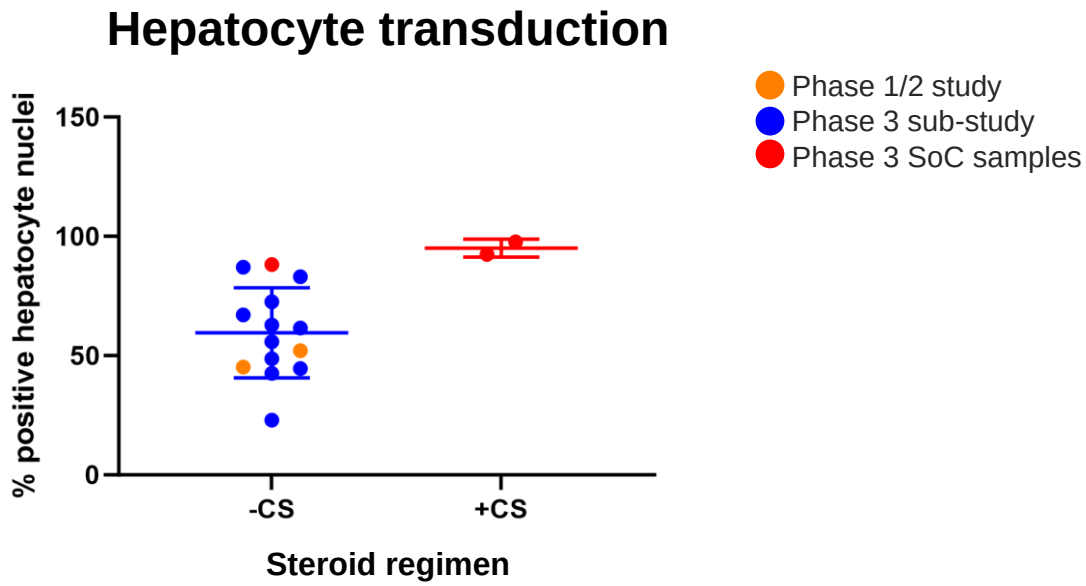
Efficient hepatocyte transduction of AAV5-hFVIII-SQ was detected regardless of prophylactic corticosteroid treatment





AAV5-hFVIII-SQ, valoctocogene roxaparvovec; Bx, biopsy number; CS, corticosteroids; FVIII, factor VIII; SoC, standard of care; wk, week.

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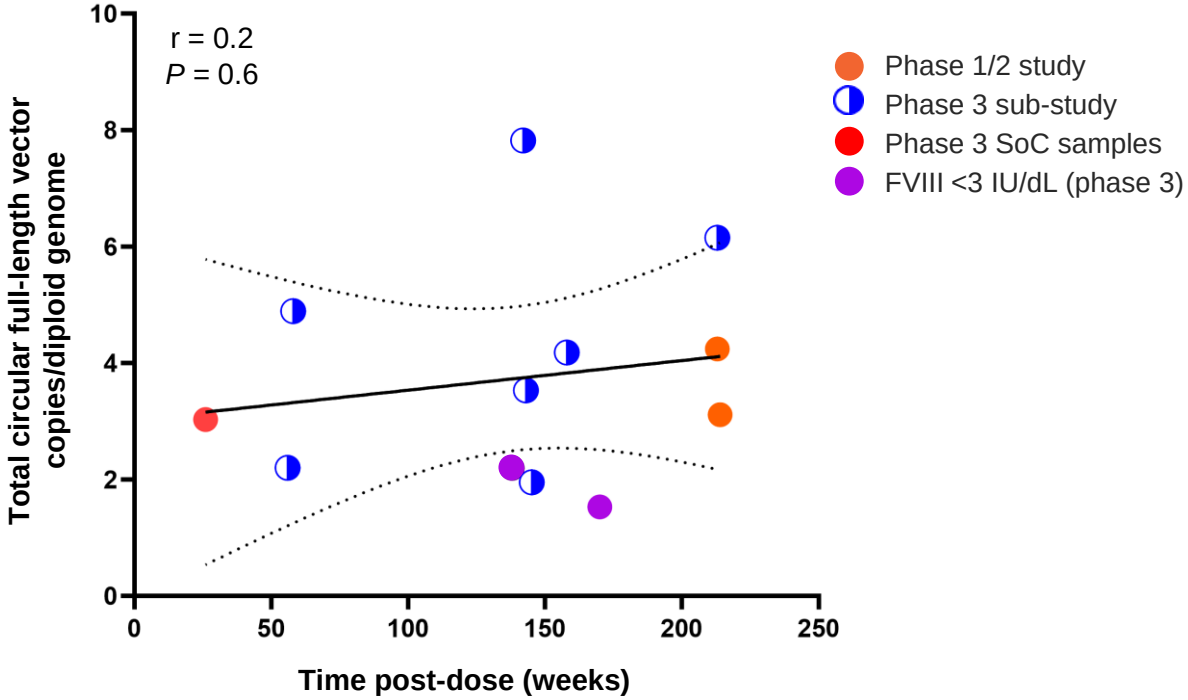
Circular full-length episomes persist through 4 years of post-gene therapy follow-up

- AAV vector genomes persist as episomal DNA and are accountable for long-term gene expression[1]
- Circular full-length episomes did not decrease over time
  - Mean ± SD circular full-length episomes in the study participants were 3.6 ± 2.0 (range, 1.5–7.8) vg/diploid cell
- Phase 1/2 and phase 3 participants had similar levels of genomes



Two study participants did not have liver biopsy tissue collected for molecular analysis; n = 13.
AAV, adeno-associated virus; SD, standard deviation; SoC, standard of care; vg, vector genome.

1. Penaud-Budloo M, et al. *J Virol.* 2008;82(16):7875-85.

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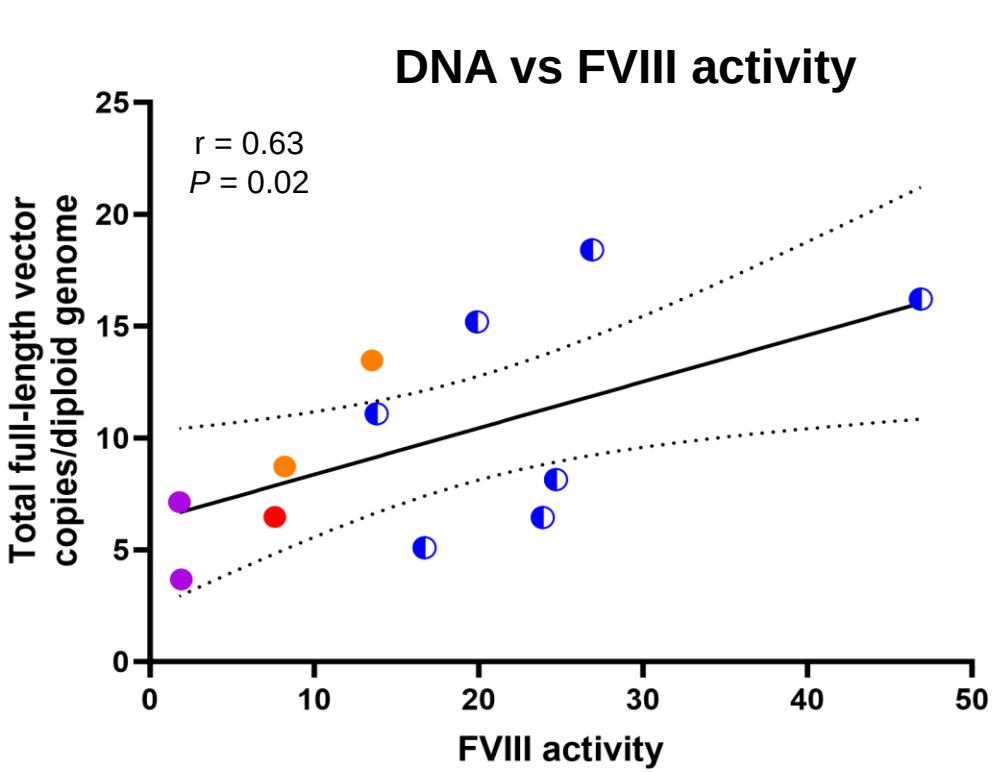
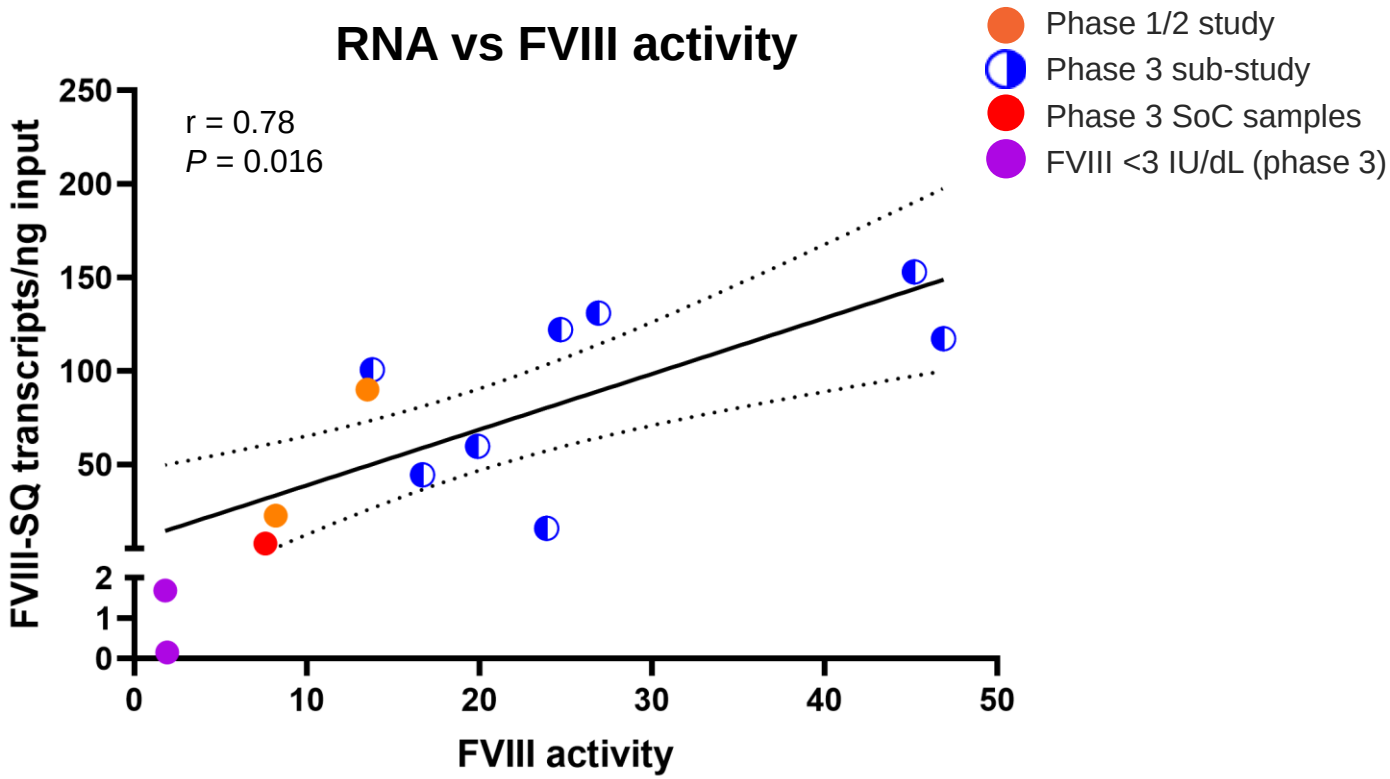
Full-length episomes and RNA transcript levels correlate with FVIII activity





FVIII activity was measured using chromogenic substrate assay.

 FVIII, factor VIII; FVIII-SQ, B-domain-deleted FVIII, SQ variant; SoC, standard of care.

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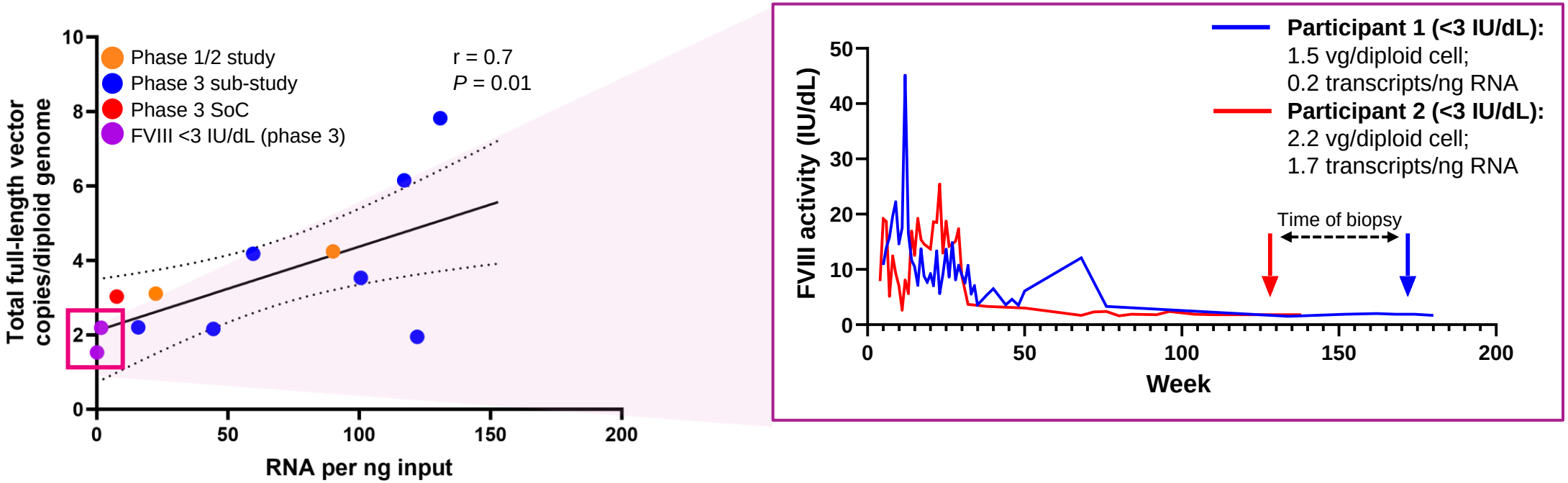
FVIII-SQ RNA expression influences FVIII response

- Mean ± SD hFVIII-SQ RNA transcript levels in participants with FVIII >3 IU/dL were 78.5 ± 50.9* transcripts/ng RNA (n = 11)
- Two participants' FVIII activity declined to <3 IU/dL
  - Low RNA transcript levels (0.2 and 1.7 transcripts/ng RNA)
  - Level of transduction and full-length vector genomes were similar to those with FVIII >3 IU/dL (2.0–2.2 vg/diploid cell)



*Range, 7.6–152.9 transcripts/ng RNA. Two study participants did not have liver biopsy tissue collected for molecular analysis; n = 13. FVIII activity was measured using chromogenic substrate assay.

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

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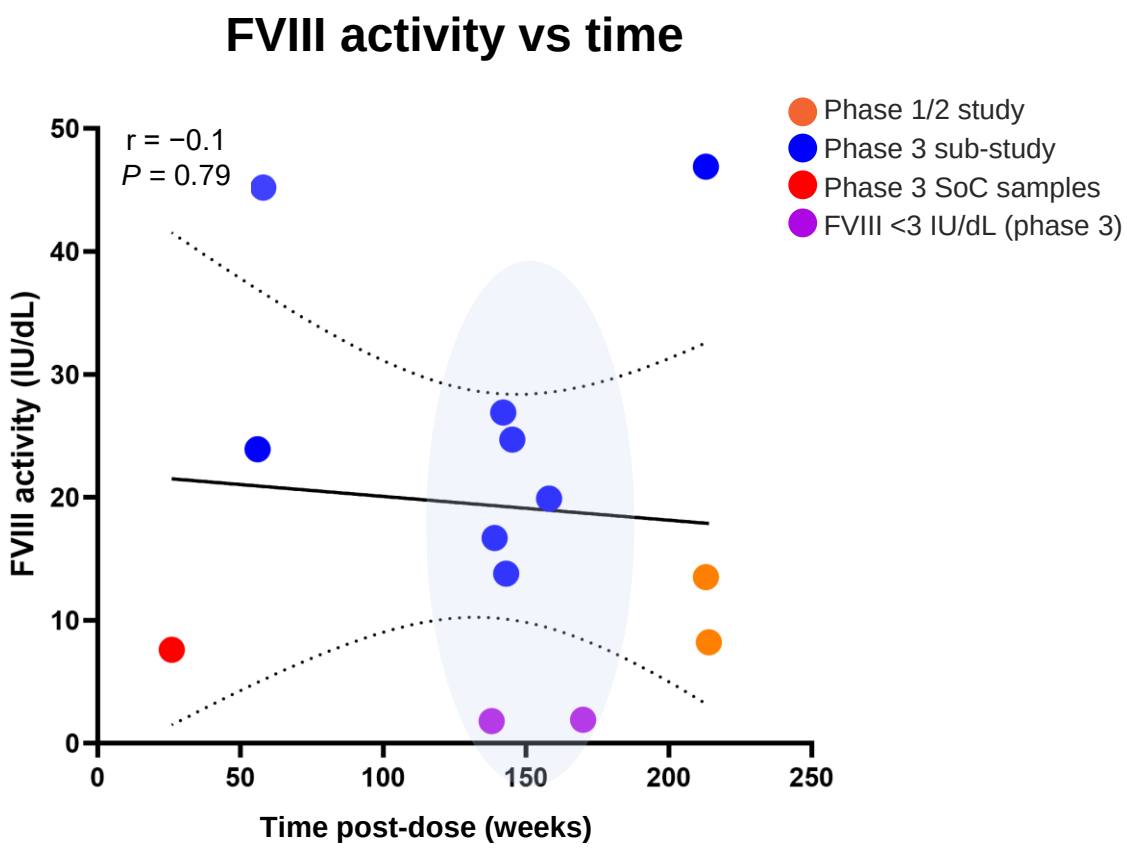
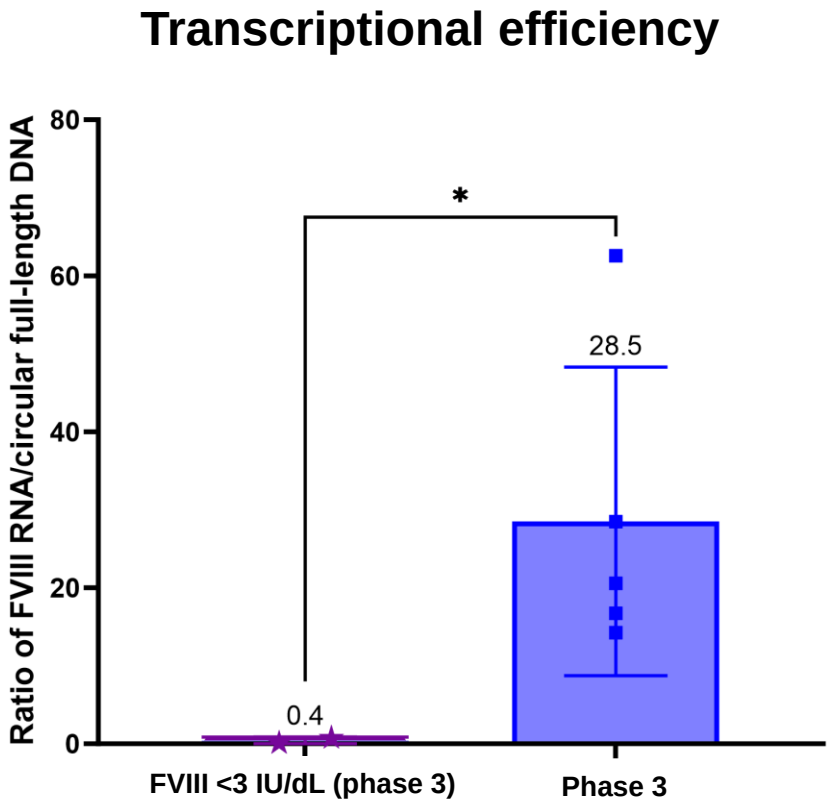
Transcriptional efficiency contributes to low FVIII activity, suggesting transgene silencing may mediate the decline of FVIII expression following AAV5-hFVIII-SQ treatment





*P <0.05. Two study participants did not have liver biopsy tissue collected for molecular analysis; n = 13. FVIII activity was measured using chromogenic substrate assay.
AAV5-hFVIII-SQ, valoctocogene roxaparvovec; FVIII, factor VIII; SoC, standard of care.

Conclusions

- Histopathology analysis shows no evidence of abnormal architecture findings or dysplasia
  - Varying levels of steatosis were detected, similar to levels observed with high prevalence in normal male populations from developed countries
  - No clinically relevant inflammation was observed
- Follow-up liver biopsy analysis of AAV5-hFVIII-SQ gene therapy suggests efficient hepatocyte transduction occurred across trials
- Full-length circular episome levels were persistent and did not decrease over time
- Decline in FVIII over time may be due to reduced transcription of episomal vector DNA to RNA in hepatocytes
- Ongoing work will assess additional factors contributing to expression variability, safety, and mechanisms of action mediating transaminitis

 AAV5-hVIII-SQ, valoctocogene roxaparvovec; FVIII, factor VIII.

Acknowledgements

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