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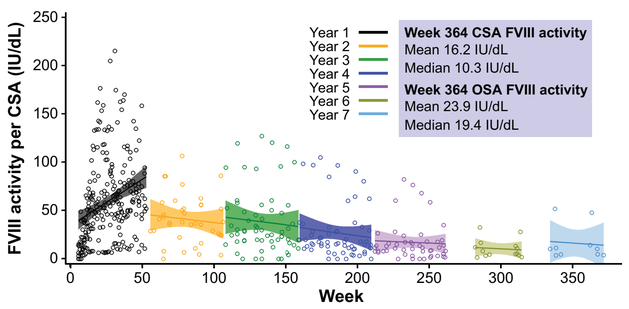
Human liver biopsy analysis shows decline in FVIII levels following AAV5-hFVIII-SQ gene therapy may be due to low RNA transcription levels despite persistence of full-length episomal vector genomes

Jane Mason[1], Bridget Yates[2], Ashrafali M. Ismail[2], Aras N. Mattis[3], Alyssa Gonzalez[2], Chan Kyu Kim[2], Kala Jayaram[2], Gili Kenet[4], Johnny Mahlangu[5], Amy L. Dunn[6], Susan Shapiro[7], Michael Wang[8], Flora Peyvandi[9], Adam Giermasz[10], Rashid Kazmi[11], Nigel S. Key[12], Margareth C. Ozelo[13], Tara M. Robinson[2], Sylvia Fong[2]

[1]Queensland Haemophilia Centre, Cancer Care Services, Royal Brisbane and Women's Hospital, University of Queensland, Brisbane, QLD, Australia; [2]BioMarin Pharmaceutical Inc., Novato, CA, USA; [3]Liver Center, Department of Medicine, University of California, San Francisco, San Francisco, CA, USA; Department of Pathology, University of California, San Francisco, San Francisco, CA, USA; [4]Amalia Biron Research Institute of Thrombosis and Hemostasis, Sackler School of Medicine, Tel Aviv, Israel; Sheba Medical Center, Tel Hashomer, Israel; [5]Haemophilia Comprehensive Care Centre, Charlotte Maxeke Johannesburg Academic Hospital, Department of Molecular Medicine and Haematology, University of Witwatersrand and National Health Laboratory Services, Johannesburg, South Africa; [6]Division of Hematology, Oncology, and Blood and Marrow Transplant, Nationwide Children's Hospital and The Ohio State University College of Medicine, Columbus, OH, USA; [7]Oxford Haemophilia and Thrombosis Centre, NIHR Oxford Biomedical Research Centre, Oxford University Hospitals NHS Foundation Trust, Oxford, UK; Radcliffe Department of Medicine, Oxford University, Oxford, UK; [8]Hemophilia and Thrombosis Center, University of Colorado School of Medicine, Aurora, CO, USA; [9]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Ca' Granda Ospedale Maggiore Policlinico, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy; [10]Hemophilia Treatment Center, University of California, Davis, Sacramento, CA, USA; [11]University Hospital Southampton and National Institute for Health and Care Research Clinical Research Facility, Southampton, UK; [12]UNC Blood Research Center, University of North Carolina, Chapel Hill, NC, USA; [13]Hemocentro UNICAMP, Department of Internal Medicine, School of Medical Sciences, University of Campinas, Campinas, SP, Brazil

Introduction

FVIII activity declines over time following AAV-mediated gene therapy

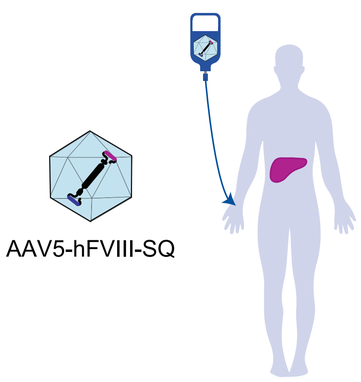


- Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is a gene therapy for hemophilia A that uses an AAV5 vector to deliver a B-domain-deleted hFVIII coding sequence controlled by a liver-selective promoter
- A single infusion has provided hemostatic efficacy for >3 years in a phase 3 study[1] and >7 years in a phase 1/2 study,[2] but FVIII activity declines over time

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

Methods

Study design

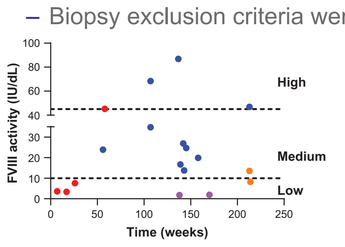


- Optional sub-study of the phase 1/2, phase 3 GENEr8-1, and phase 3b GENEr8-3 trials assessing the efficacy and safety of valoctocogene roxaparvovec in adult males with severe hemophilia A (FVIII ≤1 IU/dL)
  - Participants were administered 6x10^{13} vg/kg
  - Phase 3b trial incorporated the use of prophylactic corticosteroids (CS)*
- Standard of care (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

*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.

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



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)

- 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)

Objectives

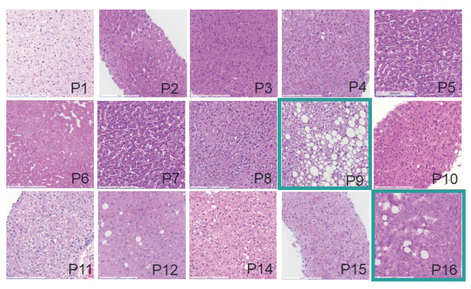
- To examine liver histopathology
- To assess rAAV5-HLP-co-hFVIII-SQ vector transduction efficiency
- To characterize and quantify episomal forms of vector DNA
- To quantify transgene expression (hFVIII-SQ RNA) and correlate with FVIII activity

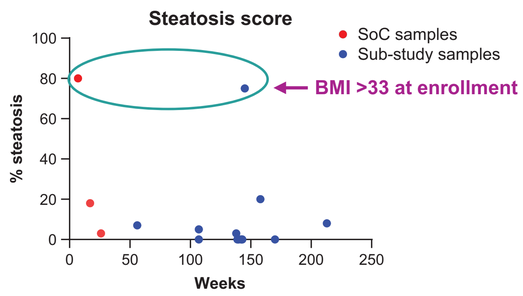
The goal of the analysis was to investigate mechanisms underlying durability and variability of response

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.

Results

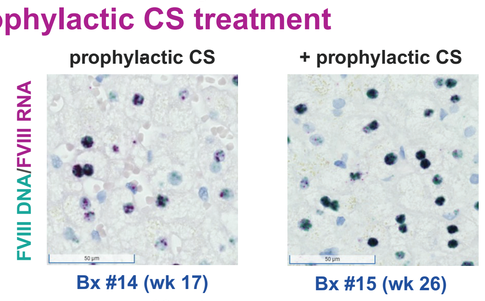
Histopathology revealed no evidence of abnormal architecture or dysplasia



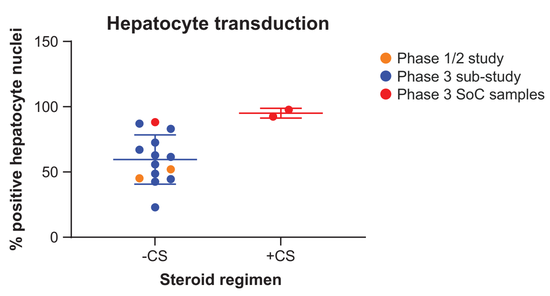


- Varying levels of steatosis were detected
  - 11/13 showed mild steatosis consistent with high prevalence in normal male populations from developed countries
  - 2/13 with severe steatosis had BMI >33 at enrollment
- No clinically relevant inflammation was observed
- Ground-glass hepatocytes observed in 1 participant with prior hepatitis B/C infection and possibly as a result of herbal supplementation
  - Ground-glass hepatocytes negative for hepatitis B surface antigen (HBsAg), FVIII, or glucose-regulated protein 78 (GRP78)

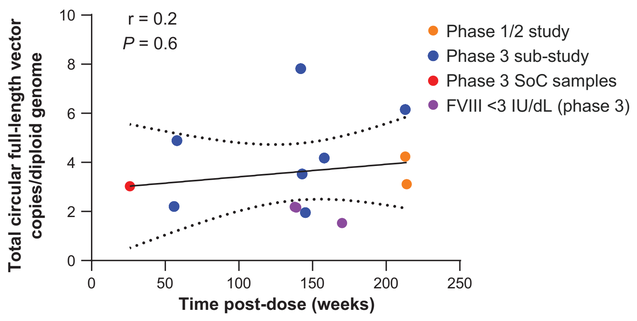
Efficient hepatocyte transduction of AAV5-hFVIII-SQ was detected regardless of prophylactic CS treatment



Bx, biopsy number; CS, corticosteroids; wk, week.



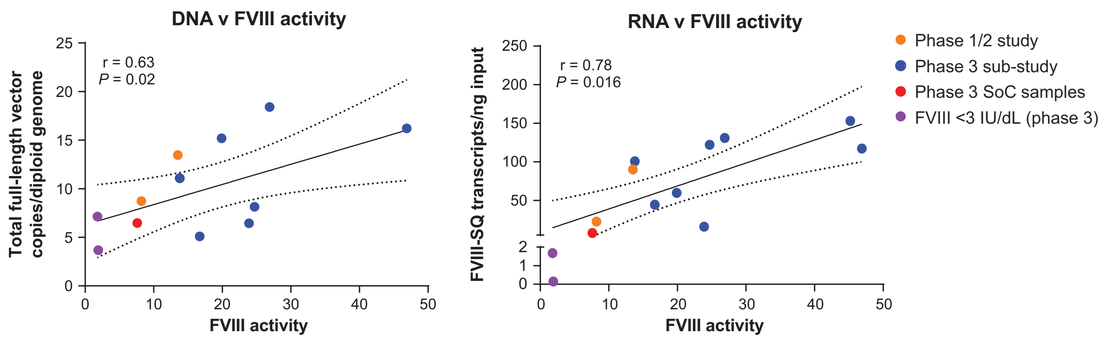
Circular full-length episomes persist through 4 years of post-gene therapy follow-up



- AAV vector genomes persist as episomal DNA and are accountable for long-term gene expression[3]
- 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 vector genomes

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

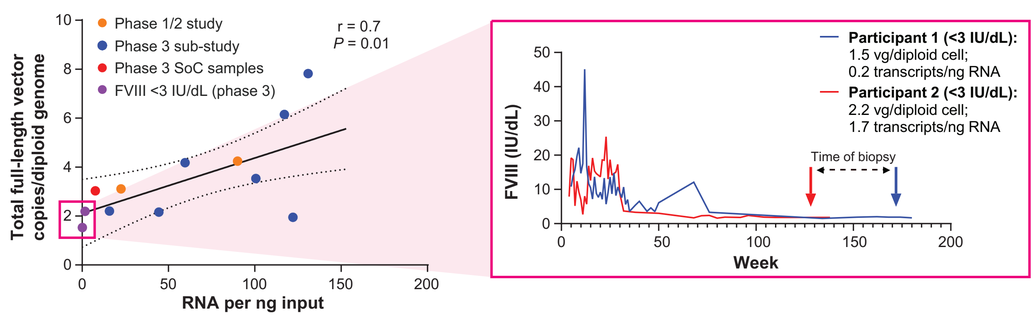
Full-length episomes and RNA transcript levels correlate with FVIII activity



FVIII activity was measured using chromogenic substrate assay.

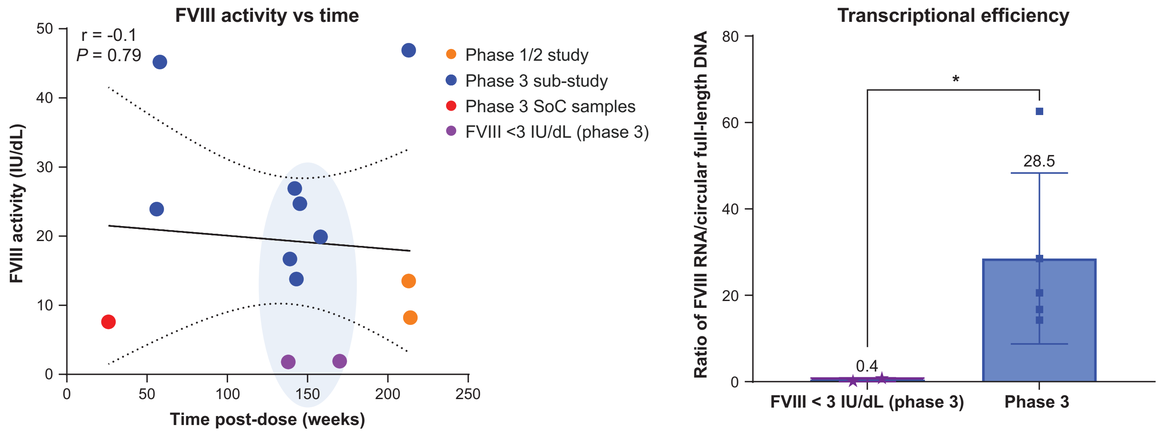
FVIII-SQ RNA expression influences FVIII response

- 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.

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.

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

References

1. Madan B, et al. *J Thromb Haemost.* 2024;22(7):1880-1893. **2.** Symington E, et al. *Haemophilia.* 2024;30(S1):96.
3. Penaud-Budloo M, et al. *J Virol.* 2008;82(16):7875-85.

Disclosures

JM declares no conflicts of interest.

Acknowledgements

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