Disclosure – conflict of interest

✓ I have the following potential conflict(s) of interest to report

- Name of commercial company: BioMarin Pharmaceutical Inc.
- Stock shareholder: BioMarin Pharmaceutical In.

Exploring actionable strategies to improve AAV5-hFVIII-SQ durability and optimize gene expression

Building Bridges in Coagulation

GTH 2024

68th Annual Meeting of the Society of Thrombosis and Haemostasis Research

WIEN

27 Feb–1 Mar

Presenter: Simon Harris

Bridget Yates, Dafna J. Groeneveld, Stephen Scheeler, Britta Handyside,

Isaac Villalpando, Suresh Agarwal, Stuart Bunting, Sylvia Fong

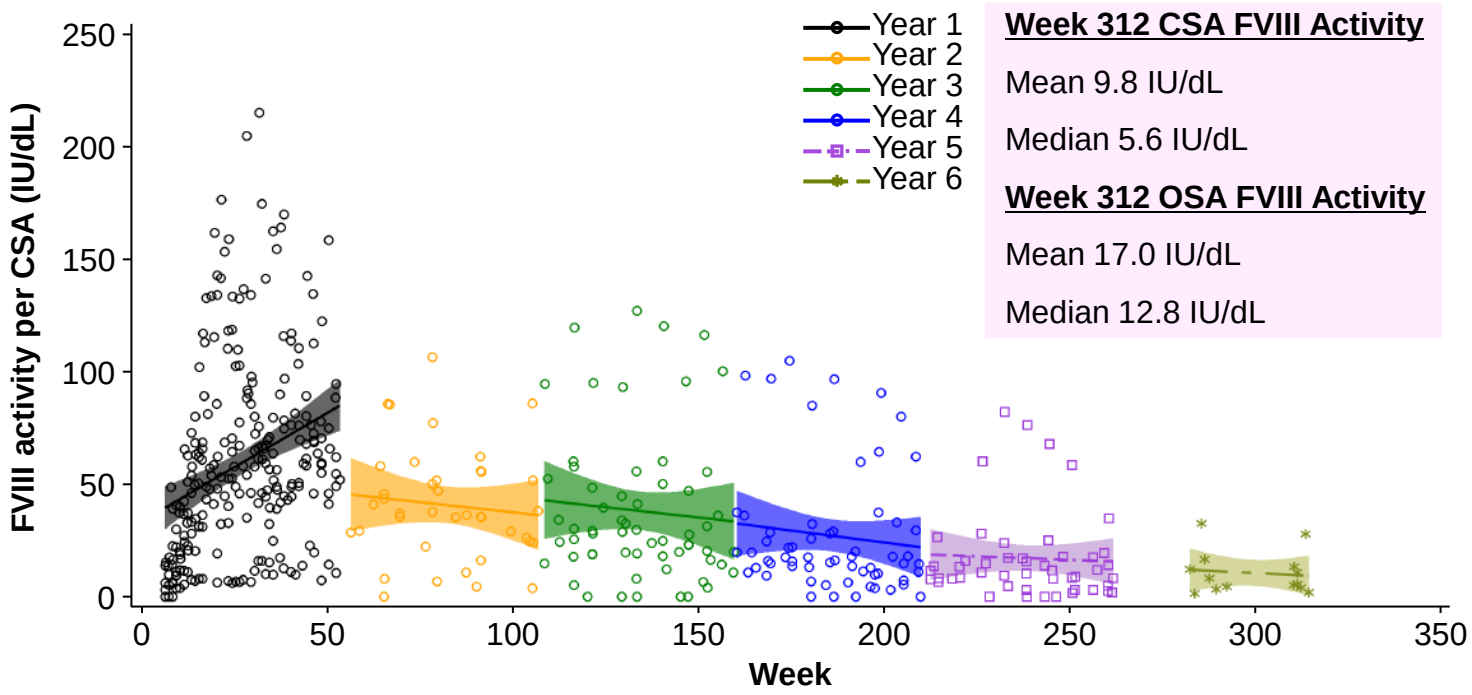
GTH

Disclosures

- **All** are employees and shareholders of BioMarin Pharmaceutical Inc.

AAV gene therapy for haemophilia A

- Efficacy
  - Haemostatic benefit relative to FVIII prophylaxis
  - Quality of life
- Safety profile
- Variability
  - Intra & inter-study
- Durability



28.02.2024

AAV: adeno-associated virus; CSA: chromogenic substrate assay; FVIII: factor VIII; OSA: one-stage assay

Laffan et al. Presentation at ISTH 2022 Congress, London, UK, 9-13 July 2022

Decline/variability in expression observed AAV-FVIII trials

- Understanding the mechanism leading loss of expression and variable response is needed to identify intervening strategies
  - Loss of expression maybe related to decrease AAV episome transcription
  - Variable response maybe related to individuals' abilities to fold and secrete FVIII proteins

Goal

- Strategies to maximize durability will provide patients longer benefit following gene therapy administration
  - Reverse decline of transgene expression
  - Increase FVIII secretion

Decline of FVIII expression

Actionable strategy to reverse decline

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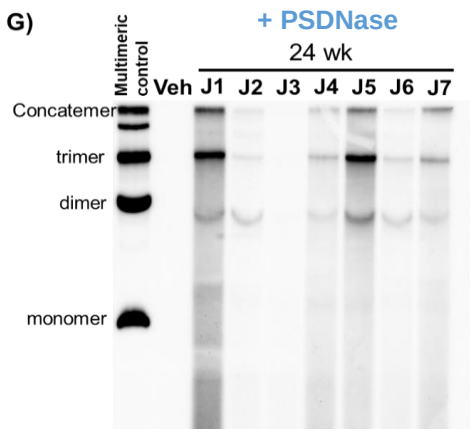
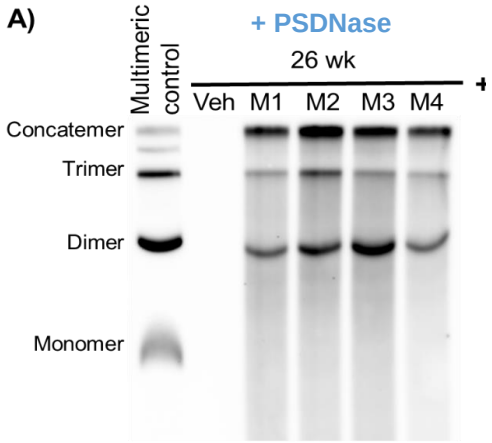
Stable episomes persist following AAV-FVIII GT

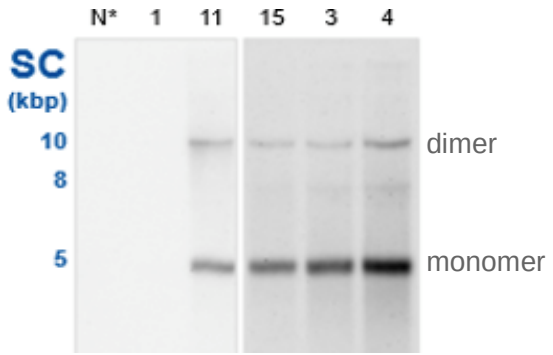
6 months



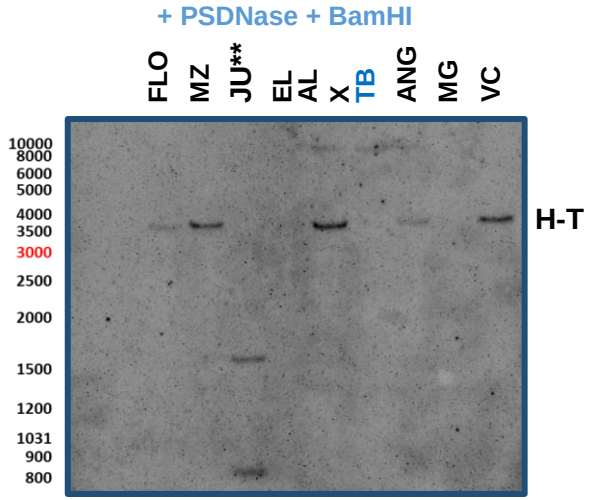
6 months



2.6 to 4.1 years



8 to 12 years

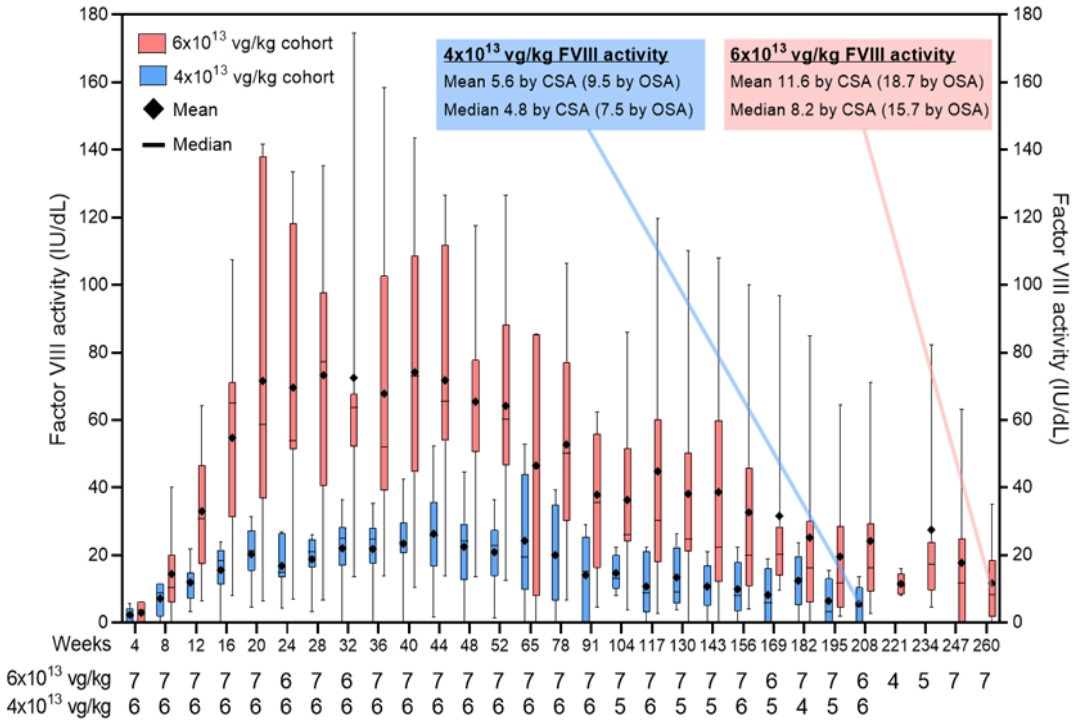


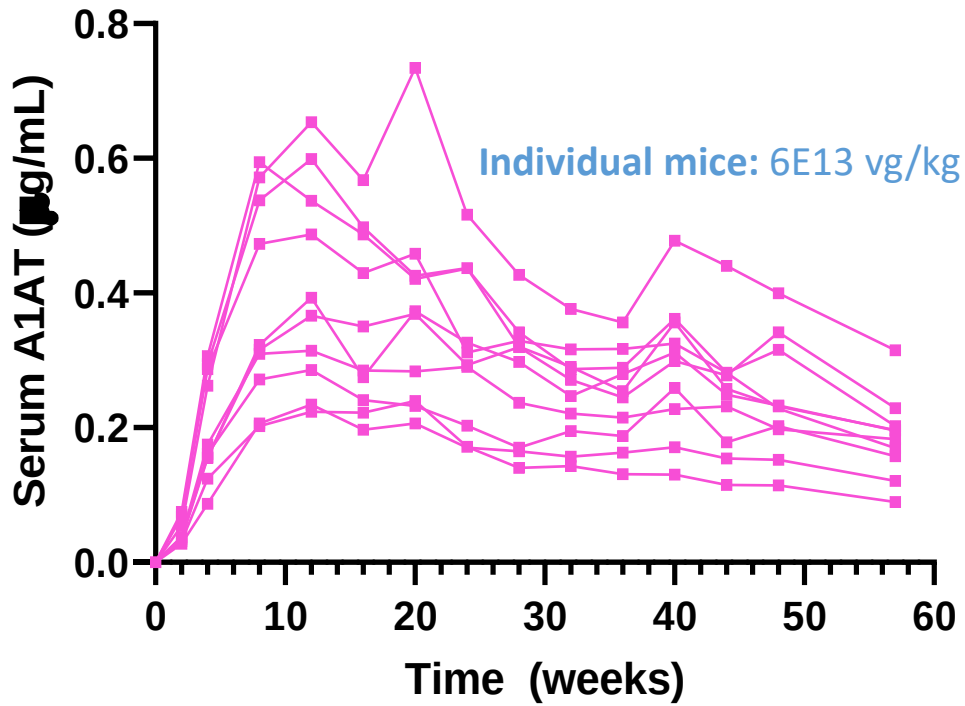
TB: Haemophilia A dog untreated control

28.02.2024

Sihn CR et al. Mol Ther Methods Clin Dev 2021;24:142-53; Fong S et al. Nat Med 2022;28:789-97; Batty P et al. Abstract presented at ISTH 2020 Congress, Milan, Italy; 12–14 July 2020

Expression profiles in mice are similar to humans





Pasi KJ et al. Haemophilia 2021;27:947-56; Handyside et al.,Vector genome loss and epigenetic modifications mediate decline in transgene expression of AAV5 vectors produced in mammalian and insect cells. Mol Ther. 2022; 30: 3570-3586

Multiple lines of evidence suggest low RNA production contributes to the decline of FVIII expression or low response to AAV-GT

Longitudinal Murine Study: Decreased interaction of active histones with episomal genomes may mediate the decline in transgene expression over time (Handyside *et al. Mol Ther* 2022)

Drug-induced suppression of FVIII expression following Accutane treatment was observed in clinic. In vitro studies showed Accutane decreased RNA transcript levels without affecting AAV vector genomes (Liu *et al. MTCMD.* 2022)

Human Biopsy Analysis: In one non-responder, hepatocytes expressed little RNA despite similar levels of vector genome (Fong *et al. Nat Med.* 2022)

Fong et al., Interindividual variability in transgene mRNA and protein production following adeno-associated virus gene therapy for hemophilia A. Nat Med. 2022; 28: 789-797; Liu et al. Application of in-vitro-cultured primary hepatocytes to evaluate species translatability and AAV transduction mechanisms of action. MTCMD. 2022; 26:61-71; Handyside et al.,Vector genome loss and epigenetic modifications mediate decline in transgene expression of AAV5 vectors produced in mammalian and insect cells; Mol Ther. 2022; 30: 3570-3586

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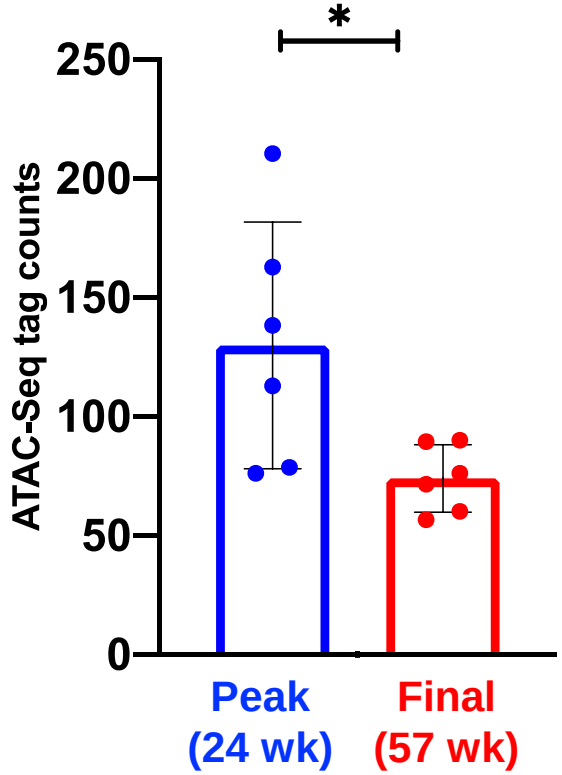
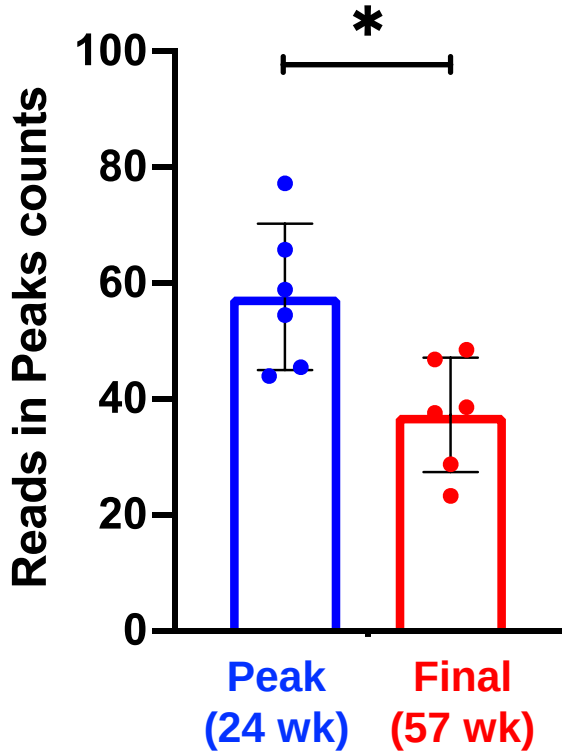
Genome accessibility may mediate decline in RNA expression in mice

Vector genome accessibility

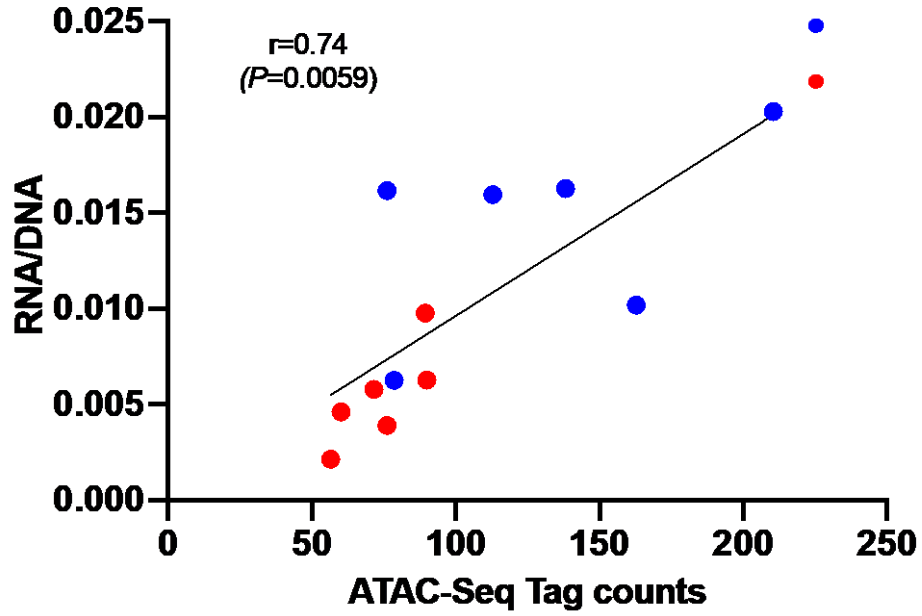


* Measured by ATAC-Seq

Interaction with active Histone H3H27ac



* Measured by CHIP-Seq

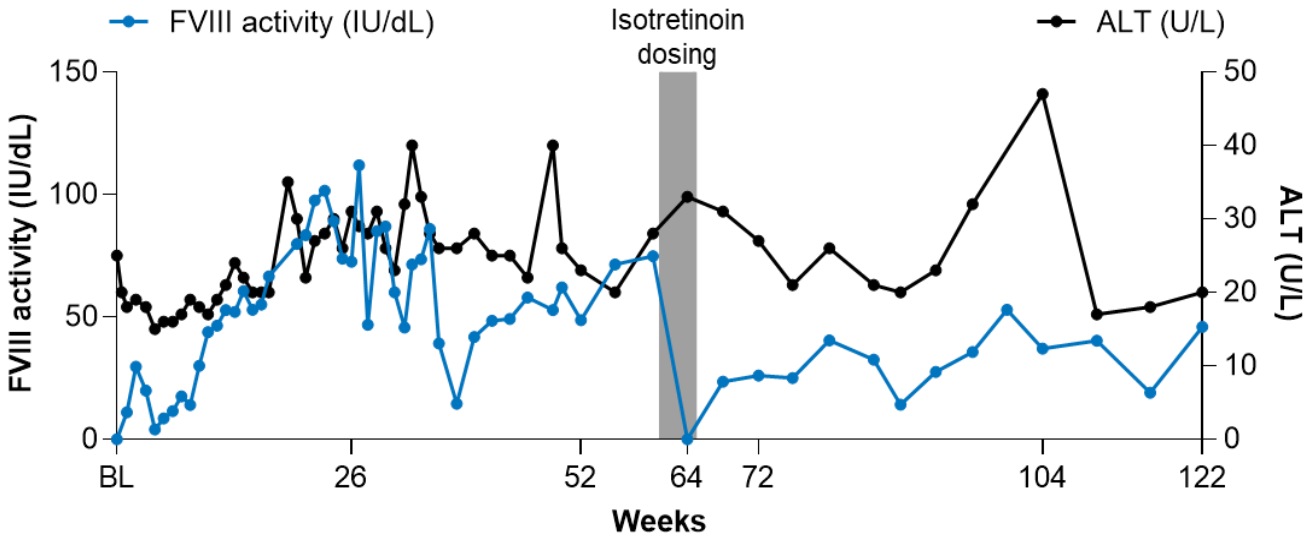


28.02.2024

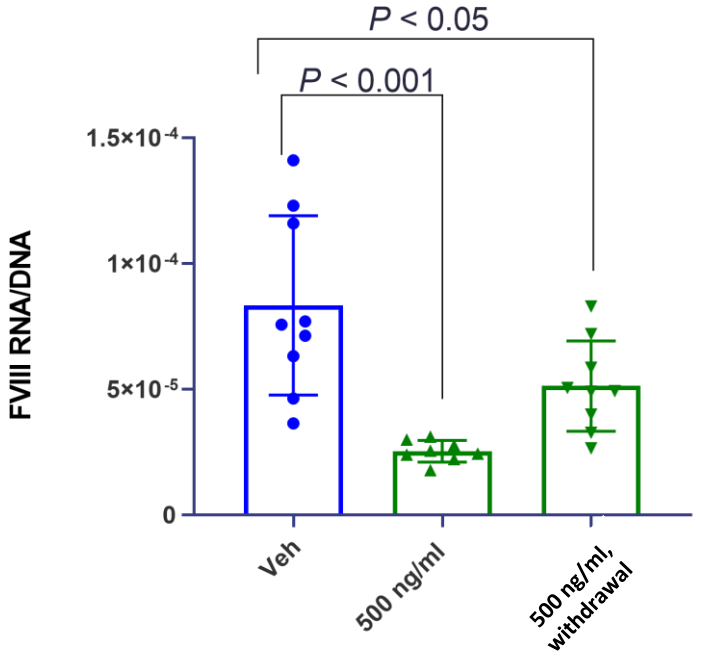
Handyside et al.,Vector genome loss and epigenetic modifications mediate decline in transgene expression of AAV5 vectors produced in mammalian and insect cells. Mol Ther. 2022; 30: 3570-3586

Transcriptional regulation contributes to decline in expression in human

FVIII plasma levels decline following Accutane Rx in clinical trial participant who received 6e13 vg/kg of AAV5-hFVIII-SQ (valoctocogene roxaparvovec)



Effect of Accutane* on AAV5-hFVIII-SQ (valoctocogene roxaparvovec) occurs at the RNA level in primary human hepatocytes

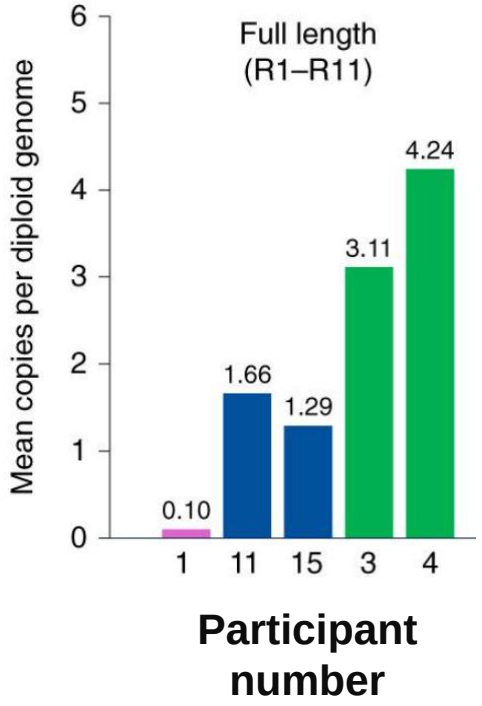


*Accutane did not induce hepatoxicity; had no effect on vector genome levels

Liu et al. Application of in-vitro-cultured primary hepatocytes to evaluate species translatability and AAV transduction mechanisms of action. MTCMD. 2022; 26:61-71

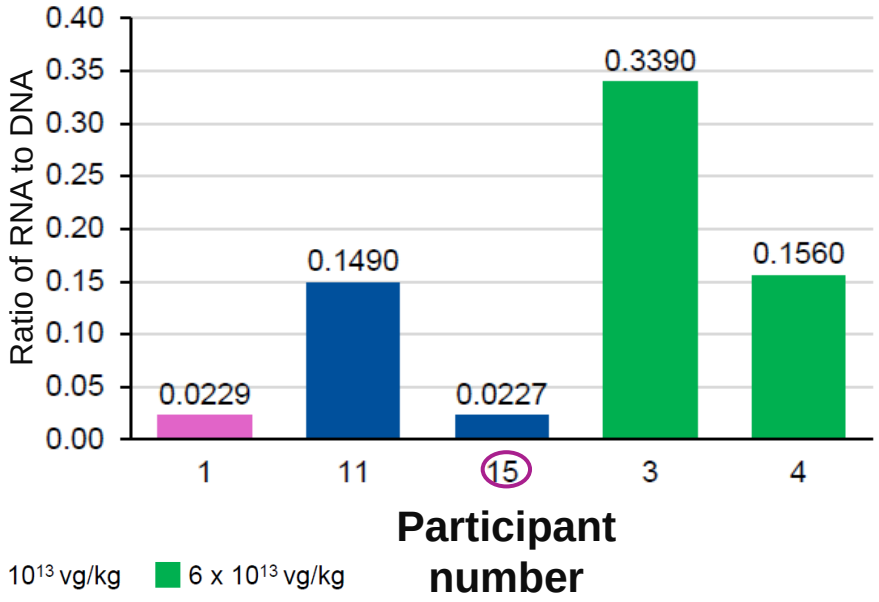
Human Biopsy Analysis: In one non-responder, hepatocytes expressed little RNA despite similar levels of vector genome

Episomal vector are similar



AAV episomes can persist over time and assimilate into chromatin with a typical nucleosomal pattern[2]

Low RNA to DNA ratio in low responder (P15)

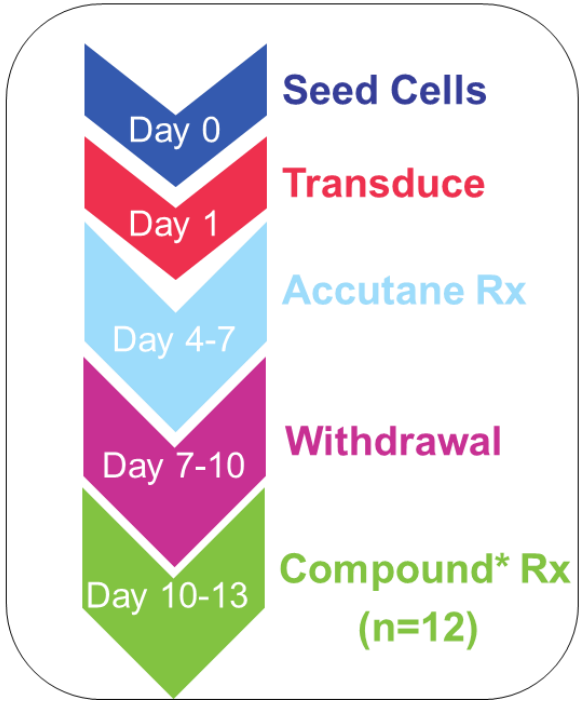


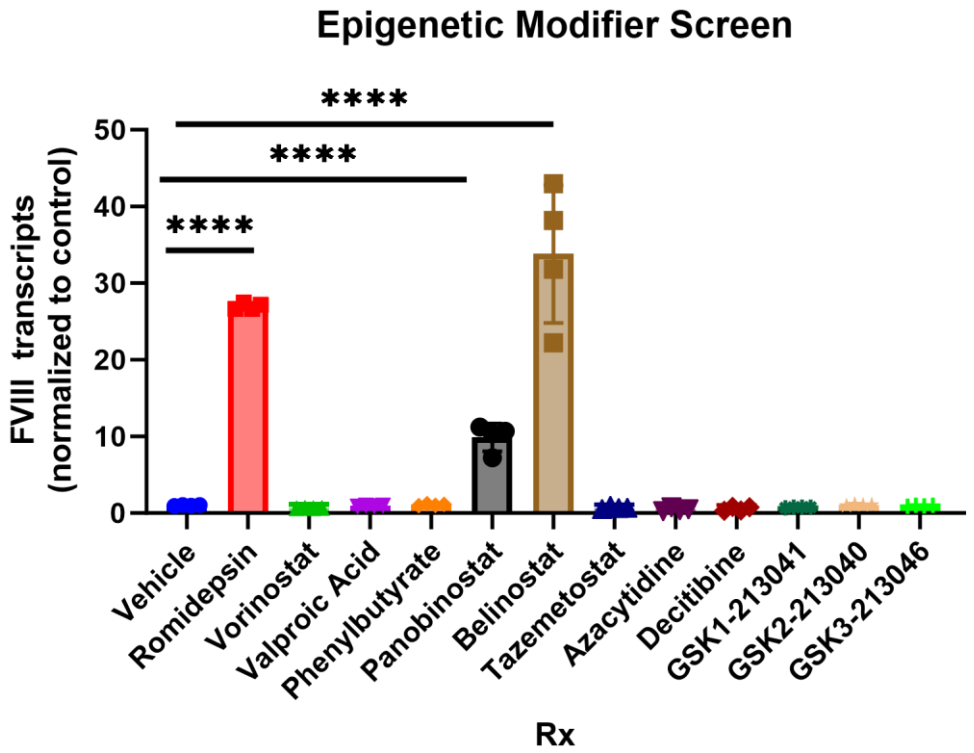
Fong et al., Interindividual variability in transgene mRNA and protein production following adeno-associated virus gene therapy for hemophilia A. Nat Med. 2022; 28: 789-7972. Penaud-Budloo M, Le Guiner C, Nowrouzi A, et al. Adeno-associated virus vector genomes persist as episomal chromatin in primate muscle. J Virol. 2008;82(16):7875-788

HDACi reverses drug-induced ROCTAVIAN silencing in vitro

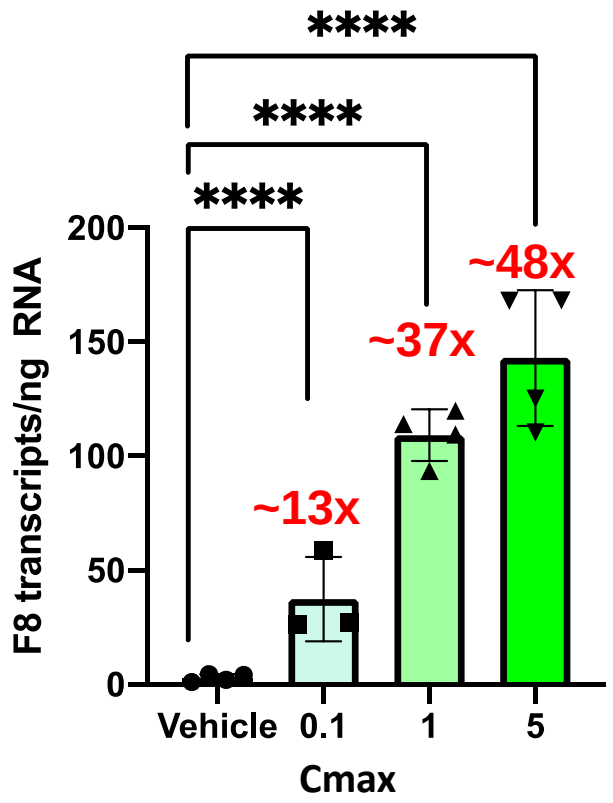
Hypothesis: Modifying the chromatin interaction with AAV-episomes using epigenetic modulators may increase accessibility of vector genomes potentially reactivating vector genome expression

Screening for Reactivation





HDACi reverses drug-induced ROCTAVIAN silencing in vitro

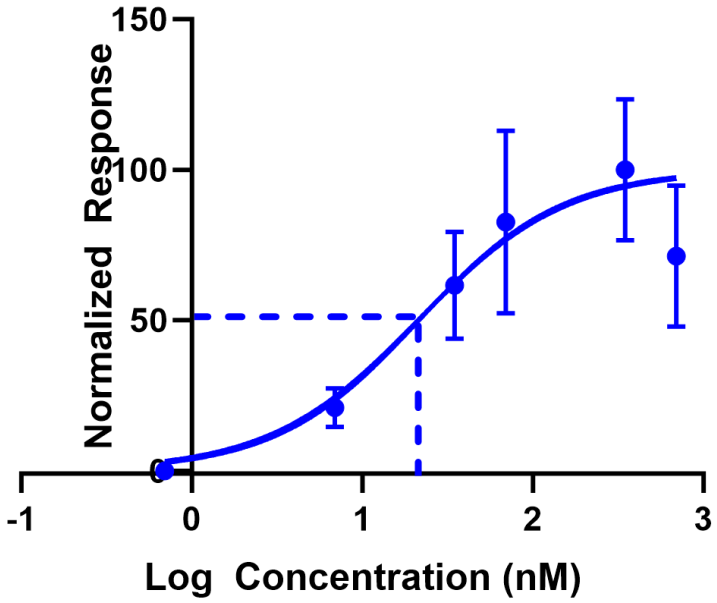


377 ng/mL= 1x Cmax

- At all doses tested, no toxicity was observed in human primary hepatocytes
- Romidepsin increased transgene expression without drug-induced silencing, though to a lesser degree

Modeling of HDACi Doses Needed to Reactivate Expression

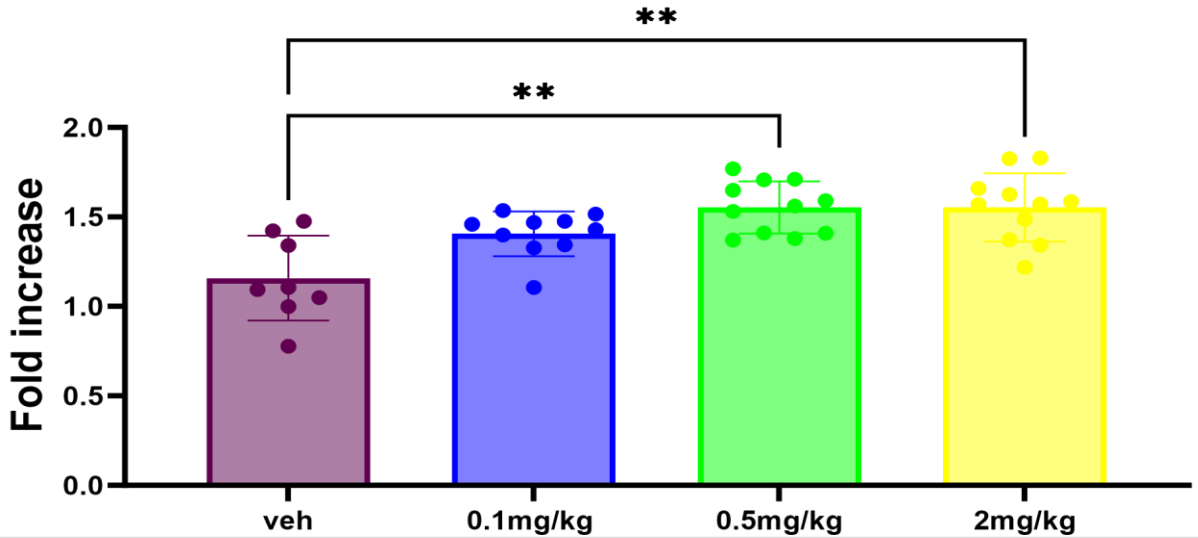
- Modeling performed to predict exposures needed for a **3-fold increase** in expression in in-vivo experiments using:
  - human and preclinical PK data (literature)
  - in vitro primary human hepatocyte data (in-house)



Reactivation: <20% of clinical dose

Pilot mouse study indicated Romidepsin can increase AAV expression

Potentiation model: Romidepsin treatment 4-weeks following in C57Bl/6 mice



- A single dose of HDACi moderately increased A1AT levels at doses ≤ 2 mg/kg (~40% of clinical dose)
  - No signs of liver toxicity measured by ALT and histopathology
  - No signs of myelosuppression (normal CBC and clinical chemistry)
- Mouse studies underway to evaluate potential reactivation of AAV5 following transgene expression decline

Variability of FVIII expression

Actionable strategy to improve FVIII secretion

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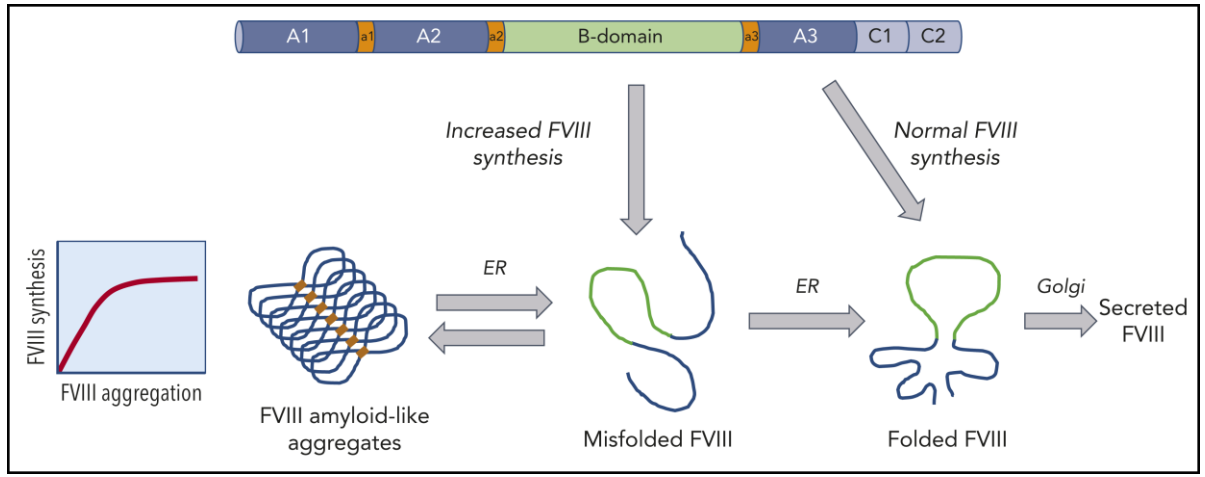
Evaluating actionable strategies to increase FVIII secretion

Increased BDD-FVIII synthesis can lead to misfolding and aggregation



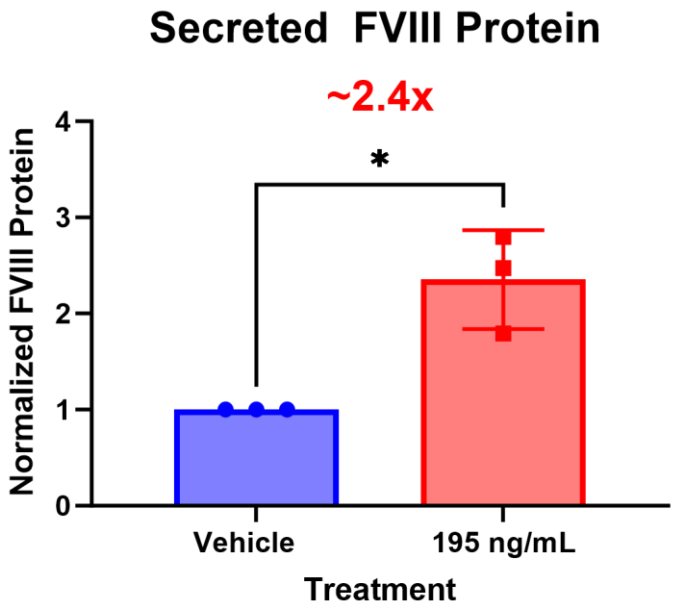
Denise E. Sabatino, Clogging up the pipeline: factor VIII aggregates, Blood, 2020

- B-domain deleted FVIII-SQ protein is inefficiently folded and secreted from the ER[1]
- Studies have demonstrated reducing ER stress with antioxidants can increase FVIII secretion both in vivo and in vitro[2]
- Cells have a capacity to fold and secrete FVIII-SQ protein and the individual capacity could lead to inter-individual variability of response[3]

Objective: Screen pharmacological chaperones to evaluate potential strategy to increase FVIII secretion

1. Malhotra 2008; 2. Malhotra et al 2008; 3. Oslowski 2011; Fong 2020 and 2022

Phenylbutyrate significantly increases BDD-FVIII protein secretion



n=3 independent experiments

195 ng/mL = 1x Cmax

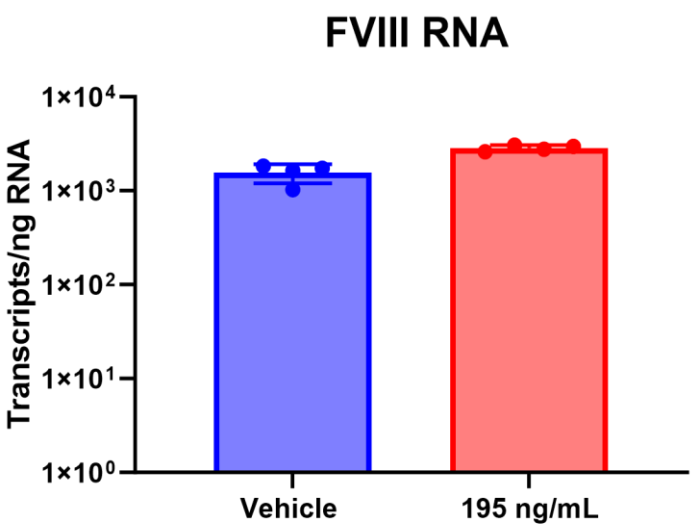
Sodium phenylbutyrate (4-PBA)

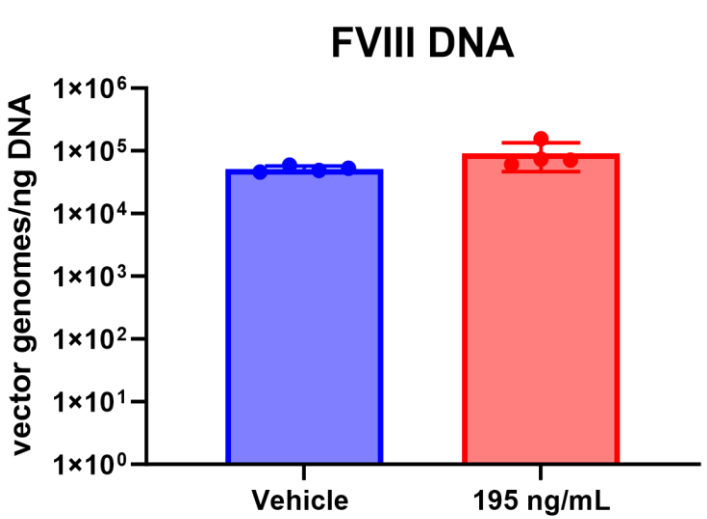
- Small molecular chaperone
- Reduces UPR/ER stress[1]
- Approved in EU and US

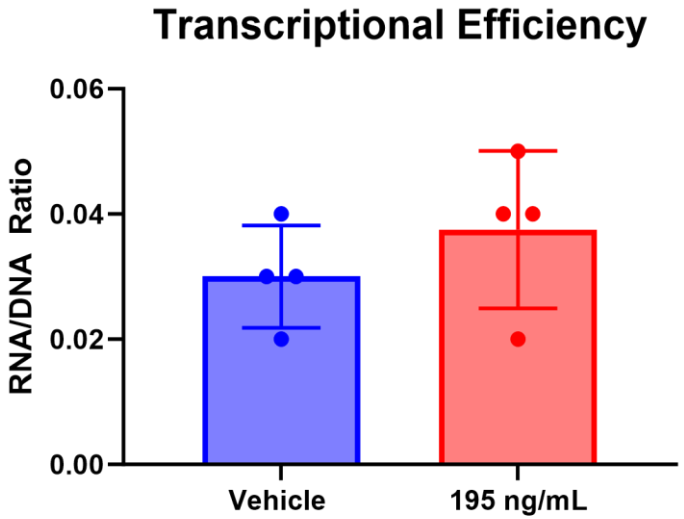
12 compounds were screened in HepG2 cells transduced with AAV5-hFVIII-SQ

Basseri, Sana et al. "The chemical chaperone 4-phenylbutyrate inhibits adipogenesis by modulating the unfolded protein response." Journal of lipid research vol. 50,12 (2009): 2486-501. Xiao, Changting et al. "Sodium phenylbutyrate, a drug with known capacity to reduce endoplasmic reticulum stress, partially alleviates lipid-induced insulin resistance and beta-cell dysfunction in humans." Diabetes vol. 60,3 (2011): 918-24. Yam, Gary Hin-Fai et al. "Sodium 4-phenylbutyrate acts as a chemical chaperone on misfolded myocilin to rescue cells from endoplasmic reticulum stress and apoptosis." Investigative ophthalmology & visual science vol. 48,4 (2007): 1683-90.

Phenylbutyrate has no effect on transgene transcriptional efficiency







195 ng/mL= 1x Cmax

Sodium phenylbutyrate had no effect on transgene expression in primary human hepatocytes

Basseri, Sana et al. “The chemical chaperone 4-phenylbutyrate inhibits adipogenesis by modulating the unfolded protein response.” Journal of lipid research vol. 50,12 (2009): 2486-501. Xiao, Changting et al. “Sodium phenylbutyrate, a drug with known capacity to reduce endoplasmic reticulum stress, partially alleviates lipid-induced insulin resistance and beta-cell dysfunction in humans.” Diabetes vol. 60,3 (2011): 918-24. Yam, Gary Hin-Fai et al. “Sodium 4-phenylbutyrate acts as a chemical chaperone on misfolded myocilin to rescue cells from endoplasmic reticulum stress and apoptosis.” Investigative ophthalmology & visual science vol. 48,4 (2007): 1683-90.

Key summaries

- Two actionable strategies were identified to potentially improve patient outcomes following AAV5-hFVIII-SQ treatment
  - Epigenetic modulators (HDACi) show potential for reactivating AAV transgene expression both in vitro and in vivo at low doses
  - The use of chemical chaperones may improve FVIII-SQ secretion

- Additional mouse studies are underway to evaluate if:
  - Romidepsin can reactivate transgene expression following decline with low and infrequent dosing regimen, and
  - Sodium phenylbutyrate can increase FVIII secretion in vivo

Acknowledgements

- This study was funded by BioMarin Pharmaceutical Inc.