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

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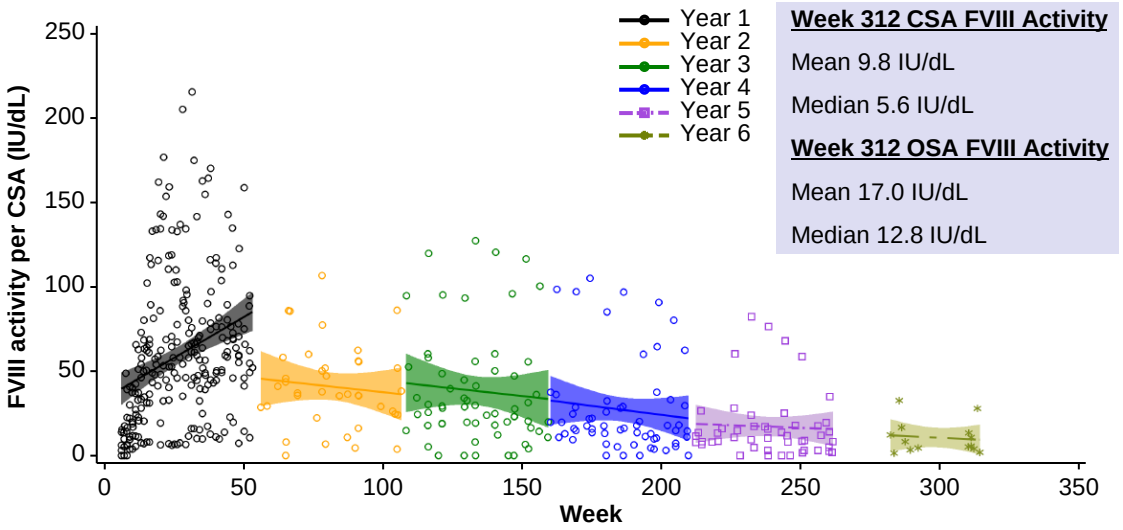
Disclosures

- Employees and stockholders 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



Week 312 CSA FVIII Activity

Mean 9.8 IU/dL

Median 5.6 IU/dL

Week 312 OSA FVIII Activity

Mean 17.0 IU/dL

Median 12.8 IU/dL

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

GT: gene therapy

Speaker's view

Decline of FVIII expression

Actionable strategy to reverse decline

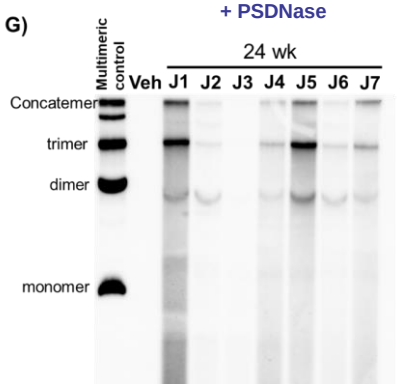
Stable episomes persist following AAV-FVIII GT

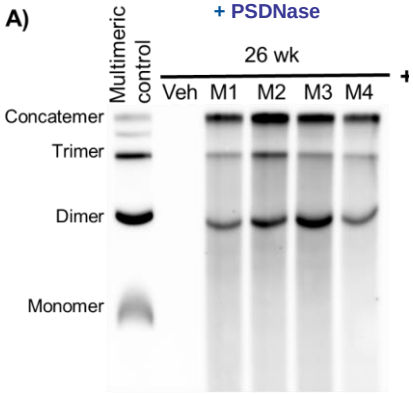
6 months

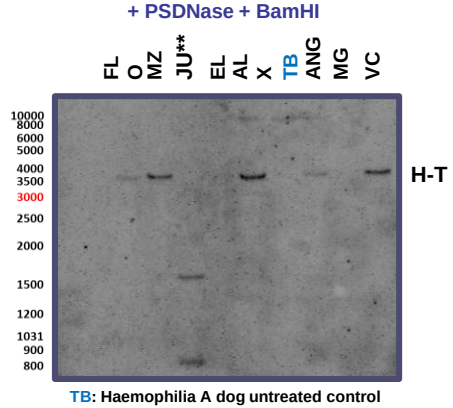
6 months

2.6 to 4.1 years

8 to 12 years

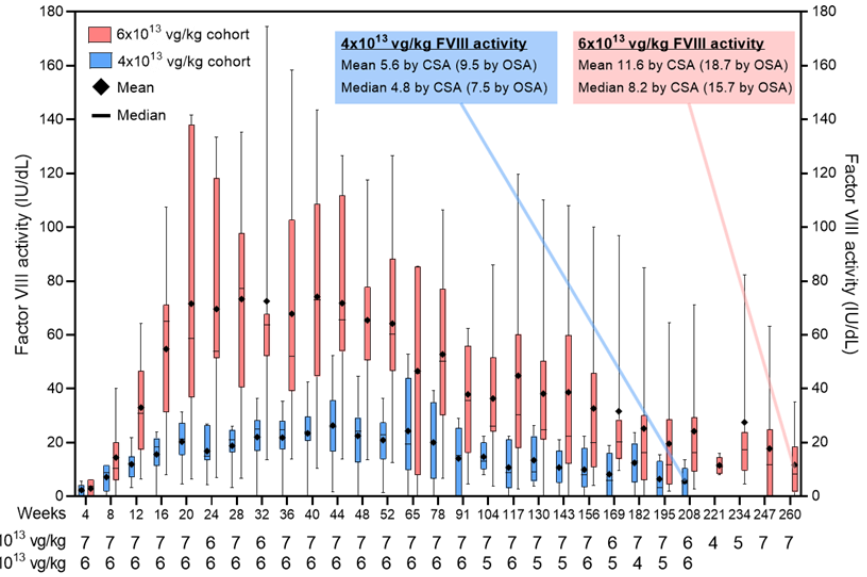


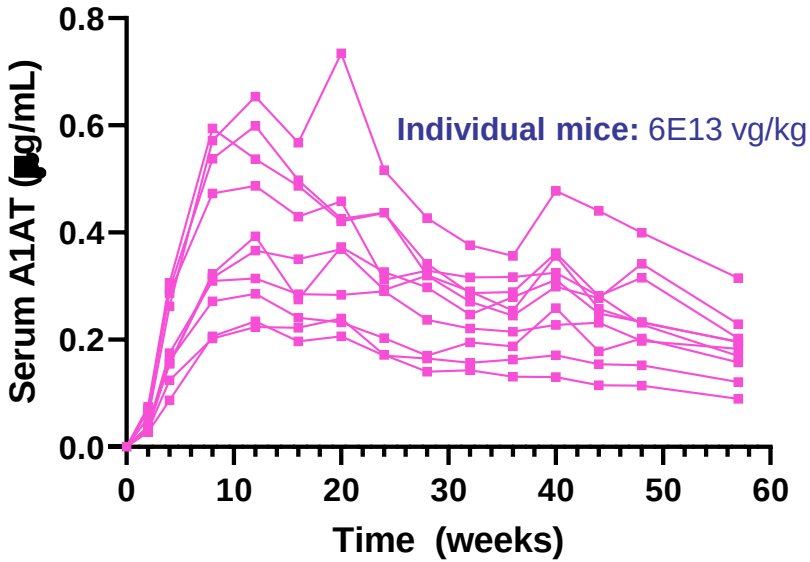




TB: Haemophilia A dog untreated control

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)

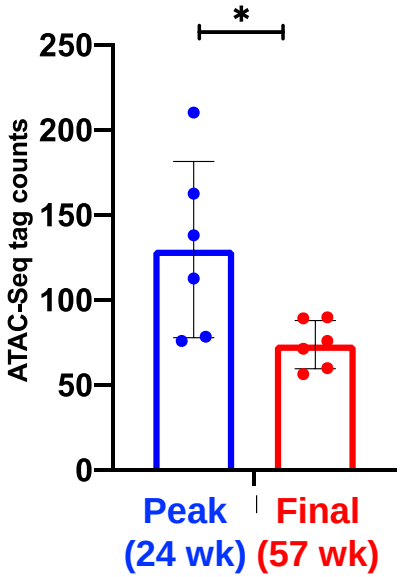
Drug-induced suppression of FVIII expression following Accutane treatment was observed in clinic. In vitro studies showed Accutane decreased RNA transcript levels without affecting AAV vector genomes (Liu *et al. MTCMD.* 2022)

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

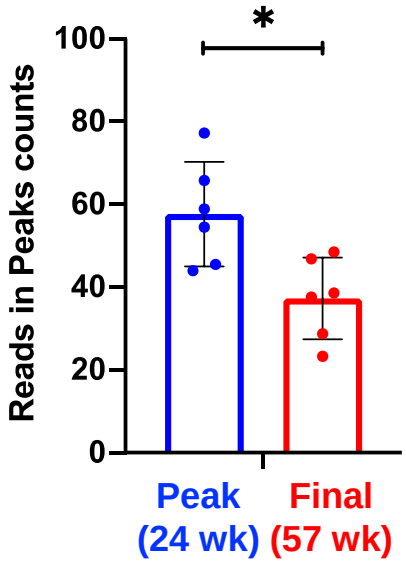
Genome accessibility may mediate decline in RNA expression in mice

Vector genome accessibility

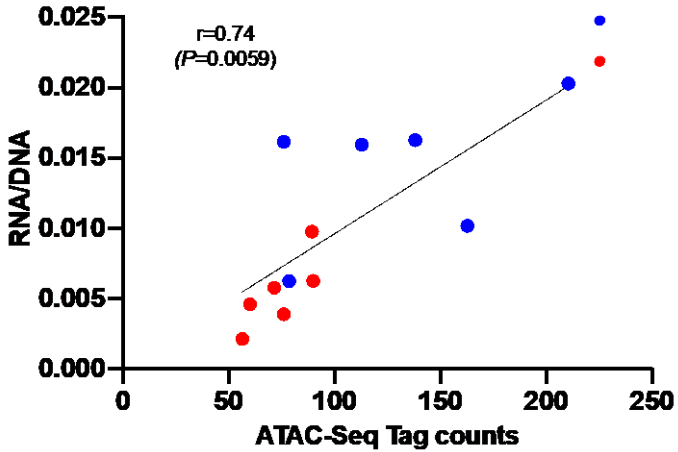


* Measured by ATAC-Seq

Interaction with active Histone H3H27ac



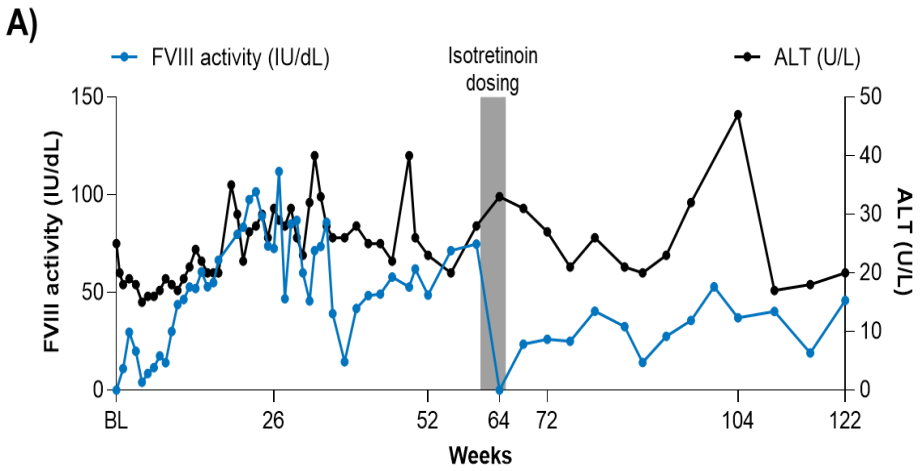
* Measured by CHIP-Seq



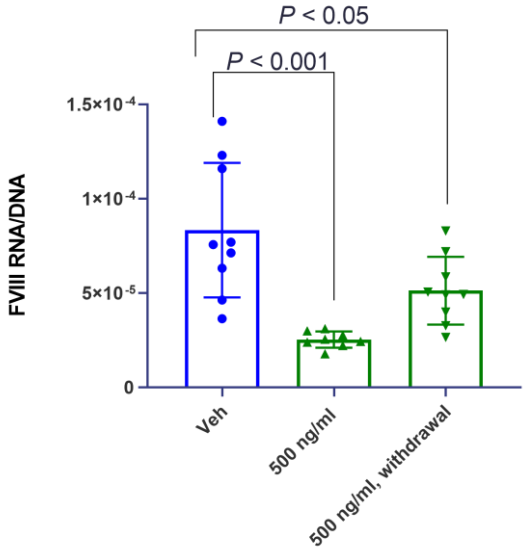
Handyside et al.,Vector genome loss and epigenetic modifications mediate decline in transgene expression of AAV5 vectors produced in mammalian and insect cells. *Mol Ther.* 2022; 30: 3570-3586

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

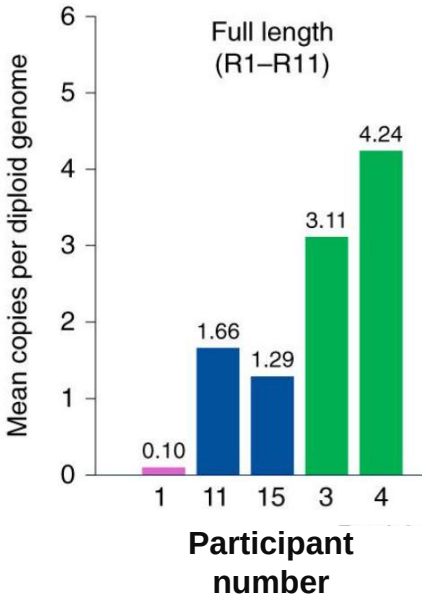


*Accutane did not induce hepatoxicity; had no effect on vector genome levels

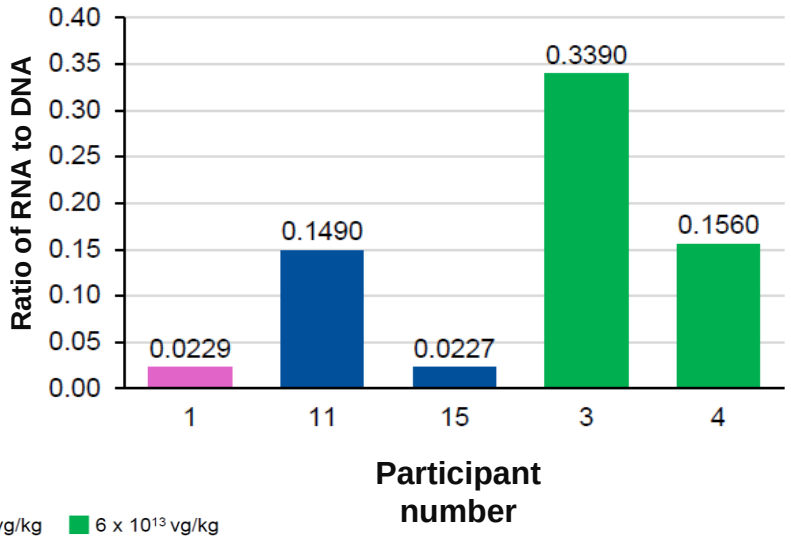
Liu *et al*. Application of in-vitro-cultured primary hepatocytes to evaluate species translatability and AAV transduction mechanisms of action. *MTCMD*. 2022; 26:61-71

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

Episomal vector are similar



Low RNA to DNA ratio in low responder (P15)



6 x 10^{12} vg/kg 4 x 10^{13} vg/kg 6 x 10^{13} vg/kg

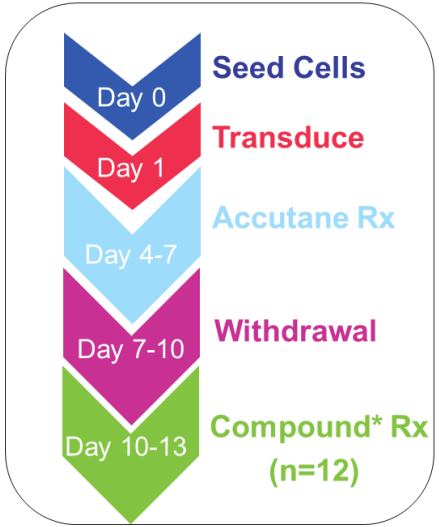
AAV episomes can persist over time and assimilate into chromatin with a typical nucleosomal pattern[2]

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
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*

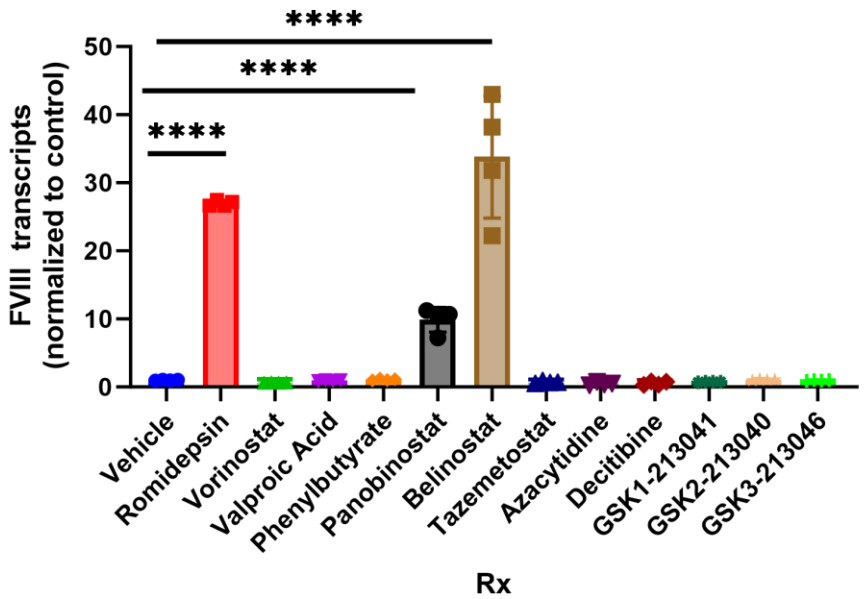
HDACi reverses drug-induced ROCTAVIAN silencing in vitro

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

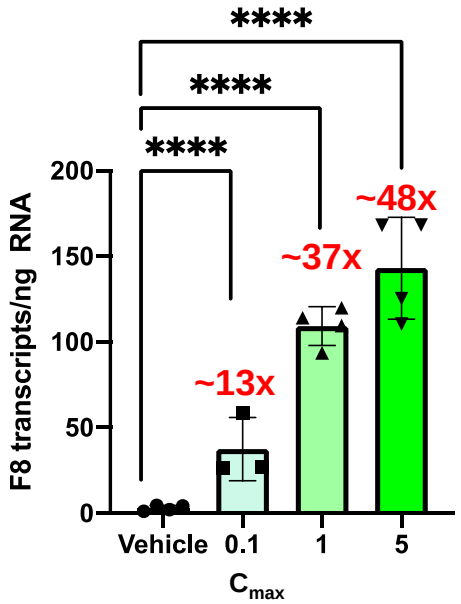
Screening for Reactivation



Epigenetic Modifier Screen



HDACi reverses drug-induced ROCTAVIAN silencing in vitro

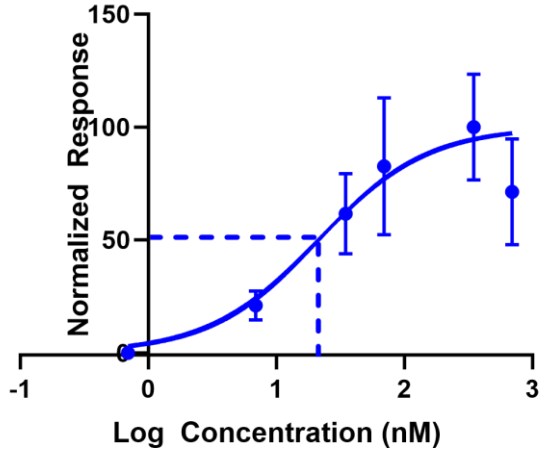


377 ng/mL= 1x C_{max}

- 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

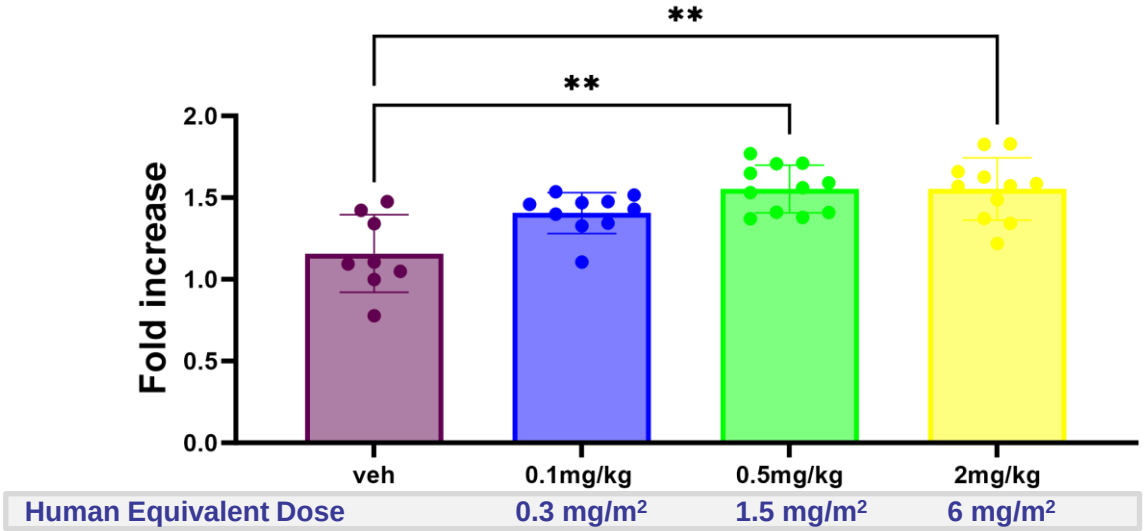
Modeling of HDACi doses needed to reactivate expression

- 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)



Pilot mouse study indicated Romidepsin can increase AAV expression

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



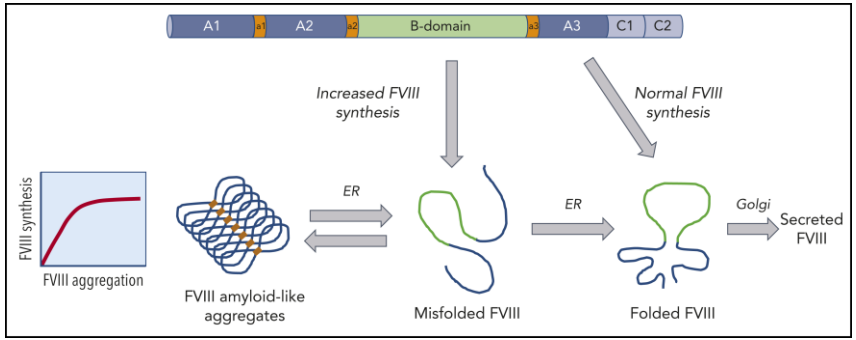
- A single dose of HDACi moderately increased A1AT levels at doses ≤ 2 mg/kg (~40% of clinical dose)
  - No signs of liver toxicity measured by ALT and histopathology
  - No signs of myelosuppression (normal CBC and clinical chemistry)
- Mouse studies underway to evaluate potential reactivation of AAV5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

Variability of FVIII expression

Actionable strategy to improve FVIII secretion

Evaluating actionable strategies to increase FVIII secretion

Increased BDD-FVIII synthesis can lead to misfolding and aggregation



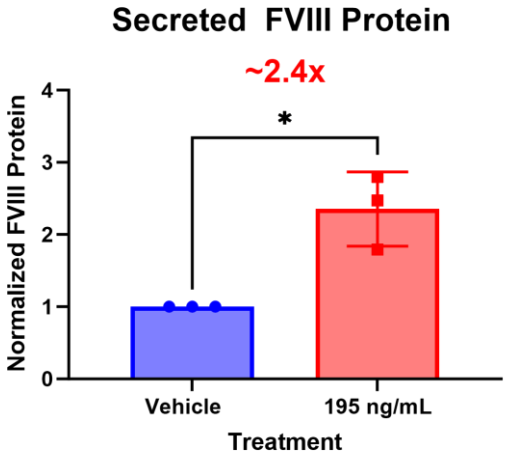
Denise E. Sabatino, Clogging up the pipeline: factor VIII aggregates, Blood, 2020

- 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

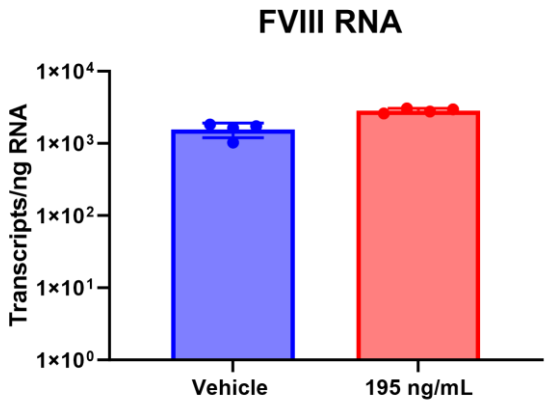
Sodium phenylbutyrate (4-PBA)

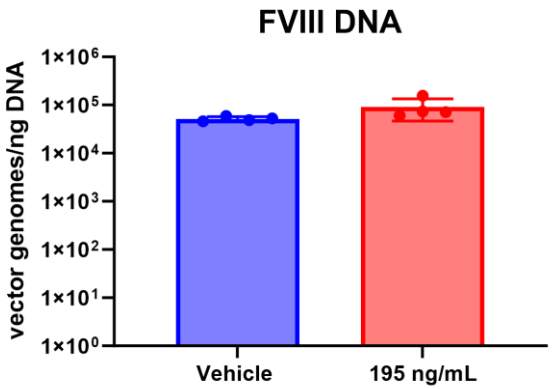
- Small molecular chaperone
  - Reduces UPR/ER stress[1]
  - Approved in EU and US

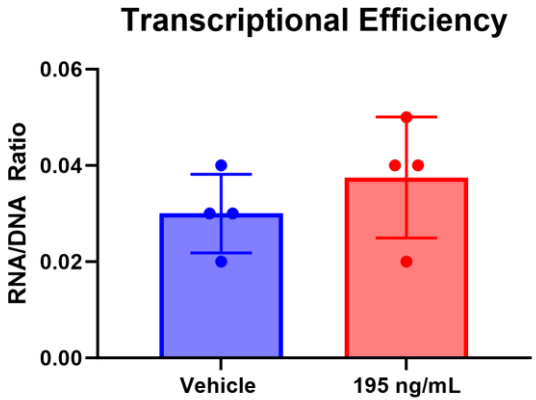
12 compounds were screened in HepG2 cells transduced with AAV5-hFVIII-SQ

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







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

    1. Epigenetic modulators (HDACi) show potential for reactivating AAV transgene expression both in vitro and in vivo at low doses

    2. 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.