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Childhood treatment with Adeno-Associated Viral gene therapy results in stable FVIII expression and improved bleeding phenotype in adult severe hemophilia A dogs

P. Batty,[1,2] A. Menard,[3] A. Mo,[1] C. Brown,[1] A.M. Ismail,[4] B. Yates,[4] L. Swystun,[1] L. Harpell,[1] A. Pender,[1] A. Winterborn,[5] S. Fong,[4] D. Lillicrap[1]

[1]Department of Pathology and Molecular Medicine, Queen's University, Kingston, Canada; [2]Cancer Institute, University College London, London, UK; [3]Kingston Health Sciences Centre, Kingston, Canada; [4]BioMarin Pharmaceutical, Novato, United States; [5]Animal Care Services, Queen's University, Kingston, Canada

INTRODUCTION

- AAV5-FVIII gene therapy is approved in the U.S. and conditionally approved in Europe for the treatment of adults with severe hemophilia A.
- The majority of FVIII is thought to be expressed by non-integrated episomal structures.
- AAV-FVIII treatment during childhood could prevent the development of arthropathy and improve quality of life.
- Long-term safety and persistence of transgene expression in AAV-FVIII-treated children is unknown.
- The inflammatory response to AAV-FVIII in children is largely uncharacterized.

PREVIOUS WORK

- We have previously reported outcomes of severe hemophilia A dogs treated at 2 weeks (n=2) or 2 months (n=3) of age using a single infusion of an AAV5-canine FVIII (cFVIII) vector.
- Dogs treated at 2-weeks demonstrated improved whole blood clot time (WBCT) despite minimal FVIII expression (<3%) after 6 months.
- Dogs treated at 2-months of age demonstrated stable FVIII expression and decreased WBCT after 6 months.

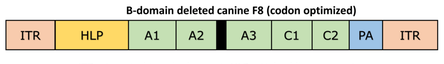

Figure 1. Structure of the AAV5-cFVIII vector

AIMS

1. To provide an update on the safety and efficacy of AAV5-cFVIII in these animals 12-18 months post-treatment.
2. To describe the early (days 0 - 21) inflammatory response to AAV5-cFVIII in infant and neonatal dogs.

RESULTS

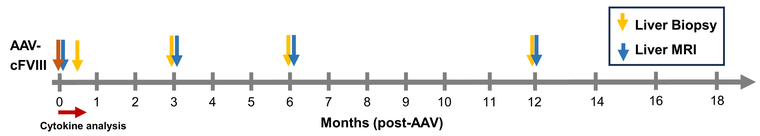

Figure 2. Study timeline

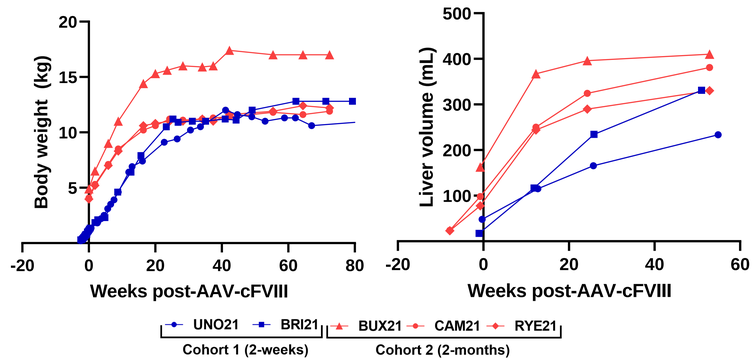

Figure 3. Fold increase in liver volume was proportional to the increase in body weight 12 months post-AAV-cFVIII.

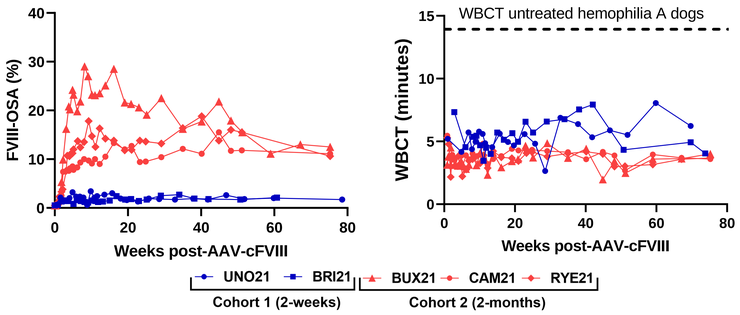

Figure 4. cFVIII expression by one-stage assay (OSA) was minimal (<3%) for dogs treated at 2-weeks and sustained for dogs treated at 2-months. Lower limit of quantification = 2%.

Figure 5. Dogs treated at 2-weeks and 2-months maintained an improved whole blood clot time (WBCT). Normal WBCT = 6 minutes. WBCT in hemophilia A dogs = 13.9 minutes

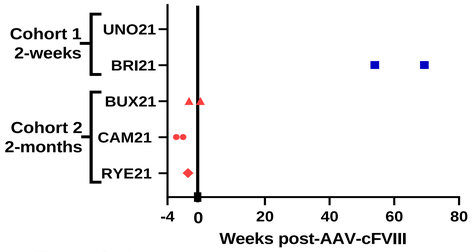

Figure 6. A reduction in bleeding events was observed for post-AAV-cFVIII treatment.

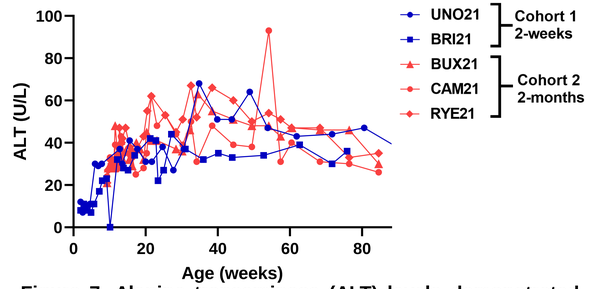

Figure 7. Alanine transaminase (ALT) levels demonstrated only minor age-related increases.

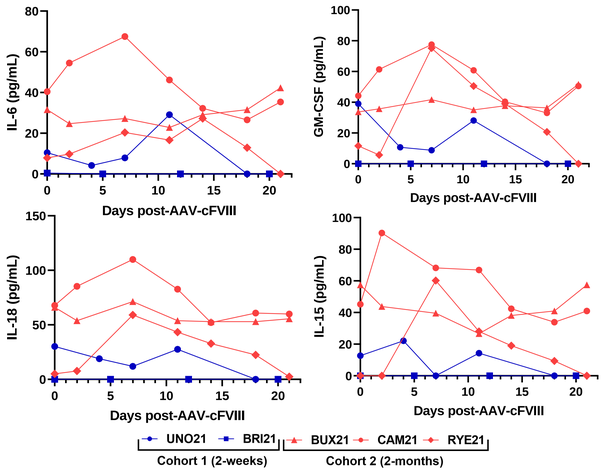

Figure 8. Transient elevations in pro-inflammatory cytokines post-AAV-cFVIII were observed for three of the dogs and resolved within 21 days.

METHODS

- Hemophilia A dogs were treated as follows:

Study ID	Sex	Age (weeks)	Treatment wt (kg)	Dose (vg/kg)	Total vg
UNO21	F	2	1.0	2.0e14	2.0e14
BR121	F	2	1.2	2.0e14	2.4e14
BUX21	M	9.3	4.9	2.0e14	9.8e14
CAM21	F	9.3	4.1	2.0e14	8.2e14
RYE21	F	9.3	4.0	2.0e14	8.0e14

Table 1. Summary of AAV-cFVIII treatment. vg=vector genomes, wt=weight

- VG dose based on RAG2/FVIII DKO murine studies (Zhang et al. Mol Ther Methods Clin Dev. 2022)
- Epigenomic data suggests that 2 months of age in dogs is equivalent to 9 months in humans (Wang et al. Cell Syst. 2020).
- Liver volume was measured by MRI and percutaneous liver biopsies were performed as indicated in Figure 2.
- Serum cytokine levels were measured at baseline and post-treatment (days 2-21) by cytokine array.

CONCLUSIONS

- Treatment of hemophilia A dogs with AAV5-cFVIII at 2-months of age resulted in stable FVIII expression into adult life despite significant liver expansion.
- For some dogs, AAV-cFVIII treatment resulted in an early transient increase in proinflammatory cytokine levels with no evidence of transaminitis that resolved within three weeks.
- Further studies on liver biopsy samples are ongoing to evaluate cellular implications, vector genome distribution, and mechanisms of AAV persistence.

ACKNOWLEDGEMENTS

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