Mechanisms of AAV Vector Persistence Following Infant Gene Therapy in the Severe Hemophilia A Dog Model

Paul Batty, Bridget Yates, A.M. Ismail, Laura L. Swystun, Alexandre Menard, Aomei Mo, Lori Harpell, Abbey Pender, Andrew Winterborn, Sandra Salcido, Alyssa Gonzalez, Chan Kyu Kim, Sebastian Brennan, Saira Afzal, Tim Rath, Sylvia Fong, David Lillicrap

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Canine Hemophilia A

Similar Genotype

- Intron-22 inversion like *F8* mutation[1]

Similar Clinical Phenotype

- Spontaneous bleeding events
- Treated with recombinant canine FVIII
- Inhibitor-prone c. 25% incidence

Long-term follow-up >10 years[2]

(1) Hough C. et al. Thromb Haemost. 2002; 87(4):659-65.
(2) Batty P. et al. Blood. 2022; 140(25): 2672-2683

Study Rationale and Aims

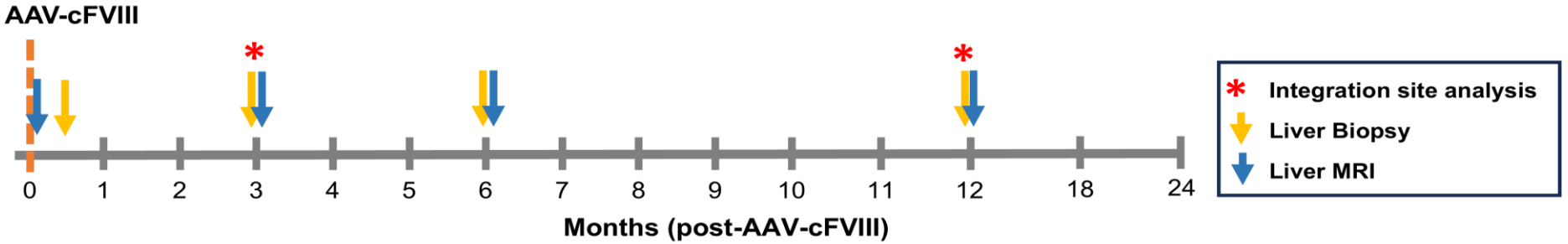
Background:

- Adeno-associated viral (AAV) gene therapy is approved for adults with severe hemophilia
- Treatment during childhood might prevent the long-term effects of joint bleeding and improve quality of life

Aims:

To evaluate the safety and efficacy of AAV-canine FVIII in neonatal and infantile hemophilia A dogs

Study Outline



Peripheral infusion of AAV5-cFVIIIco

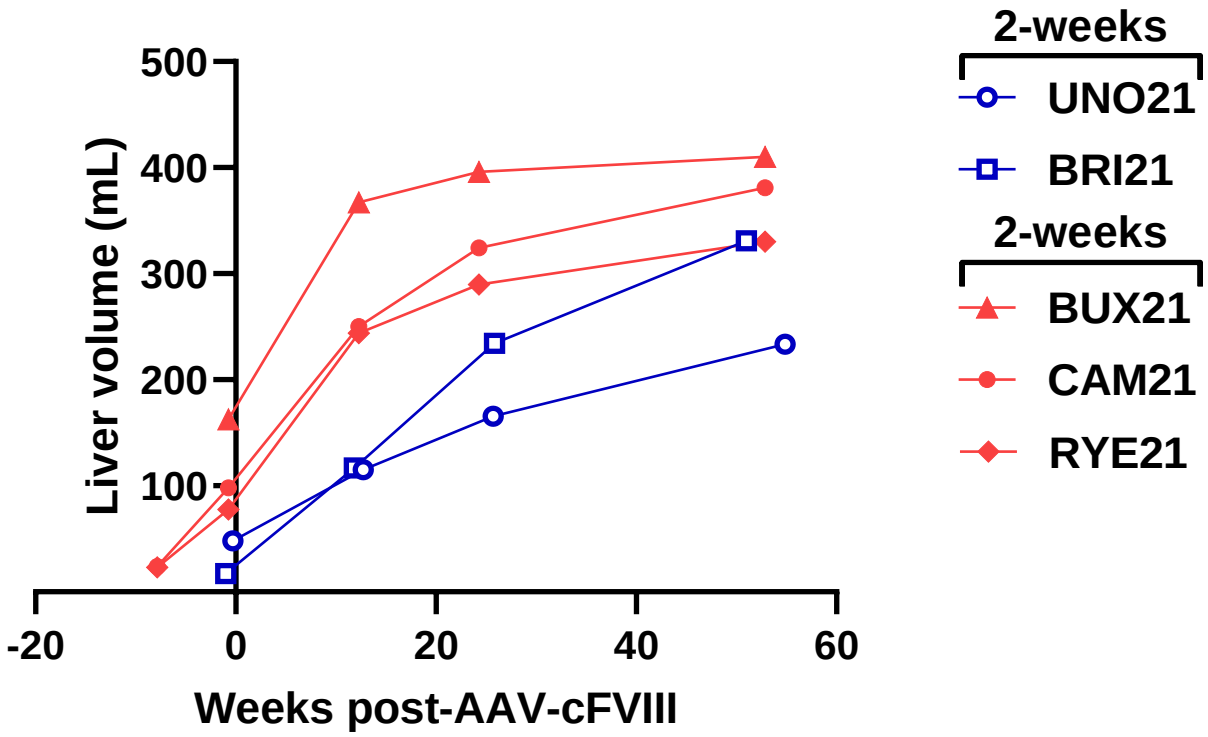
- Treatment age: neonatal (2-weeks, N=2), infant (2-months, N=3)
- Target dose: 2e14 vg/kg (2e14 – 9.8e14 vg)
- Follow up: 24 months

- Epigenomic data - 2 months of age in dogs equivalent to 9 months in humans[1]
- Integration site analysis by target enrichment sequencing (TES)

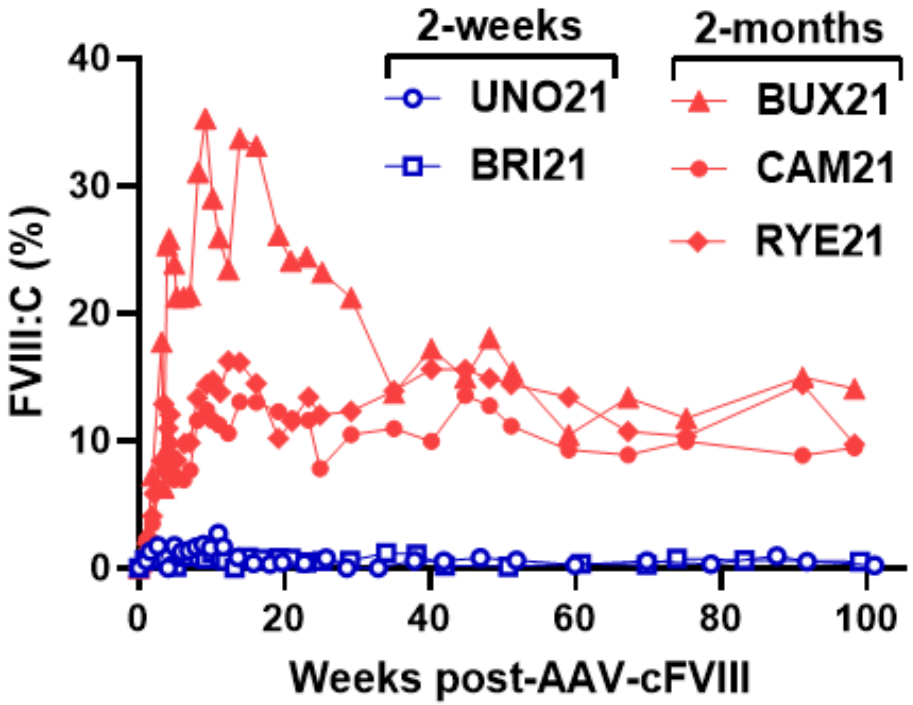
[1] Wang et al. Cell Sys. 2020;11(2):176-185

Stable FVIII expression in dogs treated at 2-months despite increased liver volumes

Liver Volume (MRI)

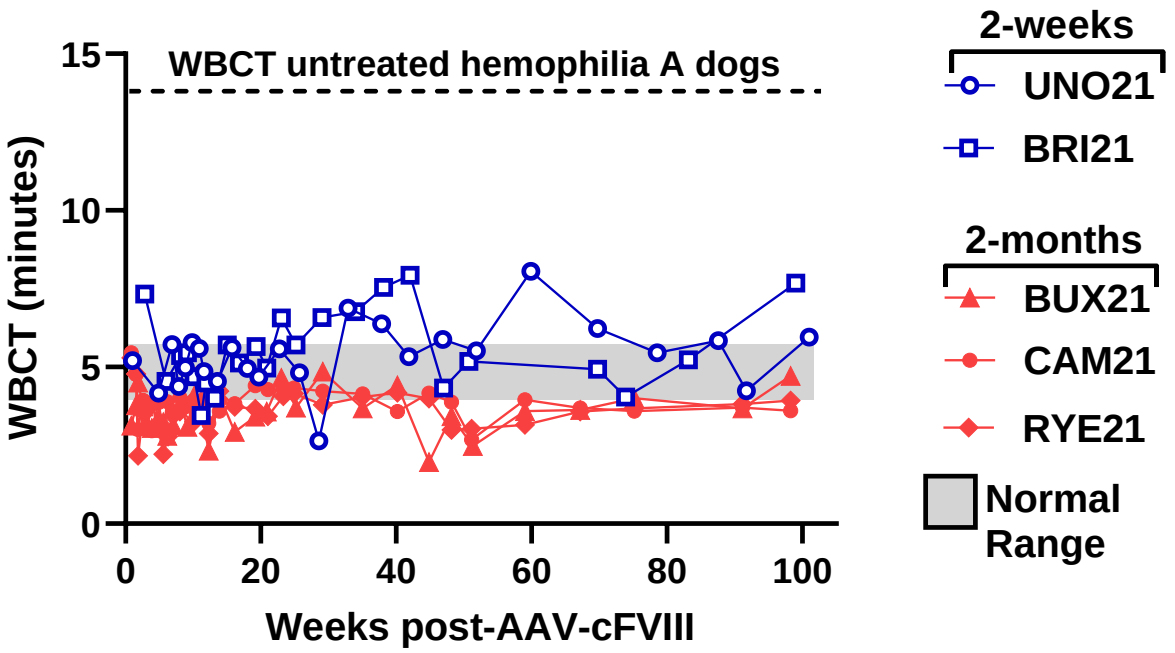


FVIII:C CSA

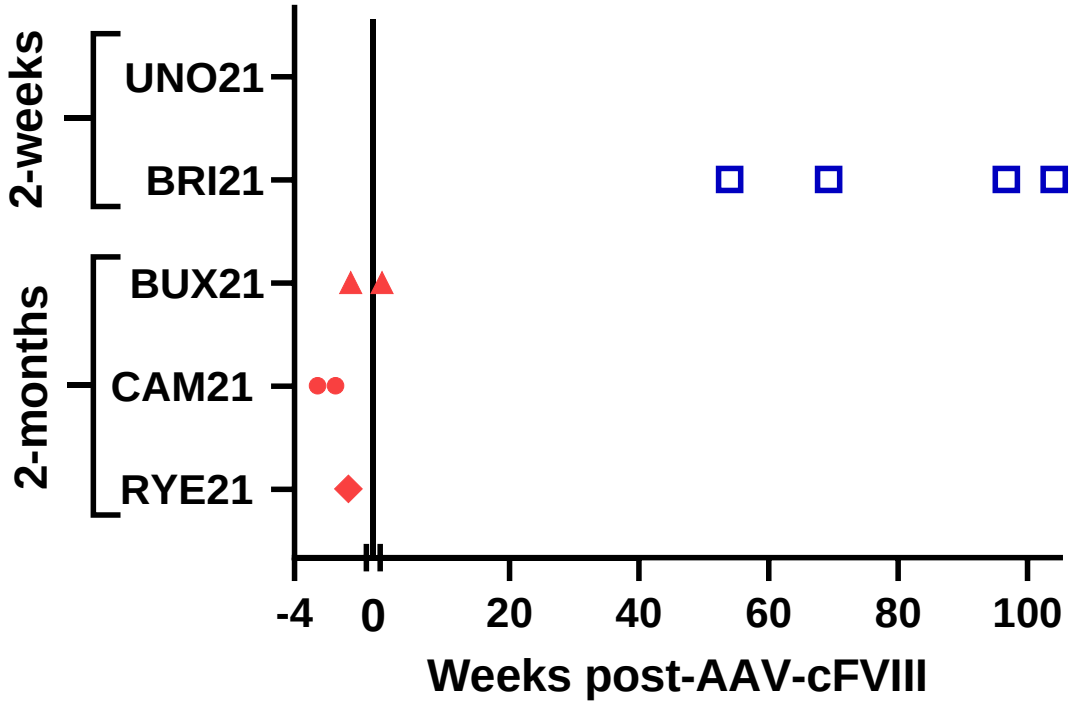


No bleeds occurred during follow-up for dogs treated at 2-months

Whole-blood clot time (WBCT)



Bleeding Events

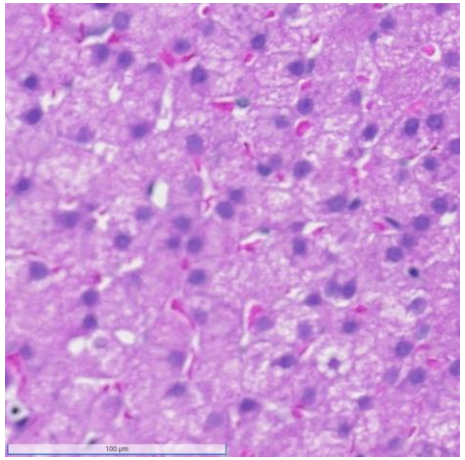


Histopathological evaluation of liver biopsies was normal

Dogs treated at 2-weeks

Dogs treated at 2-months

UNO21



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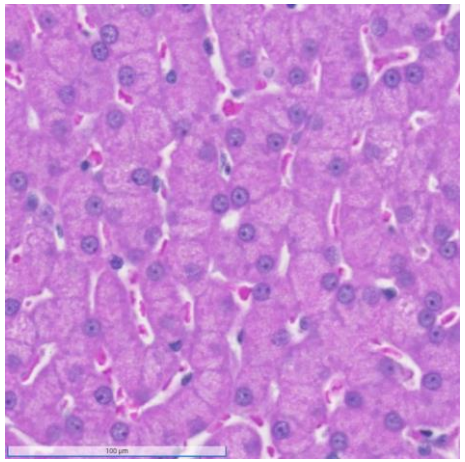
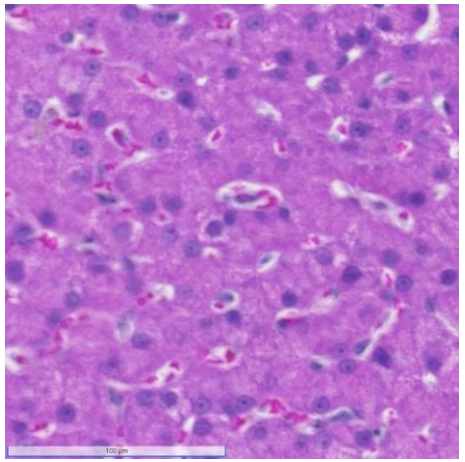
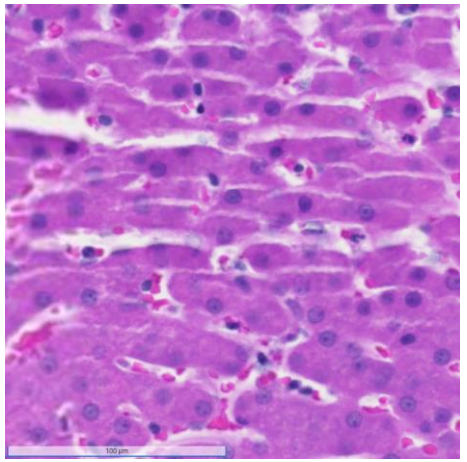


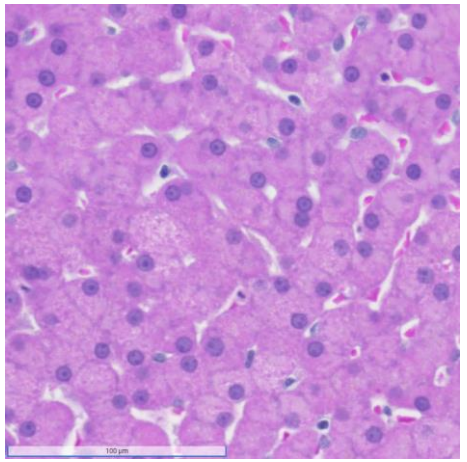
BUX21



CAM21



RYE21

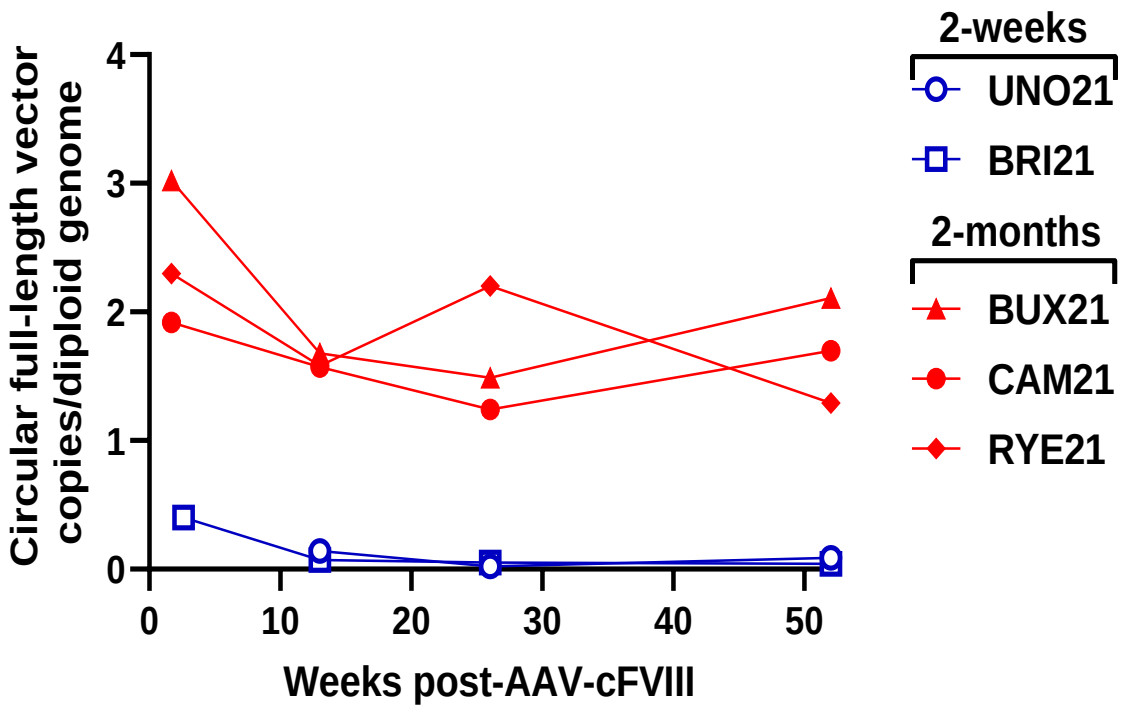


Samples obtained at 12-month liver biopsy

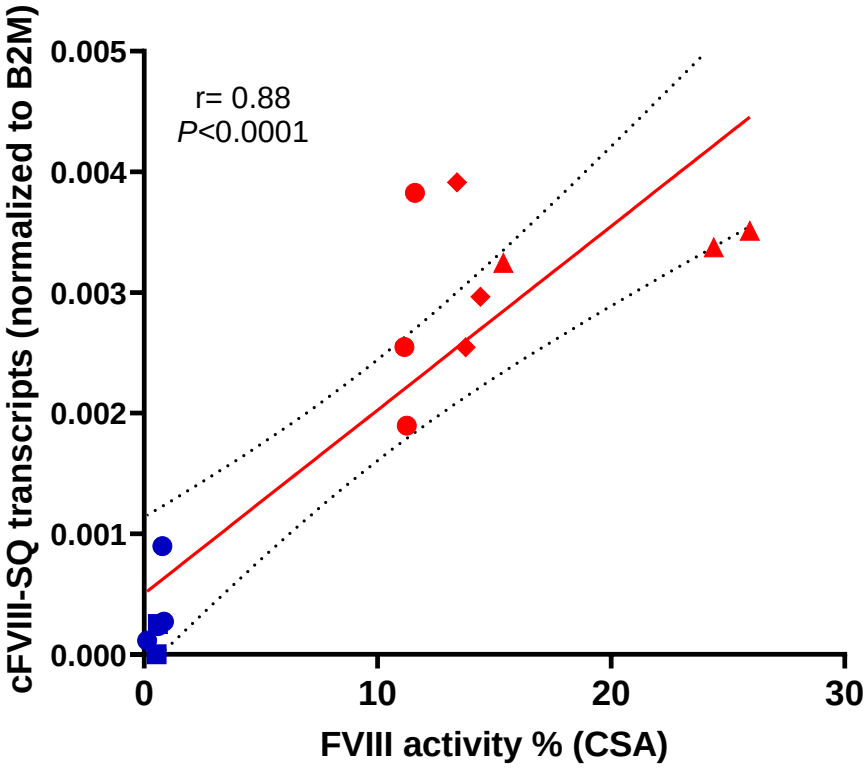
No evidence of chronic inflammation or tumor

Stable circular full-length vector genomes were observed in dogs treated at 2-months

Circular full-length vector genomes



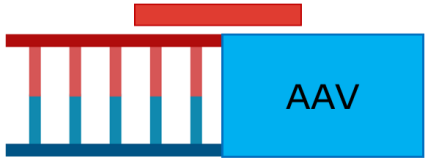
FVIII RNA correlates with FVIII expression (3, 6, and 12 months post-AAV-cFVIII)



AAV integration was seen at low frequencies in all samples

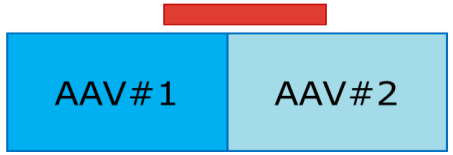
Integrated vector

Vector-Genome (VG) Junction

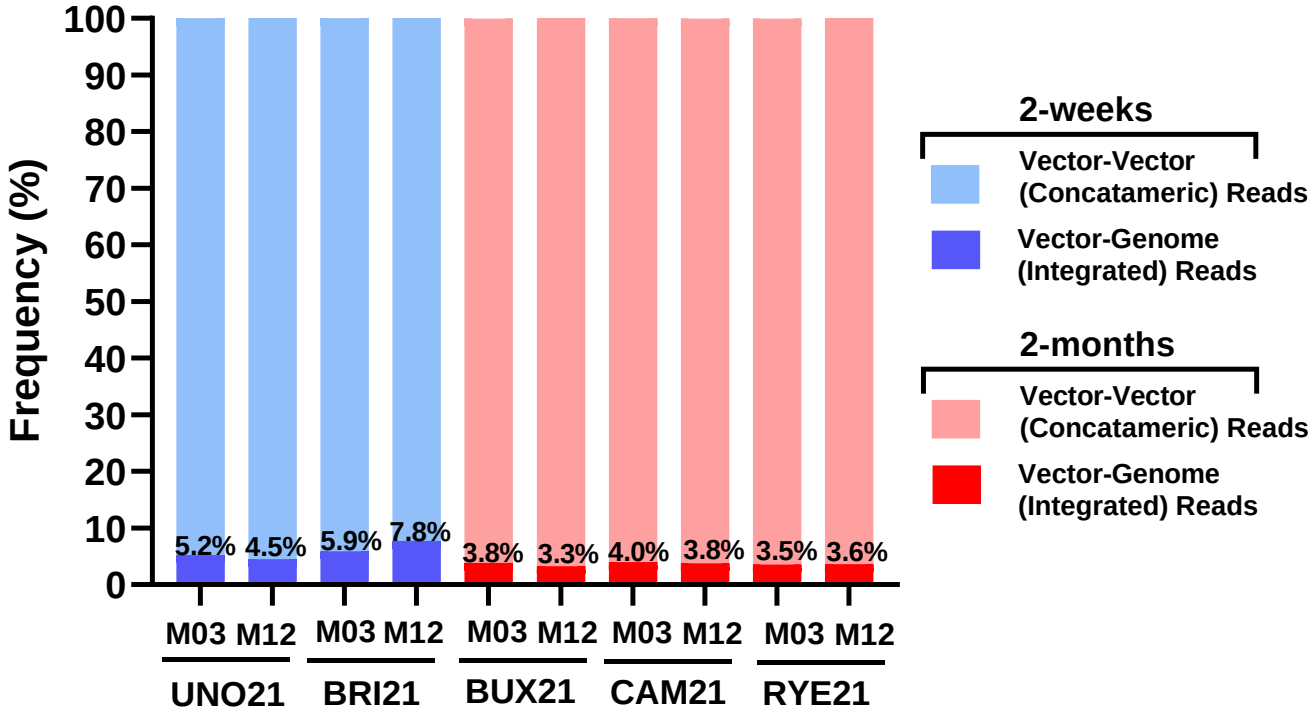


Episomal or integrated concatemerized vector

Vector-Vector (VV) Junction



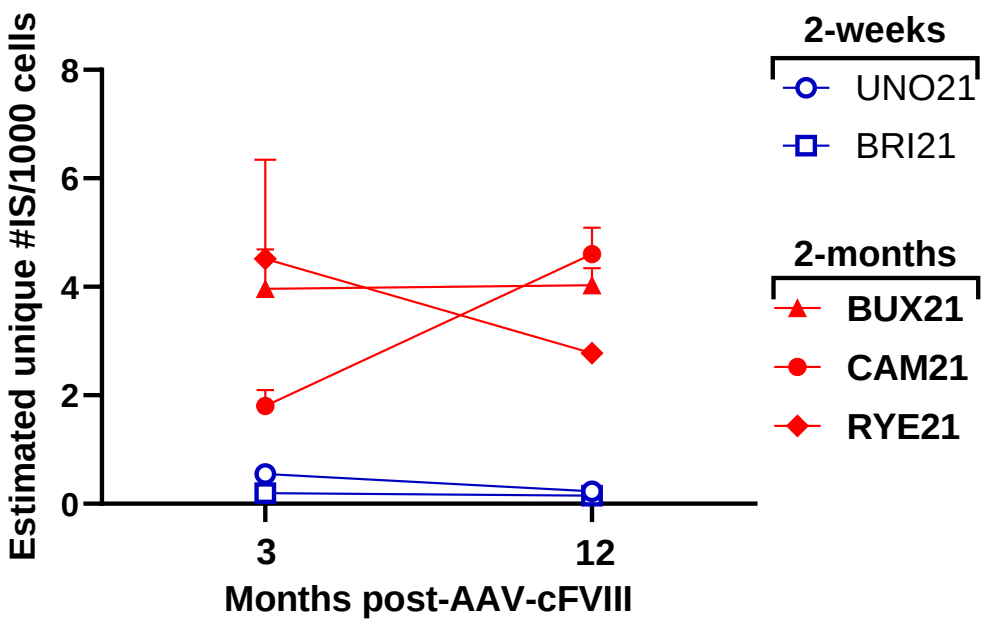
Concatameric versus integrated sequencing reads



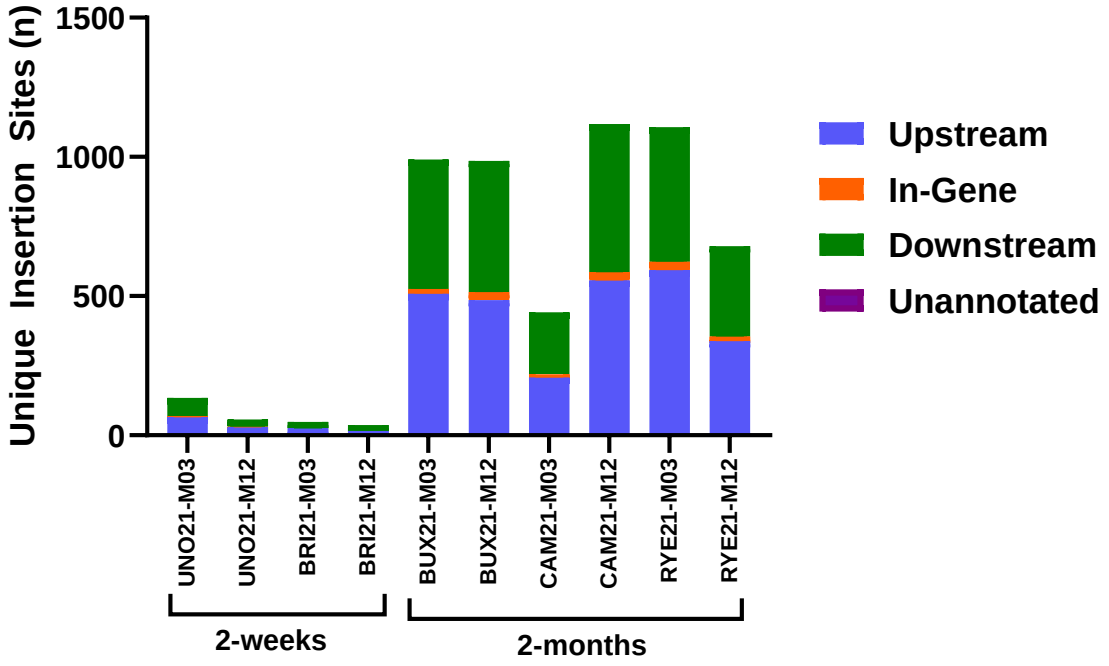
Similar to results observed in adult hemophilia A dogs (6e12 – 2.7e13 vg/kg)
(Batty et al. Blood. 2024; 143 (23):2373-2385)

AAV integration was seen at low frequencies in all samples

Estimated unique integration sites



IS location – in-gene vs intergenic



2-month IS frequency is similar to results observed in adult hemophilia A dogs despite lower dose in adult cohort (Batty et al. Blood. 2024; 143 (23):2373-2385)

Top 5 Common Integration Sites (CIS)

Rank	Count	Chromosome	Locus	Dimension (nt)	Gene(s)
Top 1	20	13	63203367	93074	*AFP*, *ALB* [1, 2]
Top 2	16	13	33380817	1027	*MIR30D* [1]
Top 3	14	1	122221447	231317	*MIR578* [1]
Top 4	13	X	124891115	9853	*CLIC2* [1]
Top 5	5	8	69727858	98019	*MIR410*

[1] CIS identified in Queen's University adult-dog treated dogs (Batty et al. Blood. 2024; 143 (23):2373-2385)

[2] CIS identified in UNC adult-dog treated dogs (Nguyen et al. Nat Biotech. 2021; 39(1):47-55)

Conclusions

Dogs treated at 2-weeks

- Low (<3%) FVIII:C, improved WBCT

Dogs treated at 2-months

- Stable FVIII expression despite liver expansion
- Normalized bleeding phenotype
- Stable full-length circular vector genome levels

Integration analysis

- Low rates of vector integration
- No evidence of clonal expansion
- No enrichment for cancer-associated genes

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  - Bridget Yates

pab7@queensu.ca & paul.batty@ucl.ac.uk

@paulbatty.bsky.social

@paul_batty1

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SEAMO
Southeastern Ontario Academic Medical Organization

CIHR IRSC
Canadian Institutes of Health Research
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