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Stable Factor VIII Expression and Improvement in Bleeding Phenotype Following Early Childhood Treatment with Adeno-Associated Viral Gene Therapy in the Severe Hemophilia A Dog Model

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Hemophilia Gene Therapy Basic Science : OC 30.2

Monday, June 26, 2023

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Disclosures for Paul Batty

Scientific Advisory Board	**BioMarin, Novo Nordisk & CSL Behring**
Speakers Bureau	**No relevant conflicts of interest to declare**
Research Support	**BioMarin**
Employee	**No relevant conflicts of interest to declare**
Consultant	**No relevant conflicts of interest to declare**
Investments / Major Stockholder	**No relevant conflicts of interest to declare**
Honoraria	**Octapharma, BioMarin, Pfizer, Novo Nordisk Institute for Nursing and Medication Education (IMNE)**
Other	**European Haemophilia Consortium Volunteer**

Learning Objectives

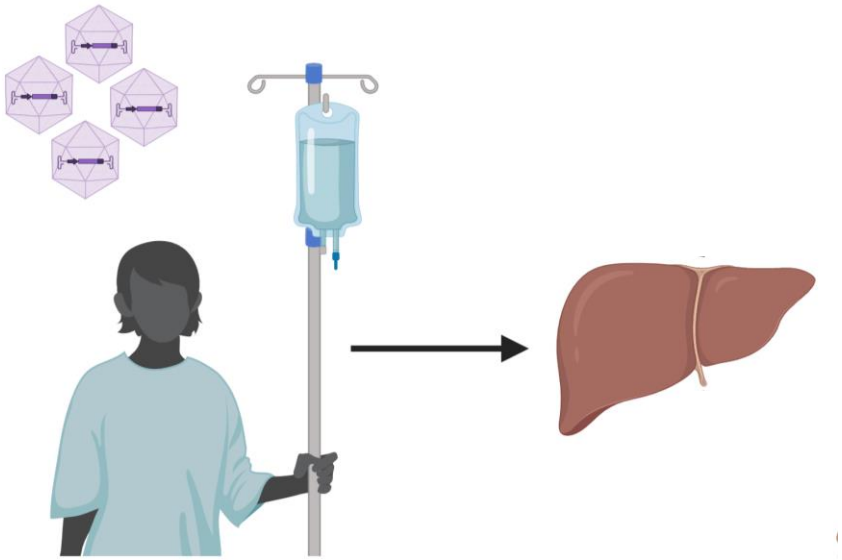
Upon Completion, participants will be able to :

1) Describe changes in liver volume and weight during early life in the hemophilia dog model

2) List laboratory tests used to evaluate outcomes of AAV-cFVIII gene therapy in the hemophilia dog model

3) Describe the differences in FVIII expression following AAV-cFVIII in hemophilia dogs treated at either 2-weeks or 2-months (human ~ 9 months) of age

Gene Therapy for Hemophilia



Hemophilia Gene Therapy

- Clinically relevant expression
  - Hemophilia A : 6 years [1]
  - Hemophilia B : > 8 years [2]
- ↓ Bleeding & Factor concentrate
- All participants ≥ 18 years

Pediatric Gene Therapy Considerations

- Benefits: ↓ Bleeding & joint disease
- Concerns: Loss of expression & safety

(1) Laffan M et al. 2022. Res Pract Thromb Haemost. 6 (S1) : ISTH 2022. **(2)** Nathwani, A.C. et al. 2018. *Blood*, **132**, 92-5856 : ASH 2018

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 Aims

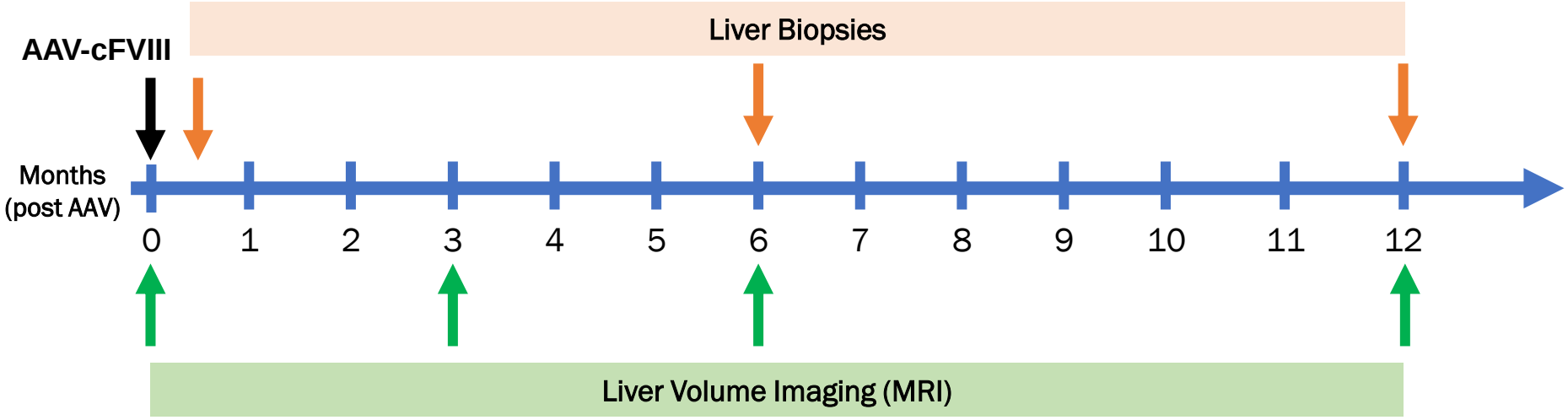
- Evaluate efficacy & safety of AAV5-cFVIII during early life (neonatal and infancy)
- Evaluate whether FVIII expression reduces as the liver grows in the 1st year of life

B-domain deleted canine F8 (codon optimized)

ITR = Inverted terminal repeat. **HLP** = Hybrid liver promoter
PA = Polyadenylation sequence

Study Outline

Dose: 2e14 vg/kg (Based on RAG2/FVIII DKO murine studies)[1]



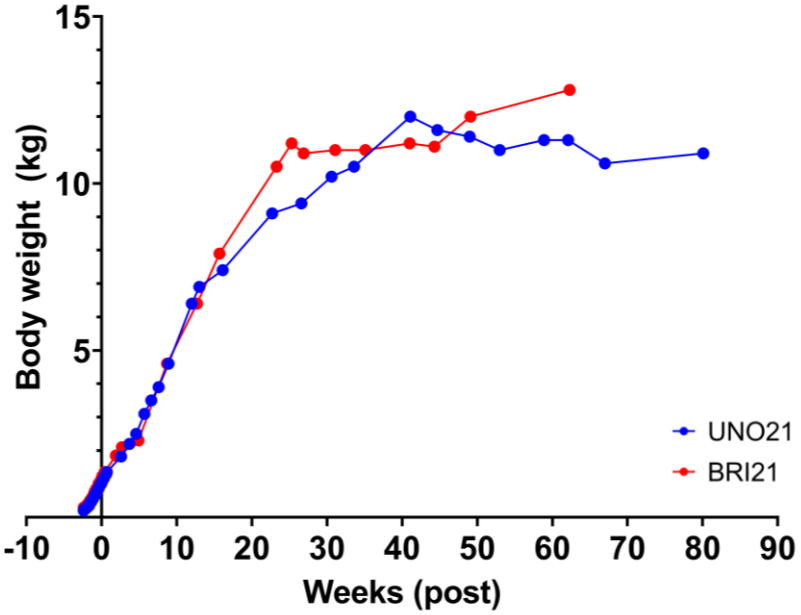
Cohort 1 (Neonatal)	Cohort 2 (Infant)
Age : 2 weeks age	Age: 2 months (human ~ 9 months) [2]

(1) Zhang L et al. Mol Ther Methods Clin Dev. 2022; 26:519-531 **(2)** Wang T et al. Cell Syst. 2020;11(2):176-185

Cohort 1 : Neonatal AAV5-cFVII Treated Hemophilia Dogs

ID	Treatment age (weeks)	Treatment weight (kg)	Sex	Construct	Dose (vg/kg)	Follow-Up* (Weeks)
UNO21	2.4	1.0	F	HLP-co-SQ-cFVIII	2.0e14	93.1
BRI21	2.3	1.2	F	HLP-co-SQ-cFVIII	2.0e14	75.3

*Data cut-off: 26/5/23



Post-AAV	Body weight increase (kg)	Body weight increase (fold)
3 Months	5.6 kg	6.1-fold
12 Months	10.4 kg	10.5-fold

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Cohort 1 : Neonatal AAV5-cFVII Treated Hemophilia Dogs

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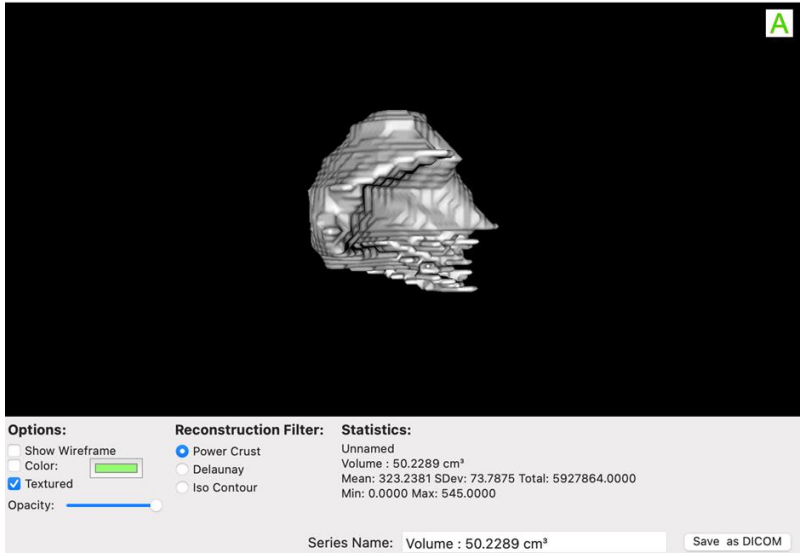
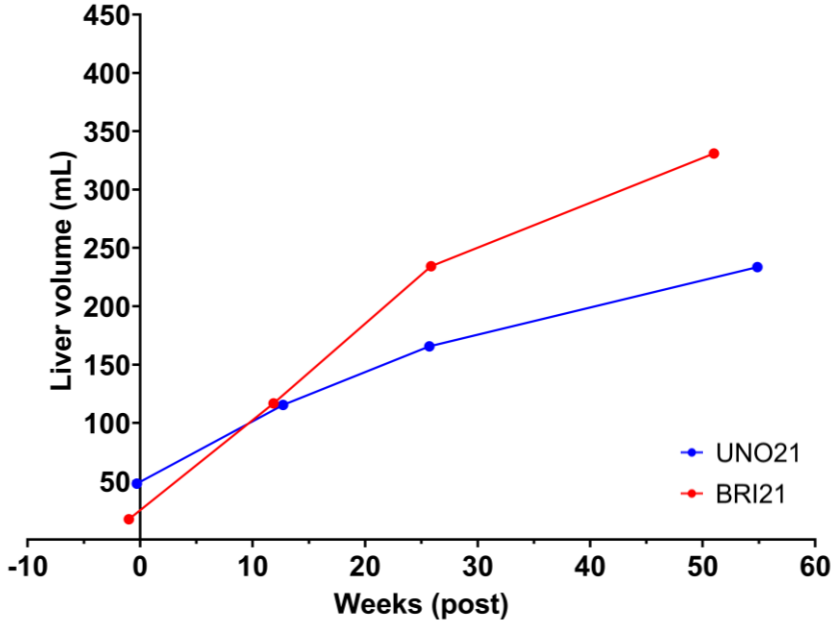
UNO21 : Baseline Liver Volume (Age 15 Days)





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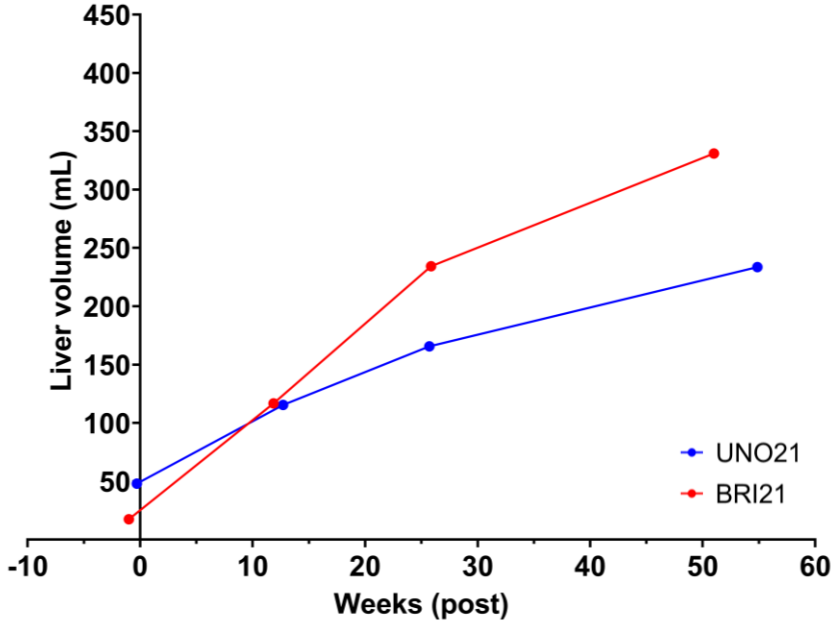
BRI21	2.3	1.2	F	HLP-co-SQ-cFVIII	2.0e14	75.3

Post-AAV	Liver volume fold increase	Body weight fold increase
3 Month	4.6-fold	6.1-fold
12 Month	12.0-fold	10.5-fold



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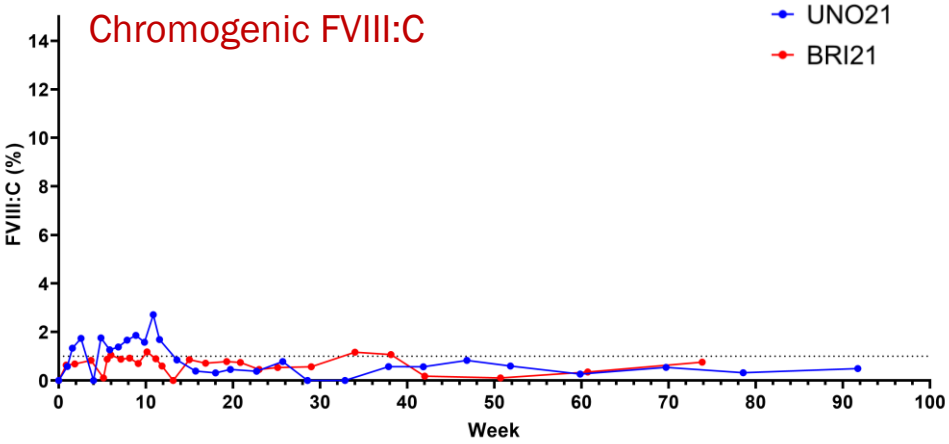
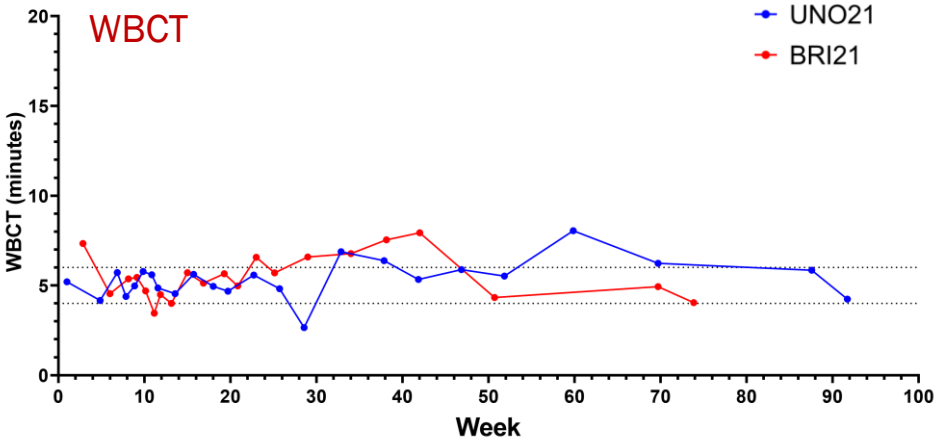
Minimal FVIII Expression Seen in AAV5-cFVII Treated Neonatal Dogs





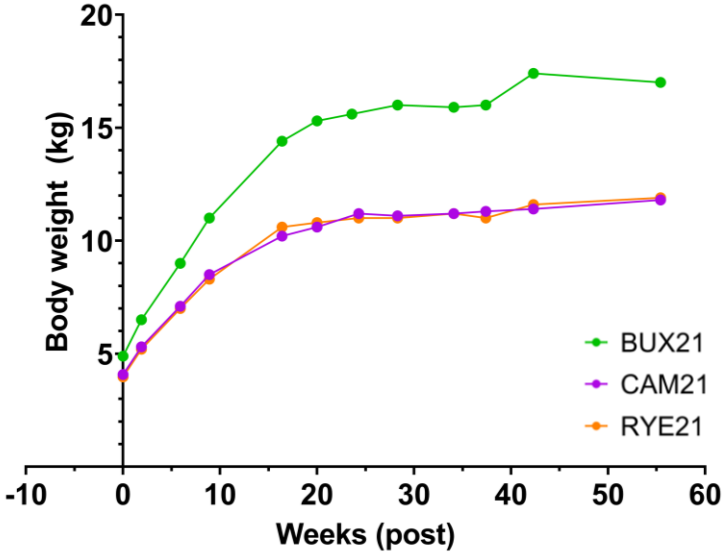
- Untreated adult hemophilia A dog Whole Blood Clot Time (WBCT): 13.9 mins (9.6 – 17.7)
- Improvement in WBCT despite minimal FVIII expression
  - Similar findings seen in adult non-responders in our previous study[1]

1) Batty P et al. Blood. 2022; 140(25): 2672-2683

Cohort 2 : Infant AAV5-cFVII Treated Hemophilia Dogs

ID	Treatment age (weeks)	Treatment weight (kg)	Sex	Construct	Dose (vg/kg)	Follow-Up (Weeks)*
BUX21	9.3	4.9	M	HLP-co-SQ-cFVIII	2.0e14	68.4
CAM21	9.3	4.1	F	HLP-co-SQ-cFVIII	2.0e14	68.4
RYE21	9.3	4.0	F	HLP-co-SQ-cFVIII	2.0e14	68.4

*Data cut-off: 26/5/23



Post-AAV	Body weight increase (kg)	Body weight increase (fold)
3 Months	6.6 kg	2.7-fold
12 Months	7.7 kg	2.9-fold

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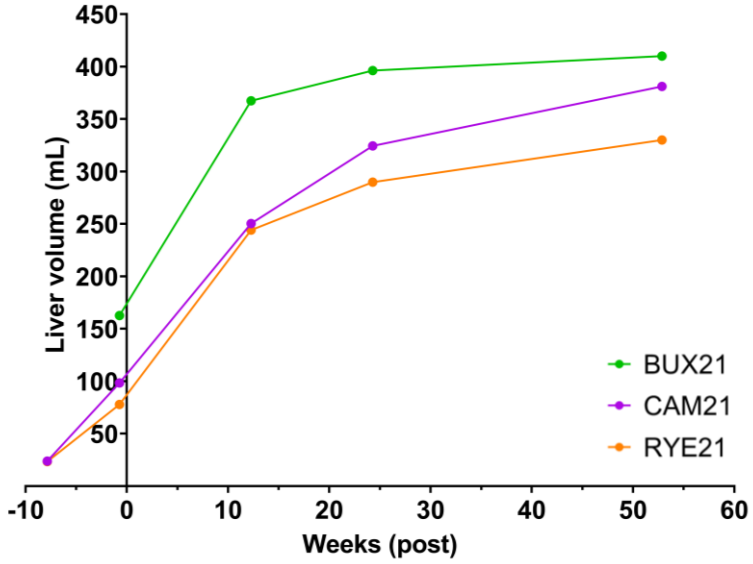
Cohort 2 : Infant AAV5-cFVII Treated Hemophilia Dogs

ID	Treatment age (weeks)	Treatment weight (kg)	Sex	Construct	Dose (vg/kg)	Follow-Up (Weeks)*
BUX21	9.3	4.9	M	HLP-co-SQ-cFVIII	2.0e14	68.4
CAM21	9.3	4.1	F	HLP-co-SQ-cFVIII	2.0e14	68.4
RYE21	9.3	4.0	F	HLP-co-SQ-cFVIII	2.0e14	68.4

Post-AAV	Liver volume fold increase	Body weight fold increase
3 Month	2.5-fold	2.7-fold
12 Month	3.9-fold	2.9-fold



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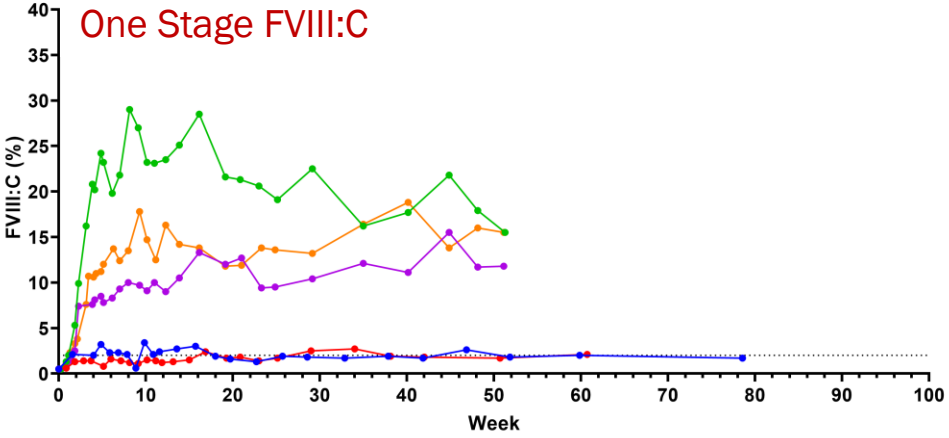
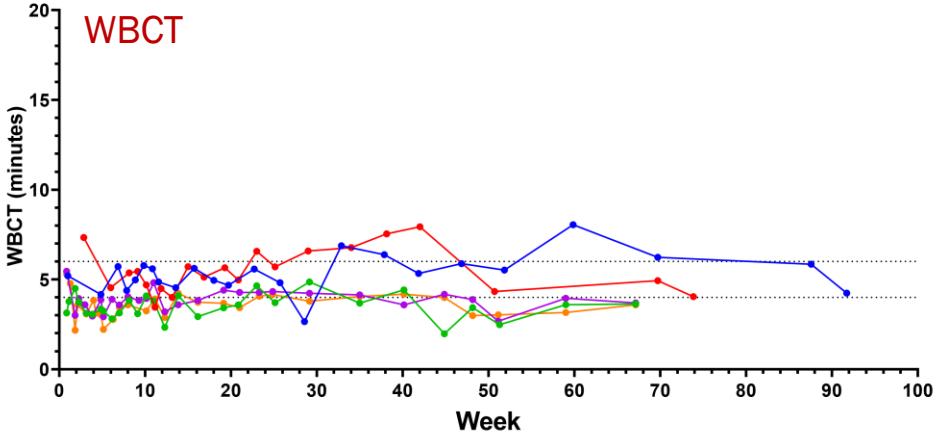
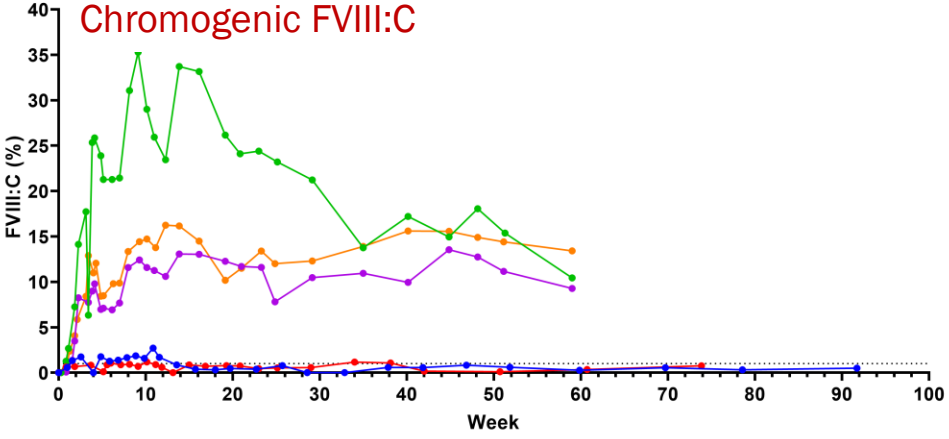
Sustained FVIII Expression Seen in AAV5-cFVII Treated Infant Dogs







Cohort 1 : Neonatal

- UNO21
- BRI21

Cohort 2 : Infant

- BUX21
- CAM21
- RYE21

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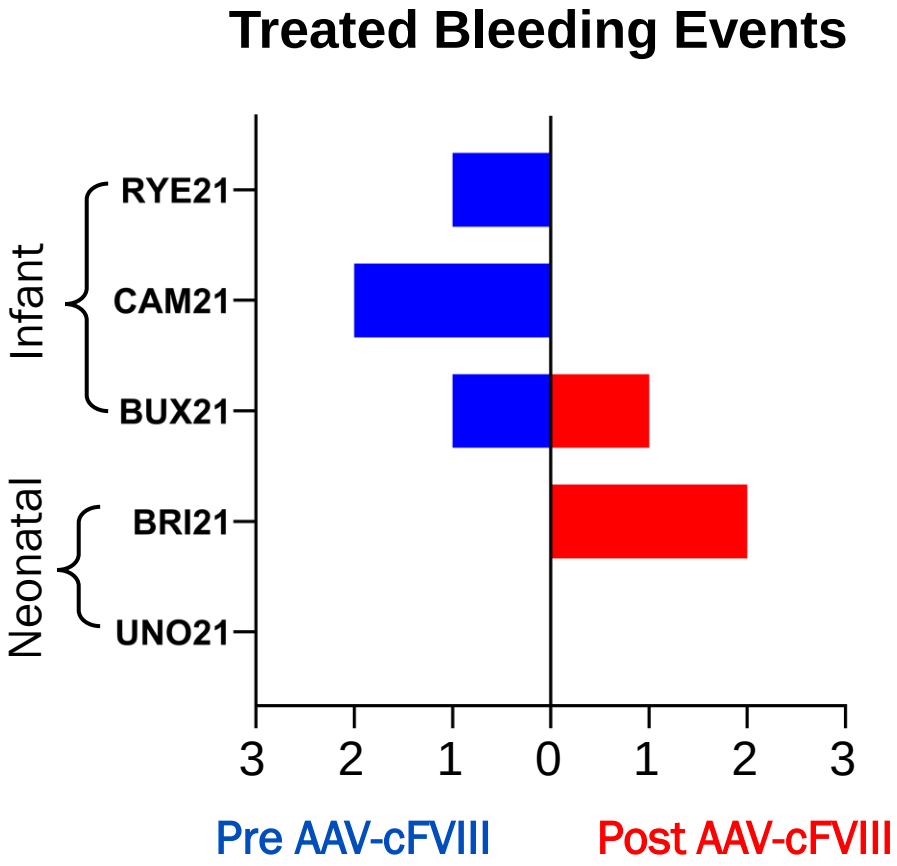
Reduction in Bleeding Events Following Infant or Neonatal AAV-cFVIII



Pre-AAV (Events = 4)

- BUX21 (n=1) Limb S (rFVIII)
- CAM21 (n=2) Limb S (TXA) & Scalp T (TXA)
- RYE21 (n=1) Scalp S (TXA)

Post-AAV (Events = 3)

- BRI21 (n=2) : Limb S (rFVIII) & Limb S (TXA)
- BUX21 (n=1): Soft tissue T (TXA) Day + 2

S = Spontaneous. T = Traumatic

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Summary

- Significant increase in liver volume & body weight seen in 1st year of life
- AAV5-cFVIII resulted in therapeutic FVIII expression into adulthood in infant treated severe hemophilia A dogs
- Improvement in WBCT & bleeding phenotype seen in both cohorts
- Percutaneous liver biopsies performed with no complications
- Analysis of AAV VG and molecular forms (episomal/integrated) ongoing

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