Innate and Adaptive Immune Responses to Adeno-associated viral Gene Therapy in the Severe Hemophilia A Dog Model

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OC 01.5 : Gene Therapy I : Saturday, July 9, 2022

ISth 2022 CONGRESS

Disclosures for Paul Batty

In compliance with COI policy, ISTH requires the following disclosures to the session audience:

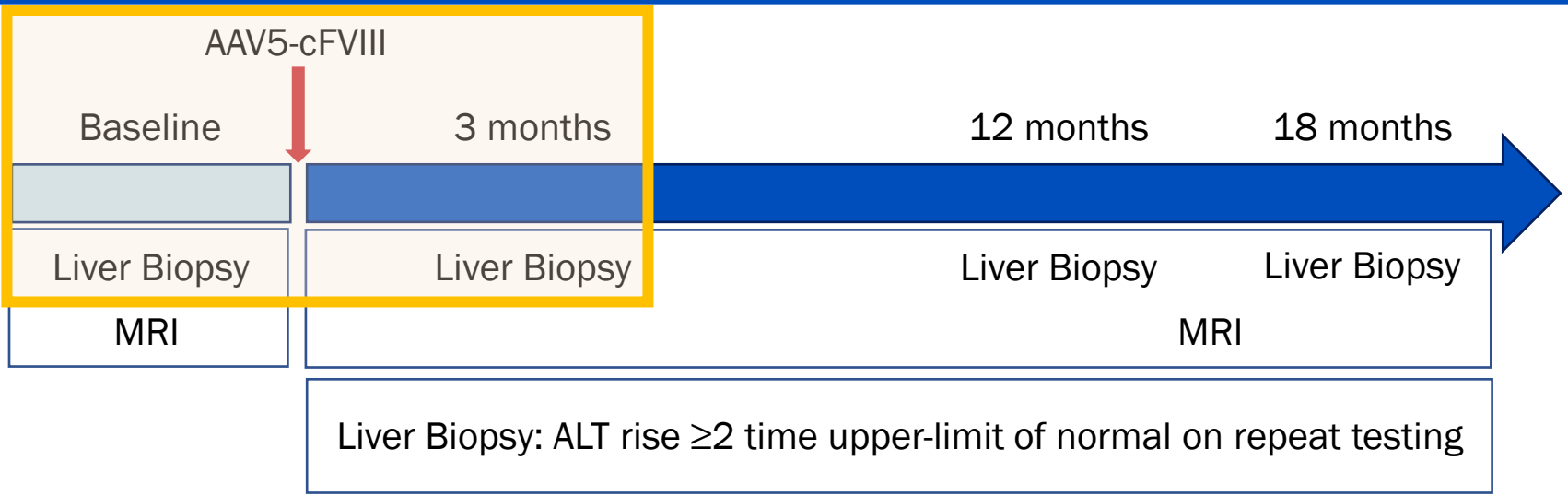
Research Support/P.I.	**Octapharma, BioMarin, Grifols**
Employee	**No relevant conflicts of interest to declare**
Consultant	**No relevant conflicts of interest to declare**
Major Stockholder	**No relevant conflicts of interest to declare**
Speakers Bureau	**No relevant conflicts of interest to declare**
Honoraria	**Octapharma, BioMarin, Pfizer, Novo Nordisk Institute for Nursing and Medication Education (IMNE)**
Scientific Advisory Board	**BioMarin**

Canine Hemophila 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; Epub ahead of print. PMID: 35405003.

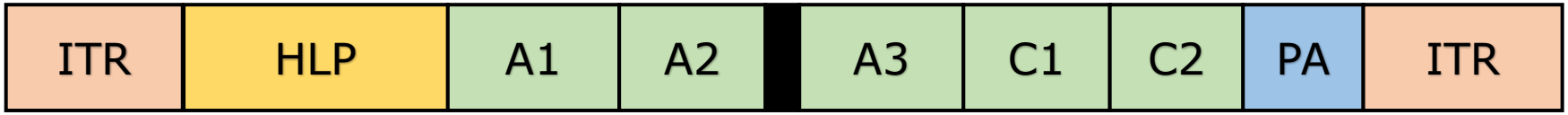
Study Aims & Outline



- Evaluate safety & efficacy of AAV5-cFVIII in a hemophilia A dog model
- Evaluate factors resulting in variable expression following AAV5-cFVIII
- Evaluate early (<3 months) innate & adaptive immune response to AAV5-cFVIII

Cohort 1 : Non-Codon Optimized AAV5-cFVIII

B-domain deleted canine F8



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

Cohort	ID	Dose (vg/kg)	Treatment Age (years)	Follow Up (years)
1	AUS2010	8.0e13	9.0	1.5 #
	BRO2018	8.2e13	0.8	**2.8 ***
	BAN2011	2.1e14	8.3	1.0 #

Response = Chromogenic FVIII:C >1%
Data capture 29/04/2022. * Follow-up ongoing. # Study end-point reached

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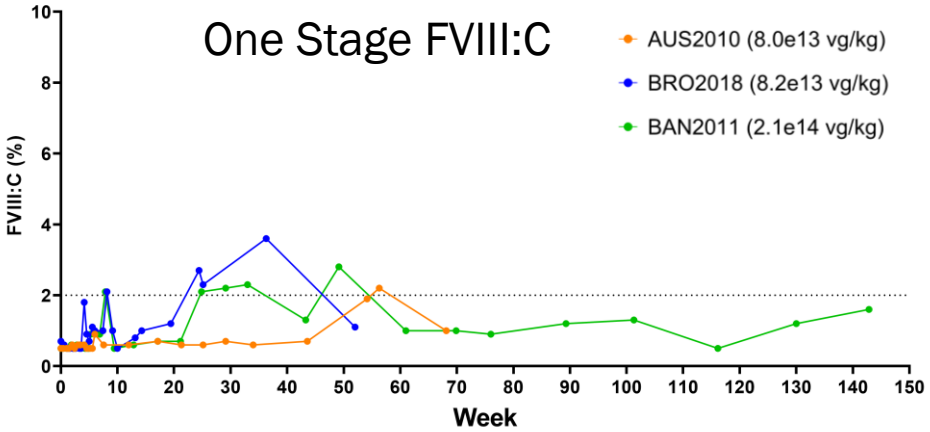
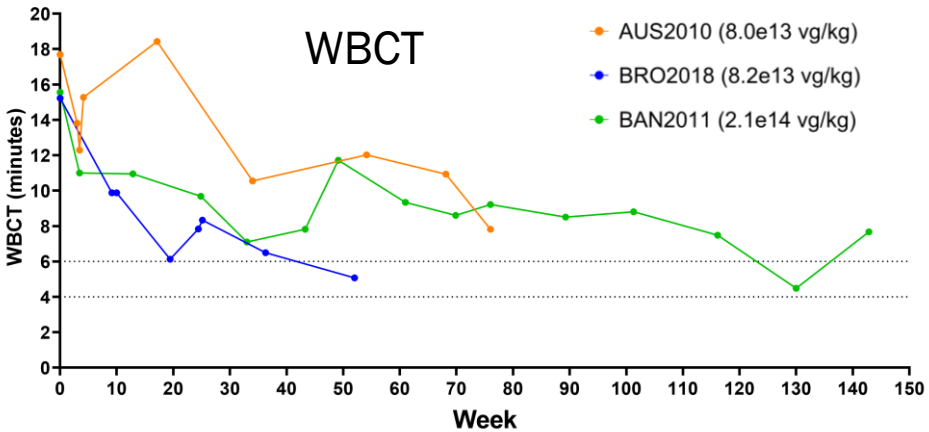
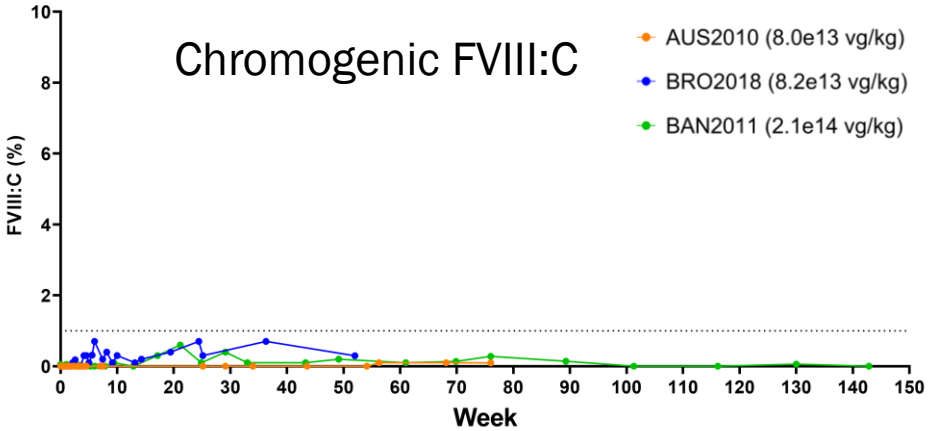
No Significant FVIII Expression with AAV5-nco-cFVIII







- Reduction in Whole Blood Clot Time (WBCT) despite no significant FVIII expression
- Similar findings seen in non-responders in our previous study[1]

1) Batty P et al. Blood. 2022; Epub ahead of print. PMID: 35405003.

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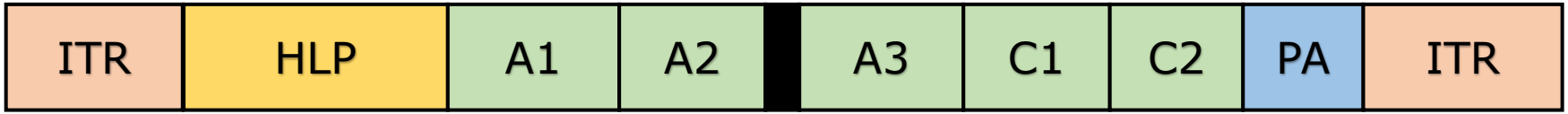
Cohort 2 : Codon Optimized AAV5-cFVIII

B-domain deleted canine F8

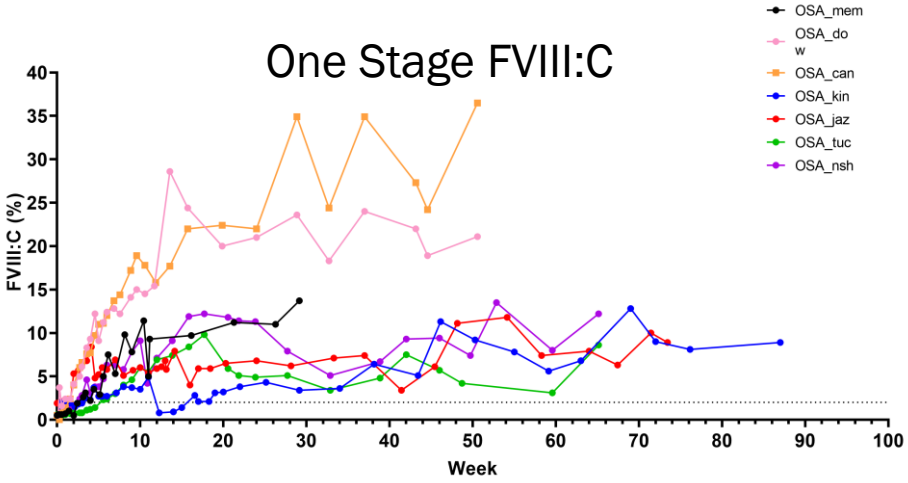


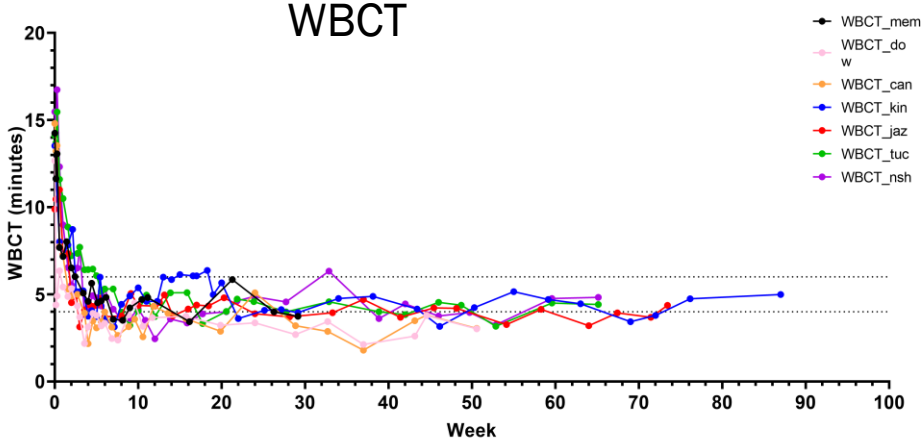
ITR = Inverted terminal repeat. **HLP** = Hybrid liver promoter
PA = Polyadenylation sequence

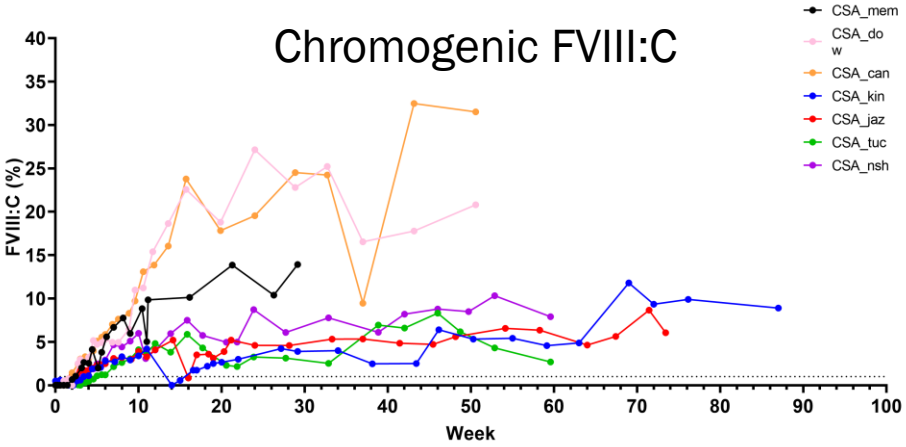
Cohort	ID	Dose (vg/kg)	Treatment Age (years)	Follow Up
2	NAS2018	6.0e13	2.4	**1.3 ***
	TUC2018	6.0e13	2.4	**1.3 ***
	KIN2015 **(V3)**	6.8e13	5.2	**1.8 ***
	JAZ2013	6.8e13	6.9	1.4 #
3	CAN2017	2.0e14	3.8	**1.0 ***
	DOW2016	2.0e14	4.9	**1.0 ***
	MEM2018 **(INH)**	2.0e14	3.0	**0.6 ***

Response = Chromogenic FVIII:C >1%. V3 = HLP-co-cFVIII-V3. INH = FVIII inhibitor history
Data capture 29/04/2022. * Follow-up ongoing. # Study end-point reached

Dose Dependent FVIII Expression with AAV5-co-cFVIII







Cohort	ID	Dose (vg/kg)
2	NAS2018 TUC2018 KIN2015 (**V3**) JAZ2013	6.0e13 6.0e13 6.8e13 6.8e13
3	CAN2017 DOW2016 MEM2018 (**INH**)	2.0e14 2.0e14 2.0e14

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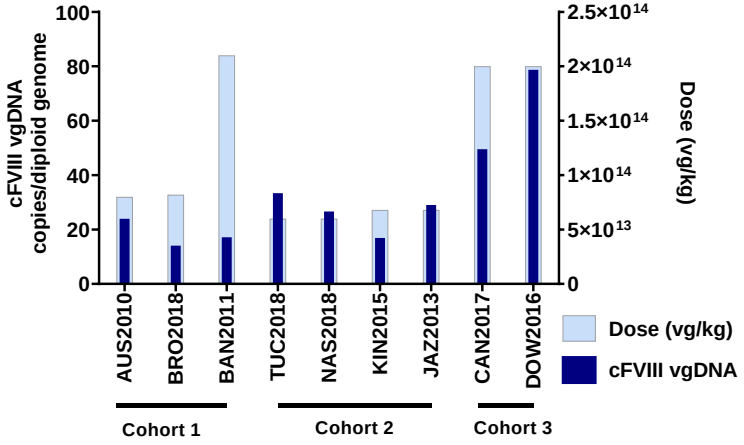
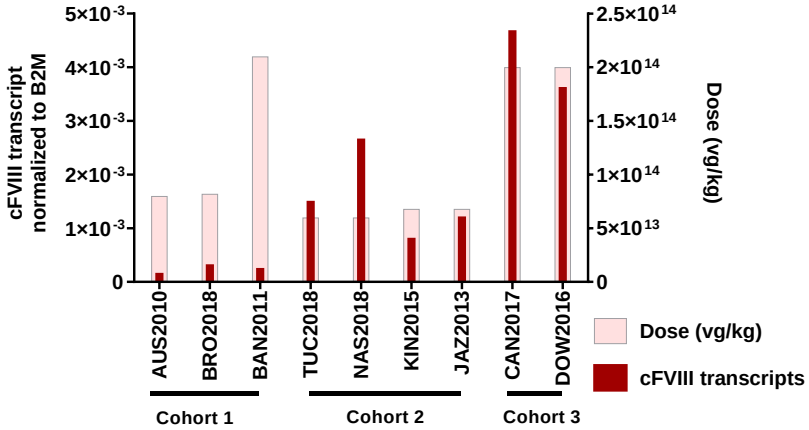
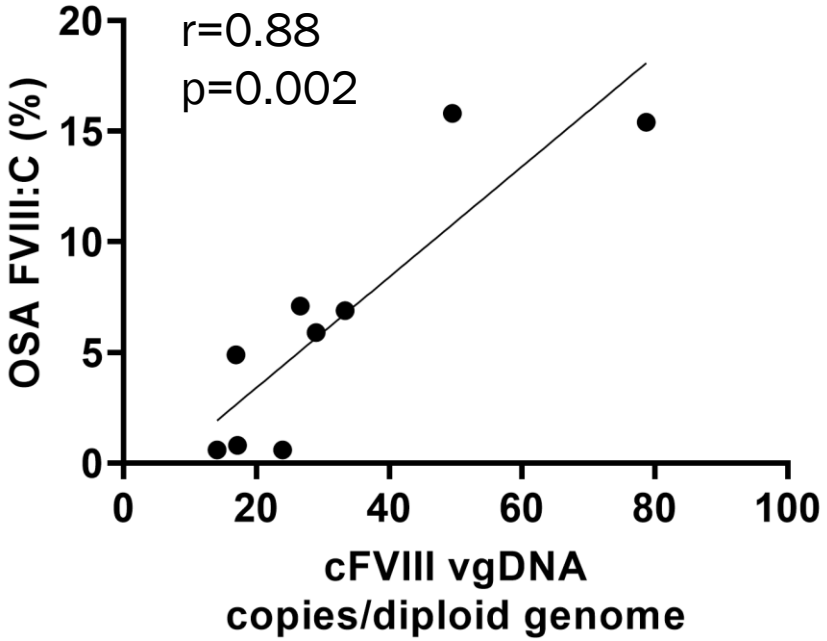
AAV5-cFVIII Detectable in All Dogs with Dose Response and Strong Correlation between Liver Vector Genomes and FVIII Expression

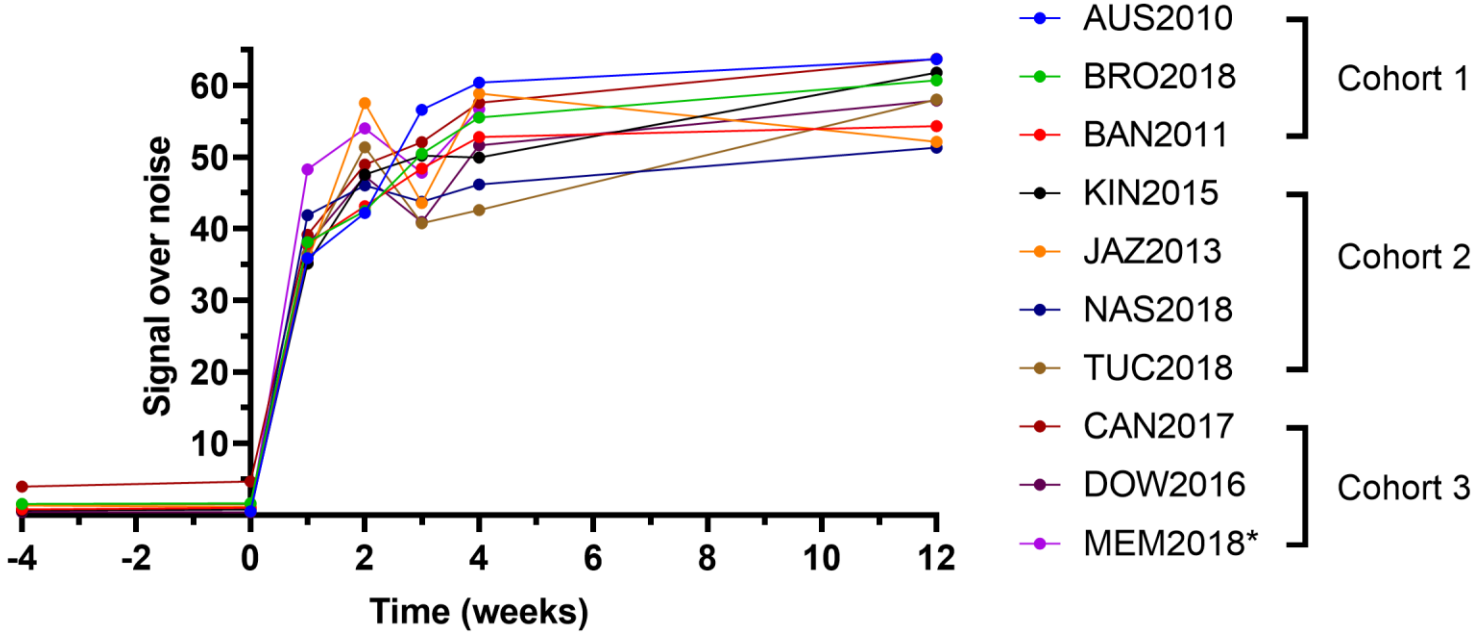






- Strong correlation between liver Vector Genome DNA & FVIII:C at 3 months

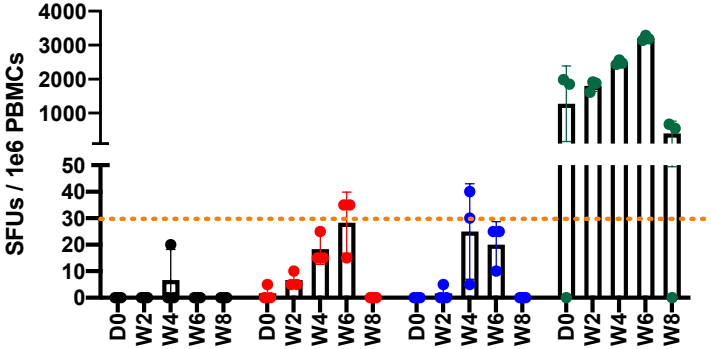
Capsid Antibodies Seen Early Post AAV-cFVIII



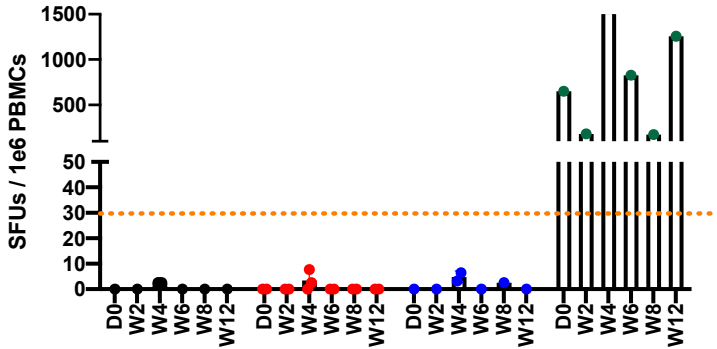
- Early capsid antibody response seen at D7 post AAV-cFVIII
- Further studies to assess AAV5 neutralizing antibodies using a cell-based transduction inhibition assay ongoing

No Significant Cytotoxic T-Cell Response* Observed

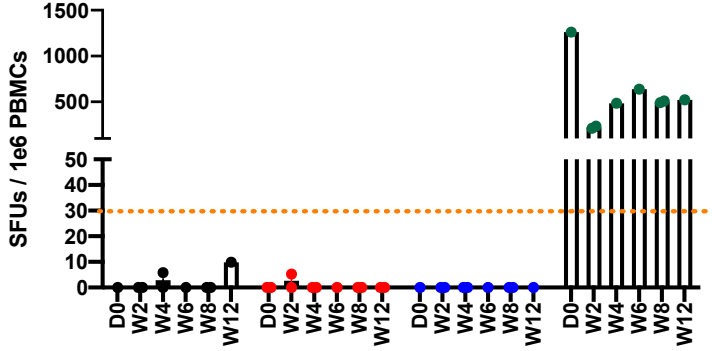
BRO2018 (Cohort 1, 8.2e13 vg/kg)



NAS2018 (Cohort 2, 6e13 vg/kg)



CAN2017 (Cohort 3, 2e14 vg/kg)



- DMSO
- AAV Peptide Pool 1
- AAV Peptide Pool 2
- Positive Control
- Limit of detection

* As measured by ELISpot INFγ assay

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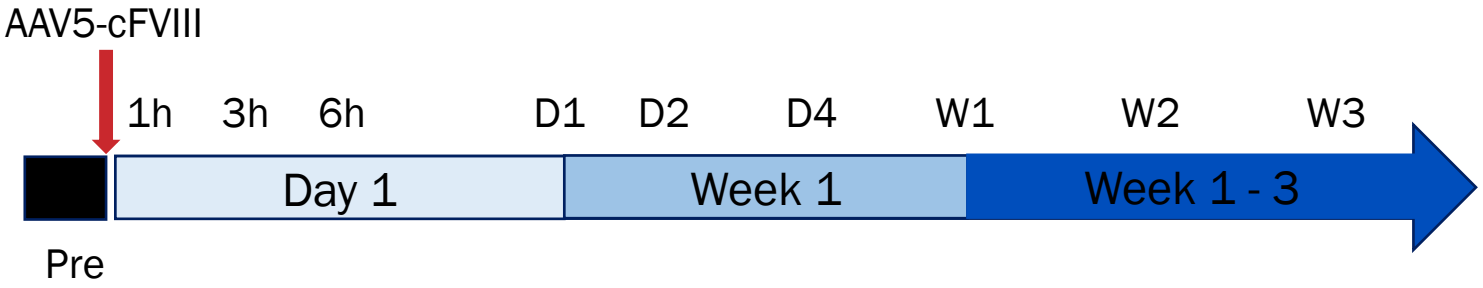
Evaluation of Early Immune Biomarkers to AAV5-co-FVIII

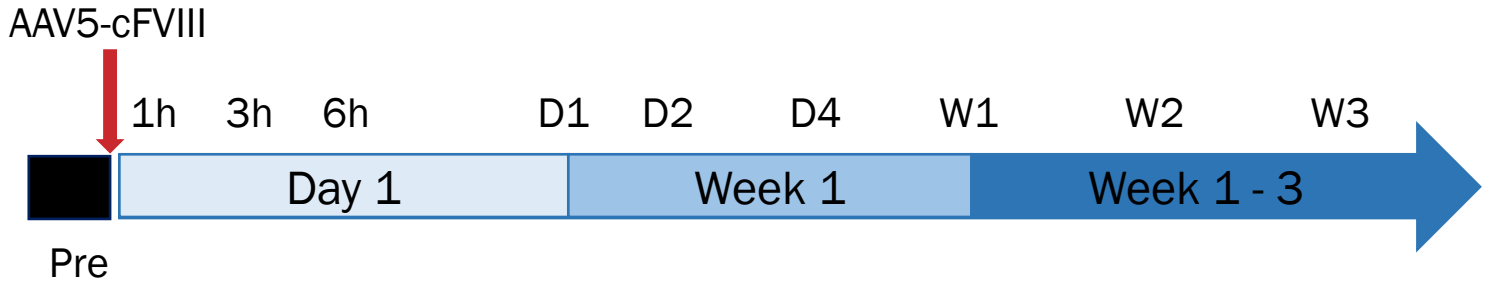


Cohort	ID	Dose (vg/kg)
2	NAS2018 TUC2018	6.0e13 6.0e13
3	CAN2017 DOW2016 MEM2018 #	2.0e14 2.0e14 2.0e14

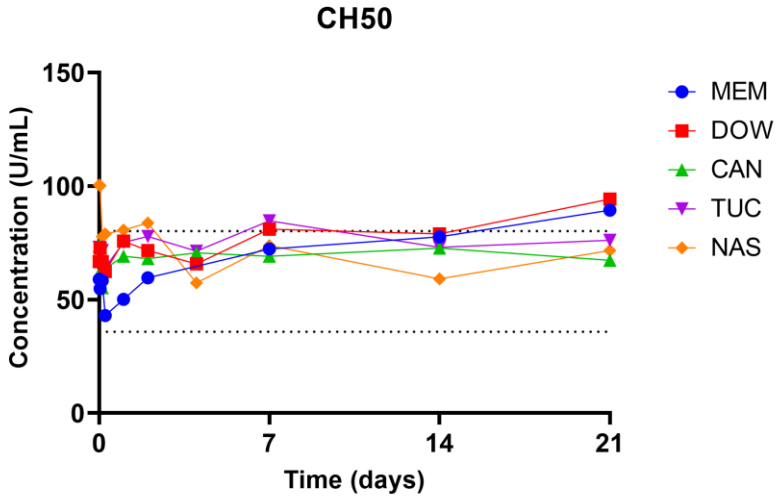
Biomakers	
Cytokines[1]	GM-CSF, IFNγ, IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, IP-10, KC-like, MCP-1, TNFα, TGF β-1, TGF β-2, TGF β-3
Complement	CH50 (Classical pathway)

1) Canine Cytokine/Chemokine 13-Plex Discovery Assay® Array (Eve Technologies)

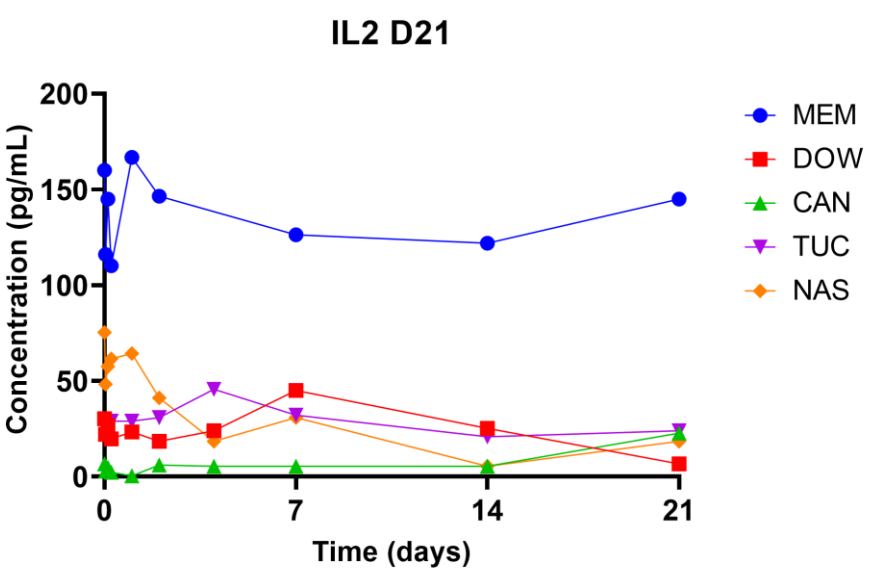
No Complement Activation (CH50) Seen Following AAV5-cFVIII

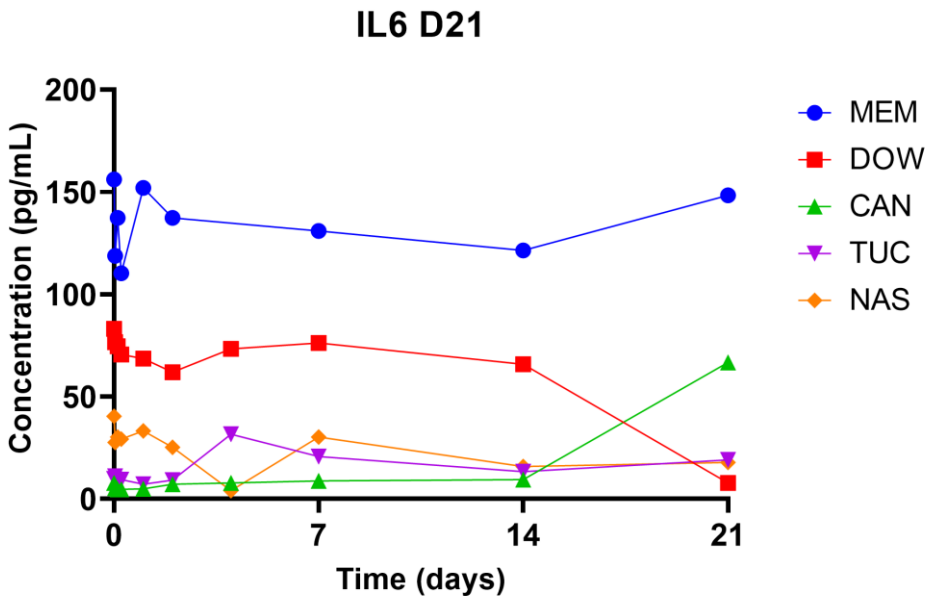


Cohort	ID	Dose (vg/kg)
2	NAS2018 TUC2018	6.0e13 6.0e13
3	CAN2017 DOW2016 MEM2018 #	2.0e14 2.0e14 2.0e14



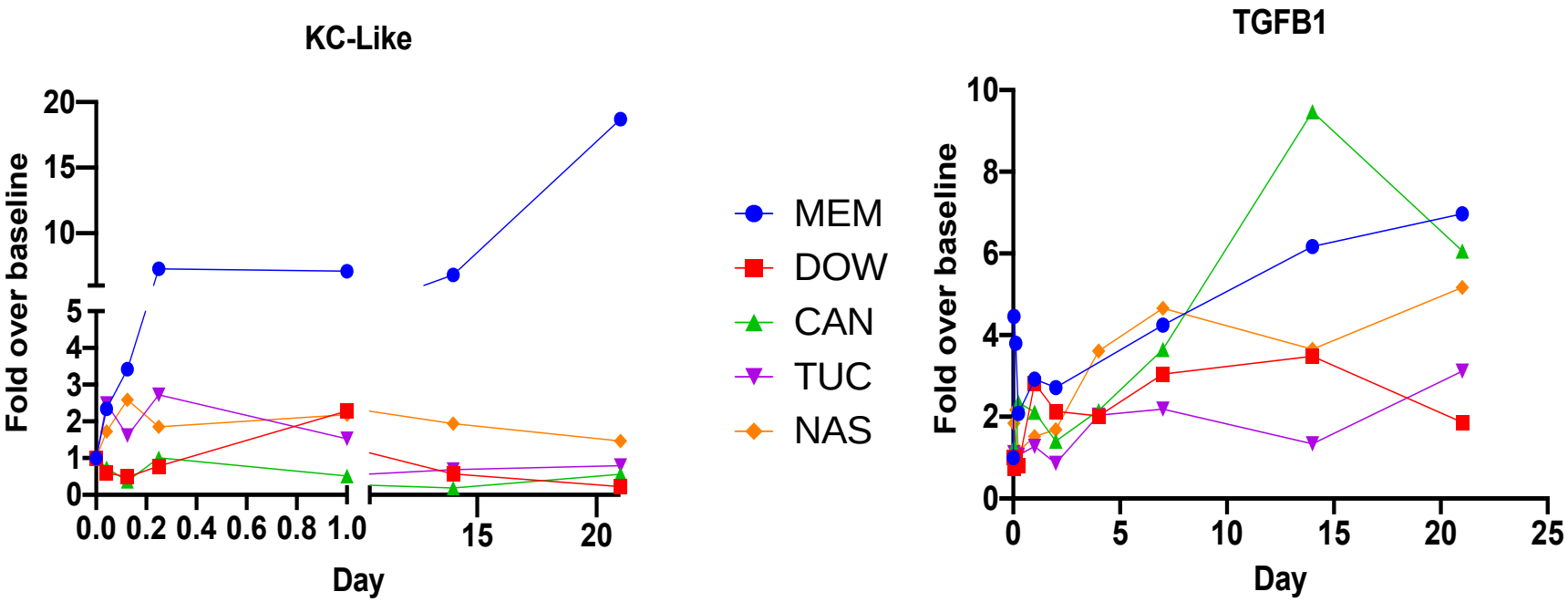
No Significant Elevation in Most Cytokines Analyzed Following AAV5-cFVIII





- Different dogs displayed individual cytokine profiles
- Higher TNF-α, IL2, IL6, IL7, IL15 & IL18 in one dog with an inhibitor history
- No change in most cytokines levels

Minor Elevation of Cytokines Observed Following AAV5-cFVIII



- Minor transient elevation in KC-like within 24 hours seen in 4/5 dogs
- Mild TGFb1 increases with time up to day 21
- No elevation in liver enzymes (ALT, AST or ALP) up to day 21

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Summary

- Codon Optimized AAV5-cFVIII resulted in therapeutic cFVIII:C expression with dose response
- Mechanistic studies are ongoing into WBCT improvement without cFVIII:C expression following non-codon optimized AAV5-cFVIII
- AAV5-HLP-cFVIII was detected in the liver in all animals at 3 months, with correlation between VG and cFVIII mRNA copies
- Capsid antibodies seen within 7 days of AAV5-cFVIIII
- No significant elevations of innate immune response was detected

Acknowledgments

- Haemophilia colony dogs
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- BioMarin
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