



Isth VIRTUAL
CONGRESS
2021 JULY 17-21
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SCIENCE CONQUERING DISEASE



Investigation of Early Outcomes Following Adeno-Associated Viral Gene Therapy in a Canine Hemophilia Model (OC 75.2)

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Basic and Laboratory Aspects of Gene Therapy for Hemophilia : July 21st 2021



Disclosures for Paul Batty

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

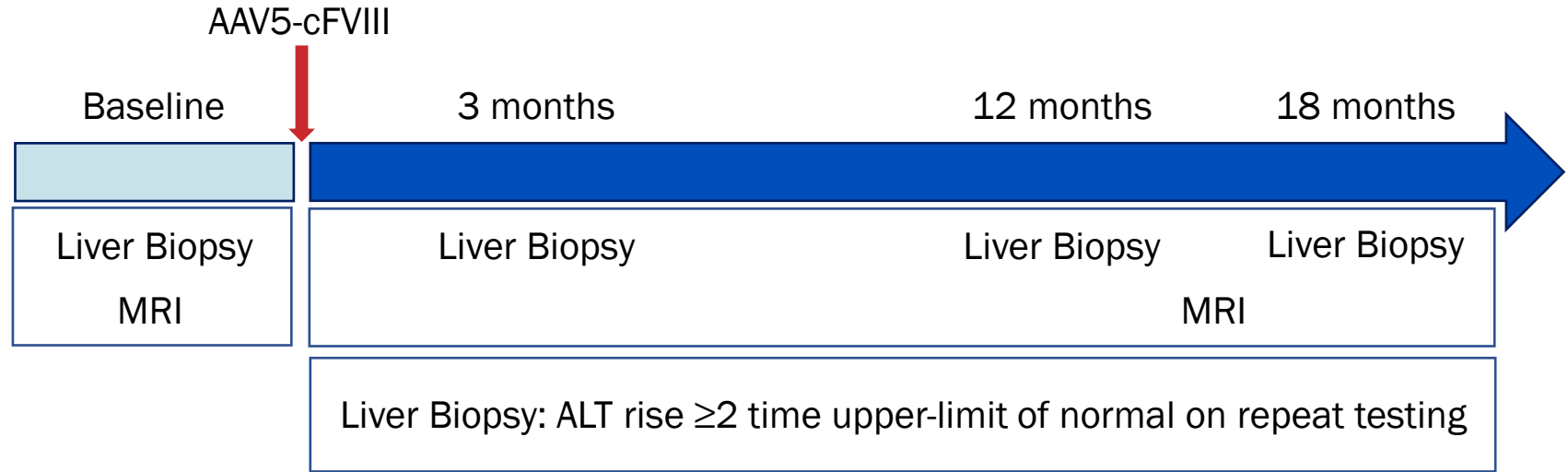
Research Support/P.I.	PB has received research funding from BioMarin, Grifols and Octapharma
Employee	No relevant conflicts of interest to declare
Consultant	No relevant conflicts of interest to declare
Major Stockholder	No relevant conflicts of interest to declare
Speakers Bureau	No relevant conflicts of interest to declare
Honoraria	No relevant conflicts of interest to declare
Scientific Advisory Board	PB has acted on an advisory board for BioMarin

Canine Hemophilia A



- Similar Genotype
 - Intron-22 inversion like *F8* mutation
- Similar Clinical Phenotype
 - Spontaneous bleeding events
 - Treated on-demand with recombinant canine FVIII
 - Inhibitor incidence c. 25%
- Long-term follow-up >10 years

Study Aims & Outline



- Evaluate safety & efficacy of AAV5-cFVIII in a hemophilia dog model
- Evaluate hepatic outcomes and mechanism(s) of elevation in ALT seen in clinical studies
- Evaluate factors resulting in variable expression following AAV5-cFVIII
- Evaluate vector genome metabolism and integration profiles following 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

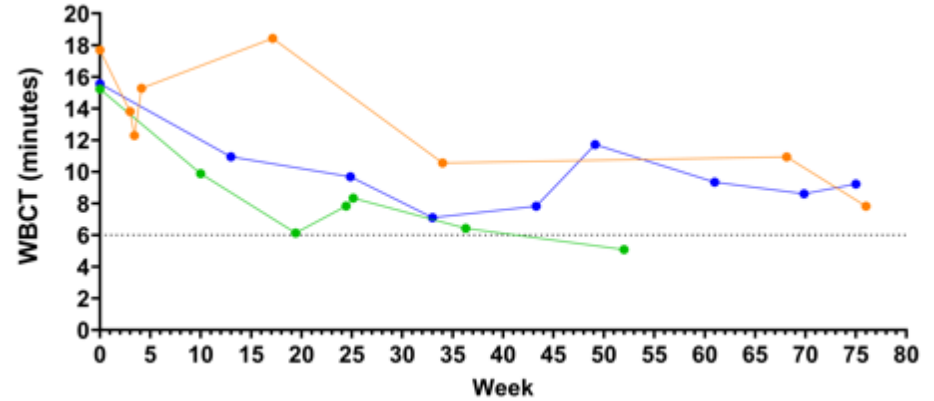
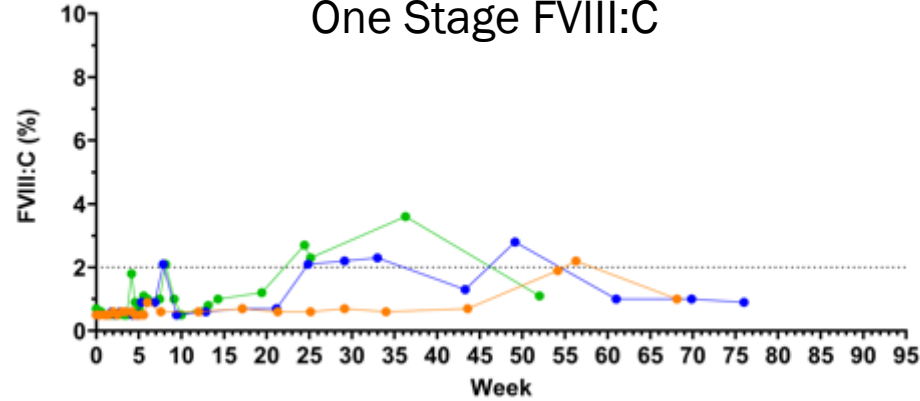
ID	Construct	Dose (vg/kg)	Age at Treatment (years)	Follow-Up (weeks)	Response
AUS2010	HLP-nco-cFVIII-SQ	8.0e13	9.0	76 #	No
BRO2018	HLP-nco-cFVIII-SQ	8.2e13	0.8	89 *	No
BAN2011	HLP-nco-cFVIII-SQ	2.1e14	8.3	52 #	No

Response = Chromogenic FVIII:C >1%

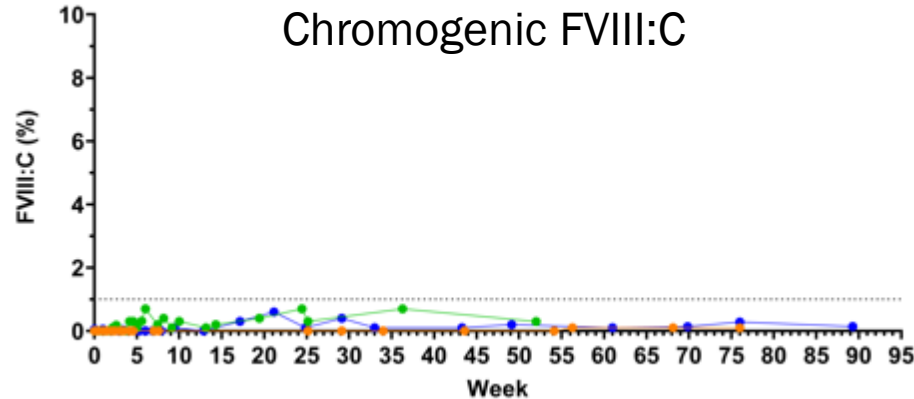
* Follow-up ongoing : Data capture 20/05/2021. # Study end-point reached

No Significant FVIII Expression with AAV5-nco-cFVIII

One Stage FVIII:C



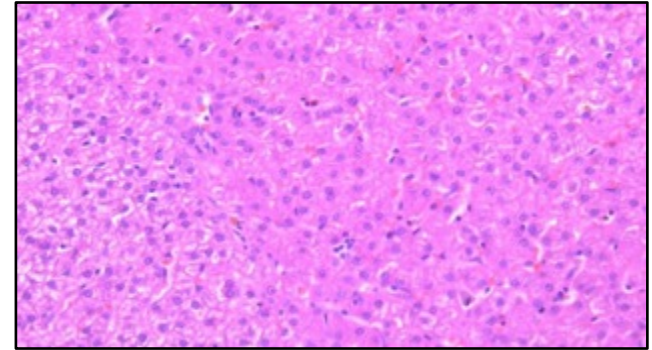
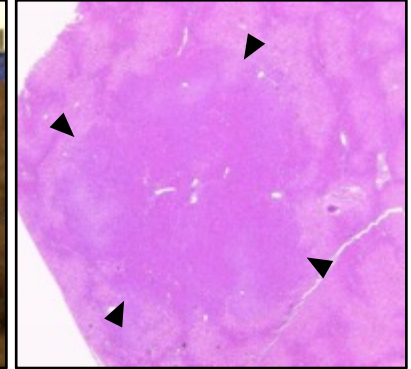
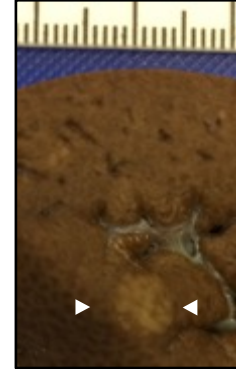
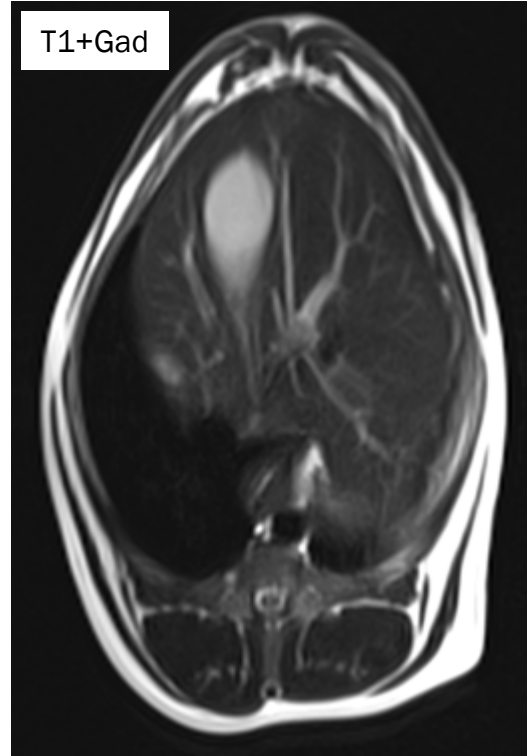
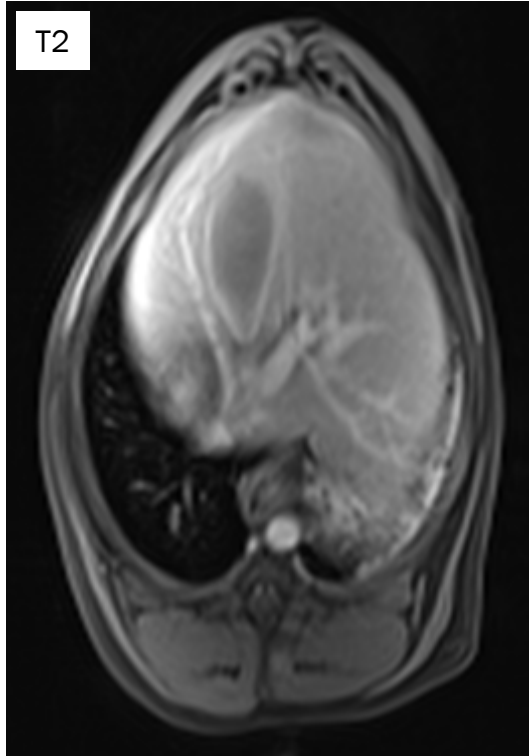
Chromogenic FVIII:C



- AUS2010 (8.0×10^{13} vg/kg)
- BRO2018 (8.2×10^{13} vg/kg)
- BAN2011 (2.1×10^{14} vg/kg)

No Liver Toxicity 12 - 18 Months Post AAV5-nco-cFVIII

BAN2011 : 12 months post AAV5-nco-cFVIII (2e14 vg/kg) : Age 9.3 years



No other systemic toxicities following AAV5-nco-cFVIII

Liver histology – Rare small (3mm) foci of hepatic nodular hyperplasia – likely age related. Otherwise unremarkable liver.

Cohort 2 : Codon-Optimized AAV5-cFVIII

B-domain deleted canine F8



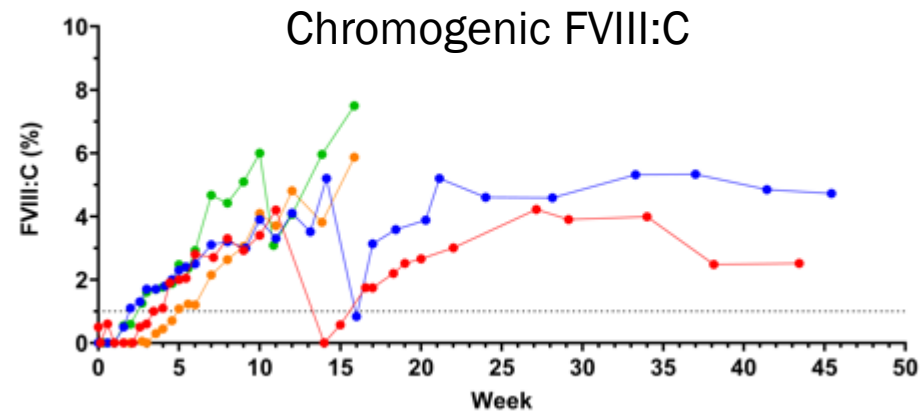
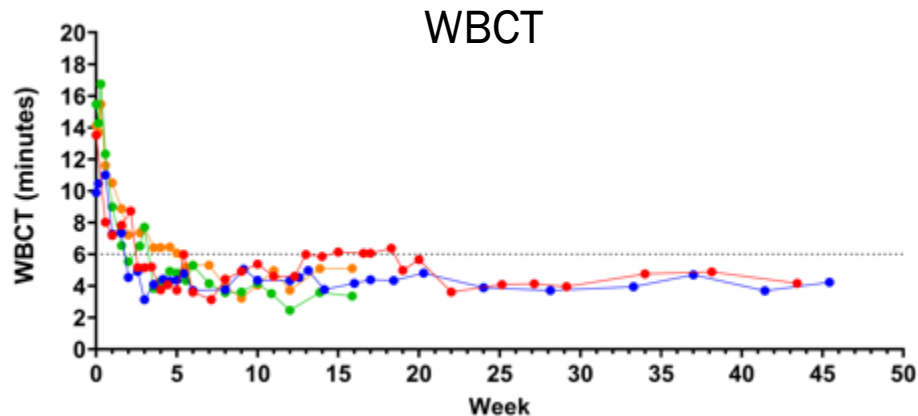
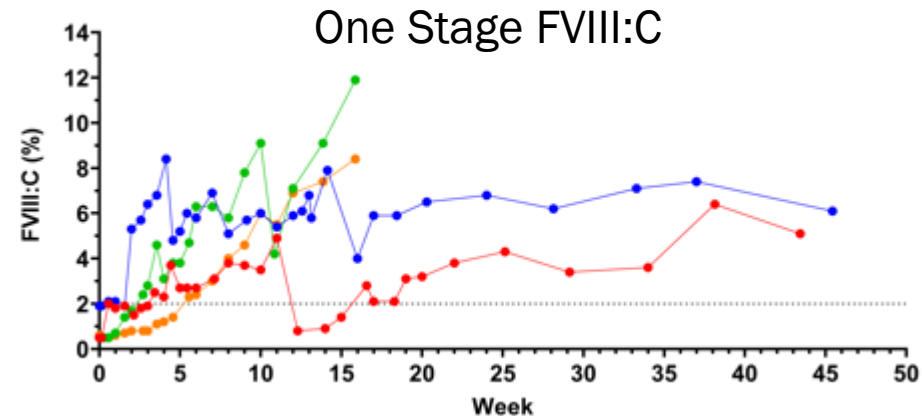
ITR = Inverted terminal repeat. HLP = Hybrid liver promoter

PA = Polyadenylation sequence

ID	Construct	Dose (vg/kg)	Age at Treatment (years)	Follow-Up (Weeks)	Current OSA FVIII:C
KIN2015	HLP-co-V3-cFVIII	6.8e13	5.2	43 *	5.1%
JAZ2013	HLP-co-SQ-cFVIII	6.8e13	6.9	45 *	6.1%
NAS2018	HLP-co-SQ-cFVIII	6.0e13	2.4	15 *	11.9%
TUC2018	HLP-co-SQ-cFVIII	6.0e13	2.4	15 *	8.4%

* Follow-up ongoing : Data capture 20/05/2021.

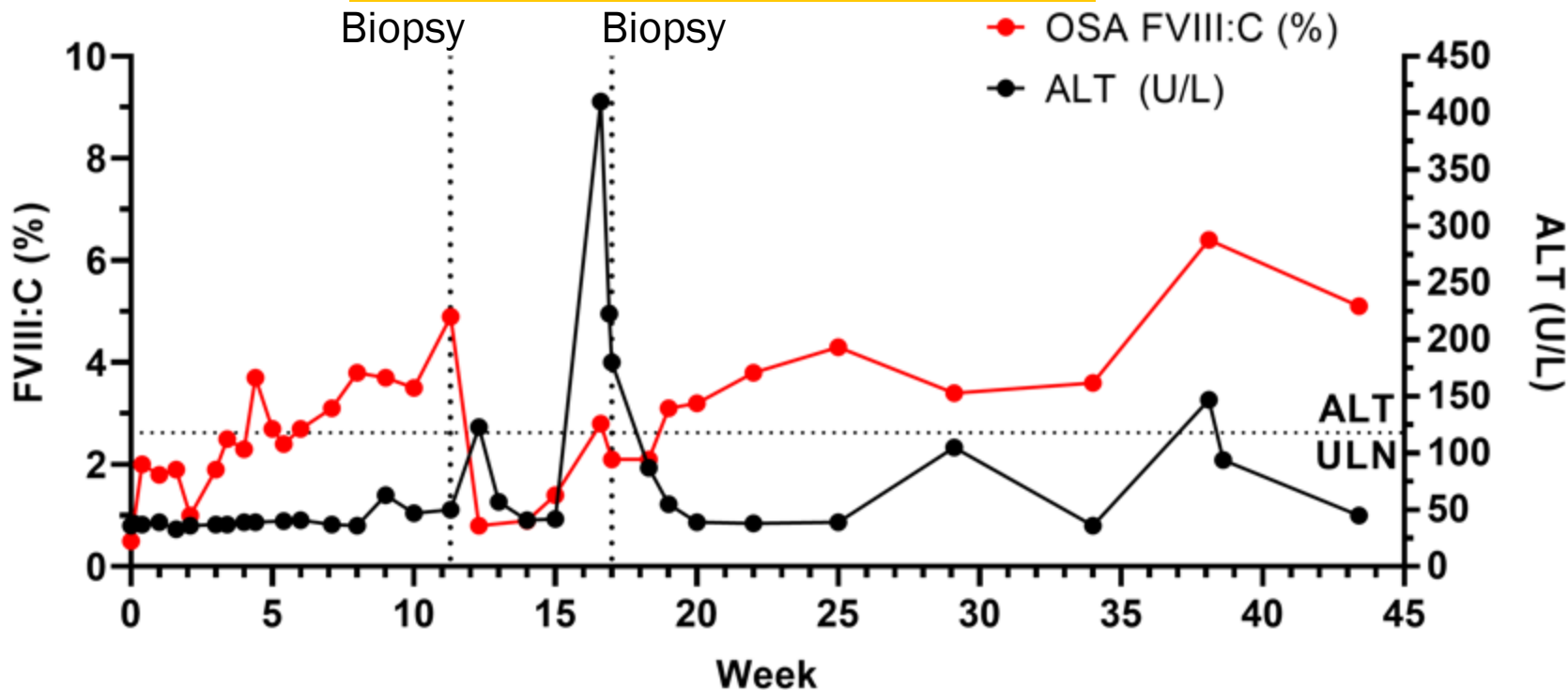
AAV5-co-cFVIII Results in cFVIII Expression and Normal WBCT



- KIN2015 (AAV5-co-V3-cFVII)
- JAZ2013 (AAV5-co-SQ-cFVIII)
- NAS2018 (AAV5-co-SQ-cFVIII)
- TUC2018 (AAV5-co-SQ-cFVIII)

Fall in cFVIII Expression with Elevation in ALT

KIN2015: AAV5-HLP-co-V3-cFVIII : 6.8e13 vg/kg



- Mild post-biopsy ALT elevation at Day 84
- Second ALT elevation at Day 116 : Biopsy performed at Day 119

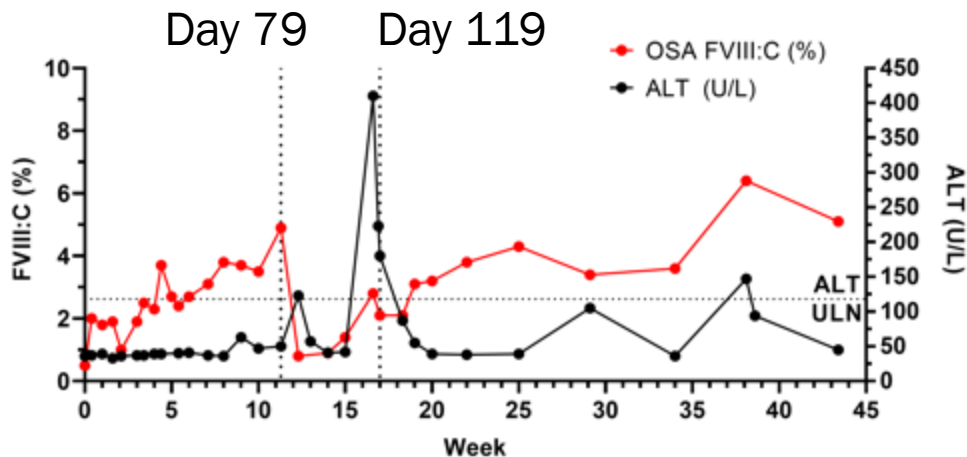
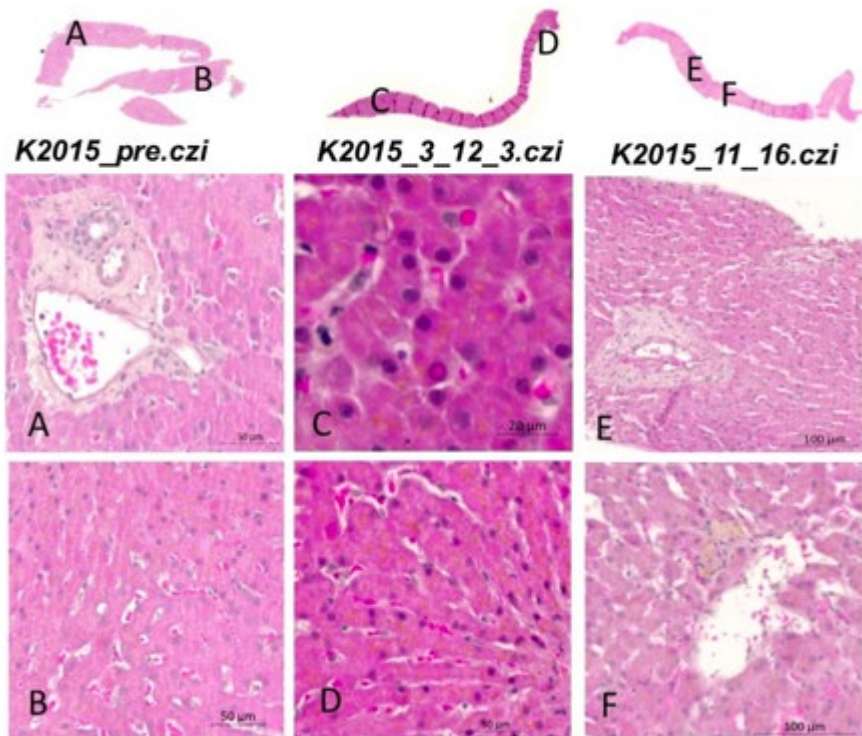
No Hepatocyte Death or Inflammatory Infiltrate

KIN2015: AAV5-HLP-co-V3-cFVIII : 6.8e13 vg/kg

Baseline

Day 79

Day 119



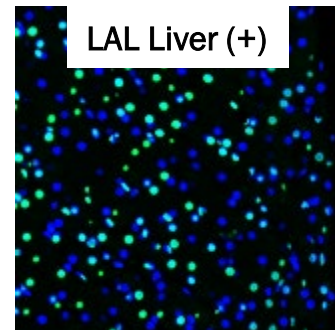
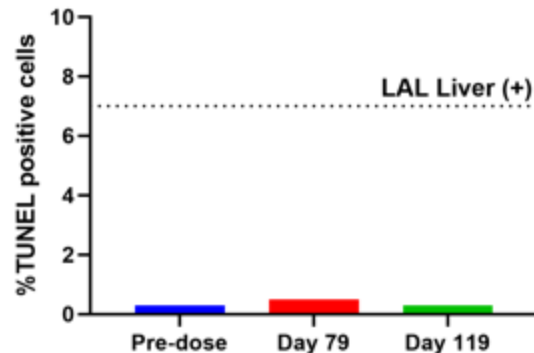
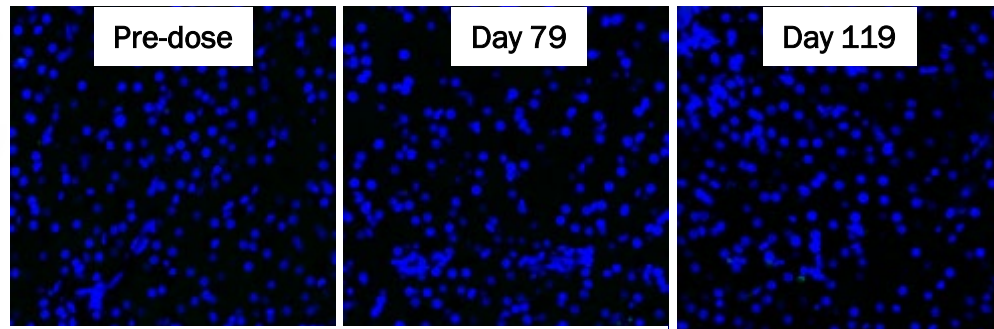
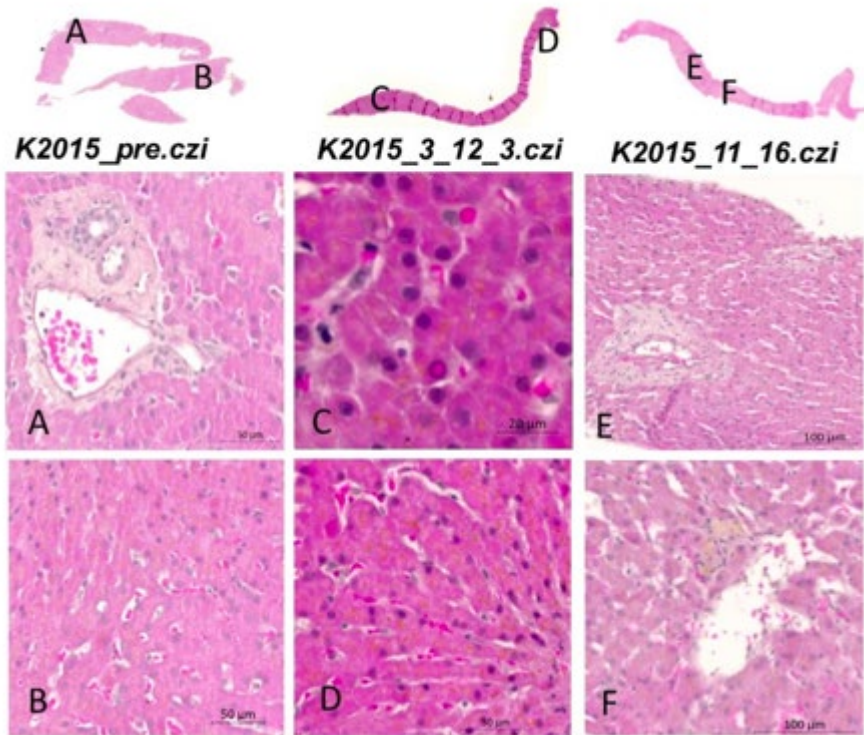
No Hepatocyte Death or Inflammatory Infiltrate

Baseline

Day 79

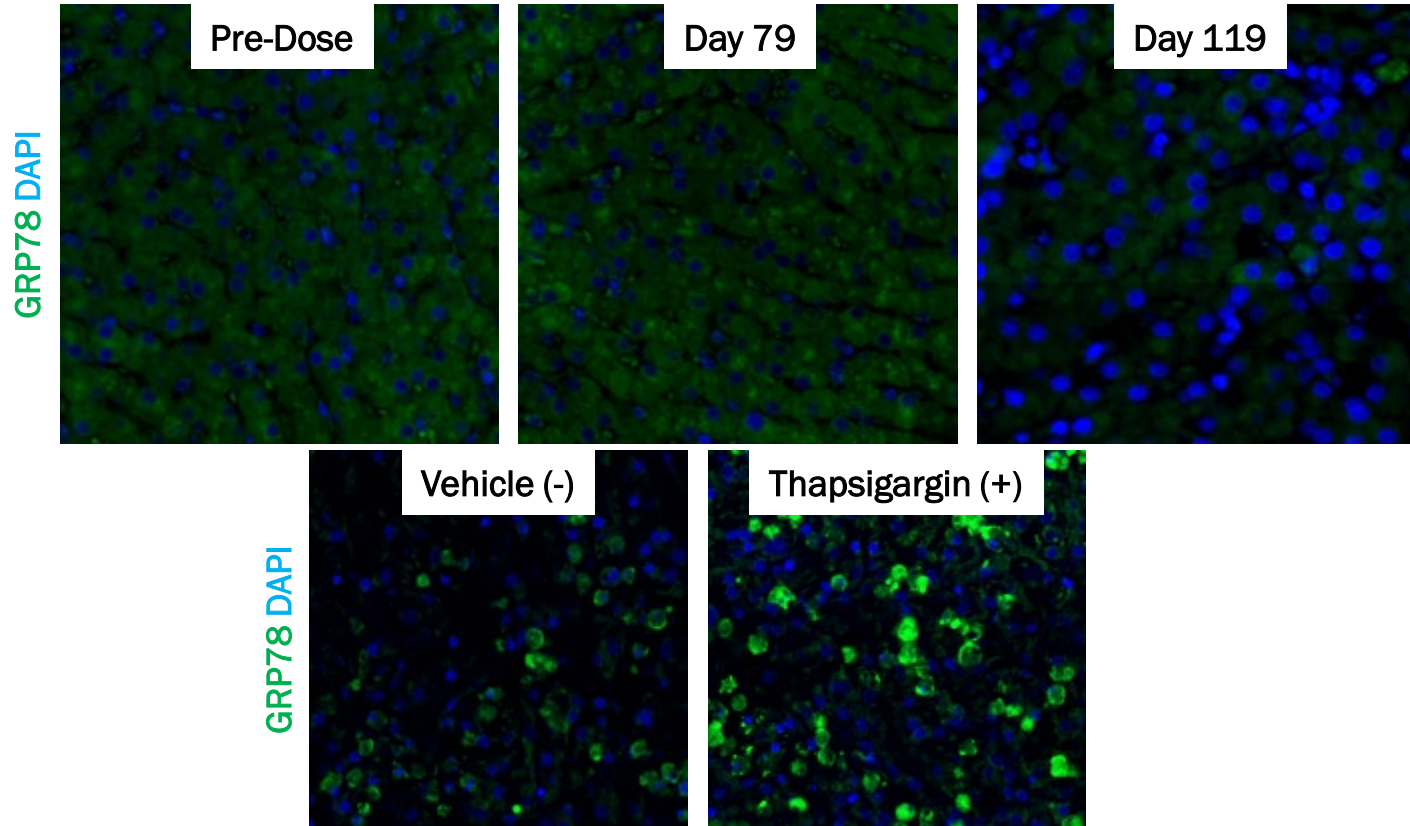
Day 119

KIN2015: AAV5-HLP-co-V3-cFVIII : 6.8e13 vg/kg



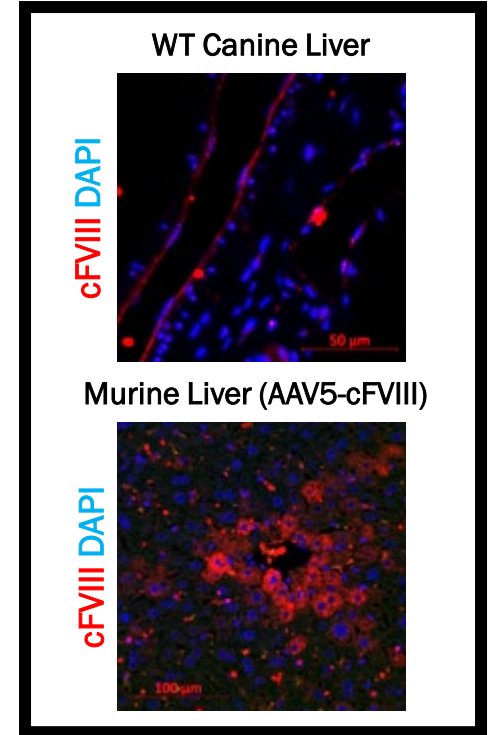
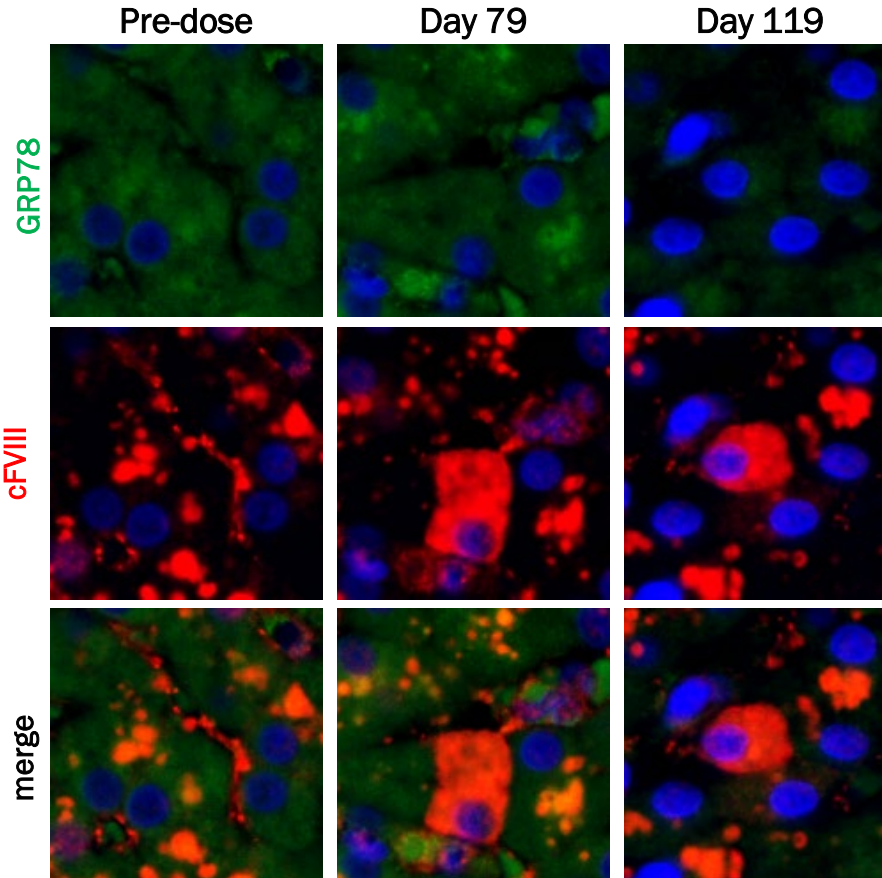
No Increase in GRP78 Expression Following AAV5-cFVIII

KIN2015: AAV5-HLP-co-V3-cFVIII : 6.8e13 vg/kg



No Increase in GRP78 in Hepatocytes Expressing cFVIII

KIN2015
AAV5-HLP-co-V3-cFVIII
6.8e13 vg/kg



Fall in FVIII:C Correlates with Decreased cFVIII mRNA

KIN2015: AAV5-HLP-co-V3-cFVIII : 6.8e13 vg/kg

Pre-Dose

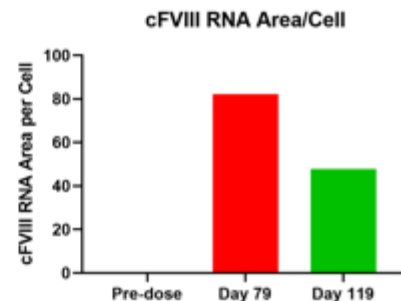
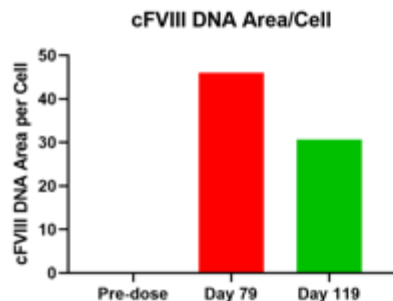
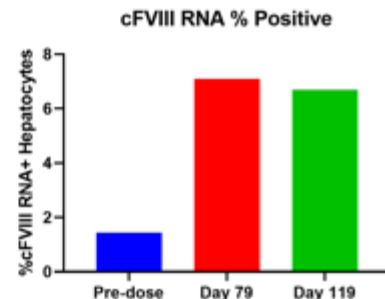
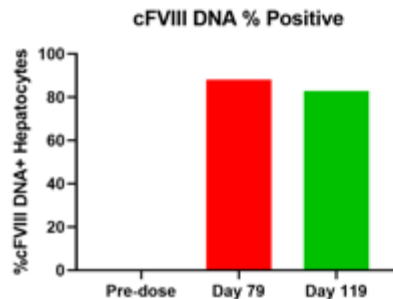
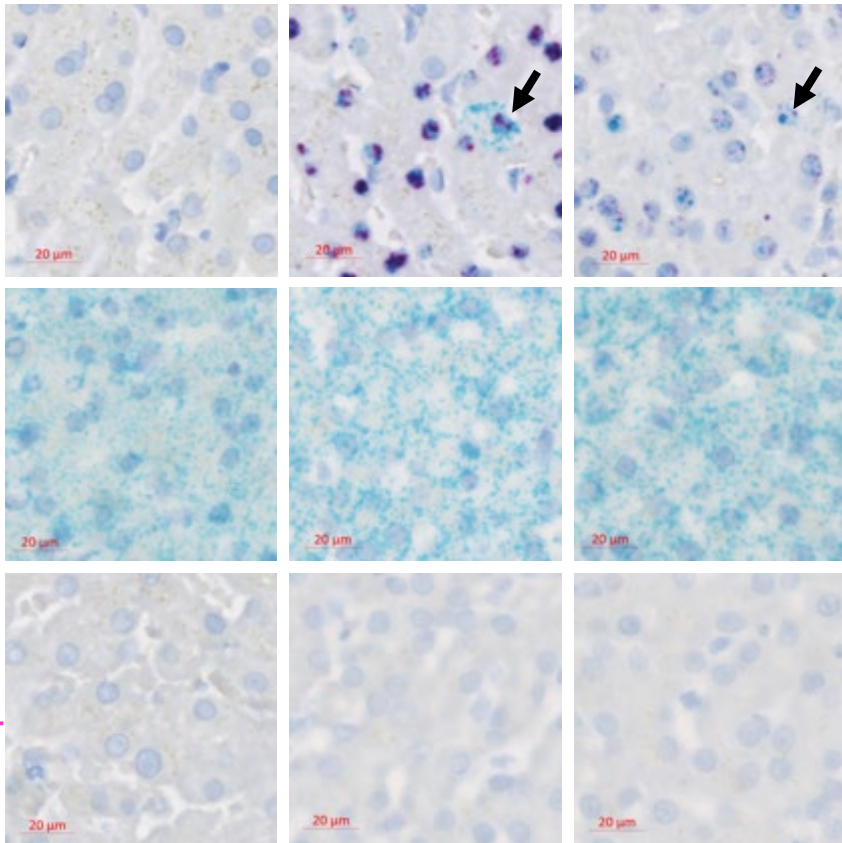
Day 79

Day 119

cFVIII DNA cFVIII RNA

UBC

dapB



Summary

- Codon Optimized AAV5-cFVIII resulted in therapeutic cFVIII:C expression
- No evidence of liver disease or malignancy after 12 – 18 months
- First animal model description of elevated ALT & transient loss of cFVIII:C following AAV-FVIII
 - No hepatocyte cell death, inflammation or upregulation of the unfolded protein response
 - Reduction in FVIII:C associated with decreased AAV vg DNA & cFVIII mRNA

Acknowledgements

- Haemophilia colony dogs
- Queen's University
 - Alex Menard
 - Lori Harpell
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 - Bridget Yates



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