Kinetic Profiles of anti-AAV5 Antibody Generation and Clearance in Neonatal, Infant, and Adult Hemophilia A Dogs

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Session Title: Gene therapy - Clinical and pre-clinical

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OC 02.1

Conflict of Interest Disclosure – Paul Batty

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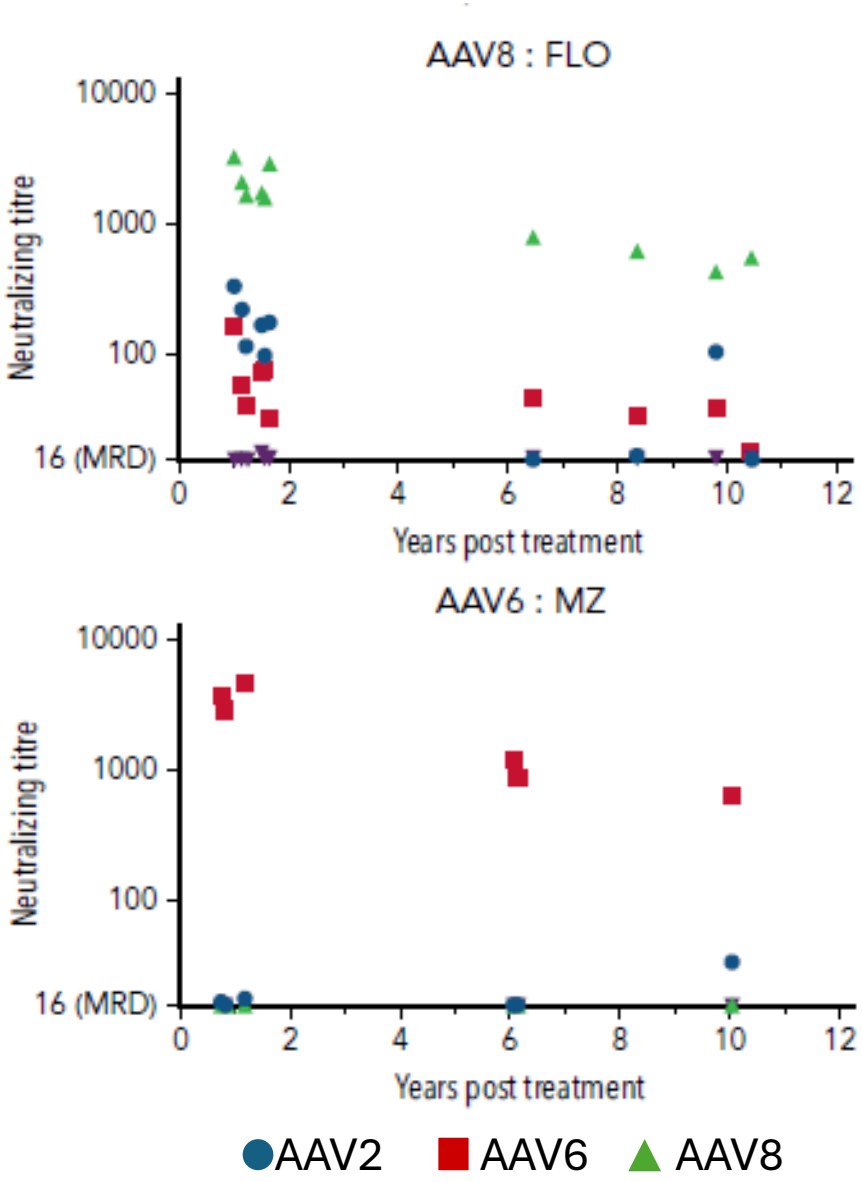
Canine Hemophilia A

- 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

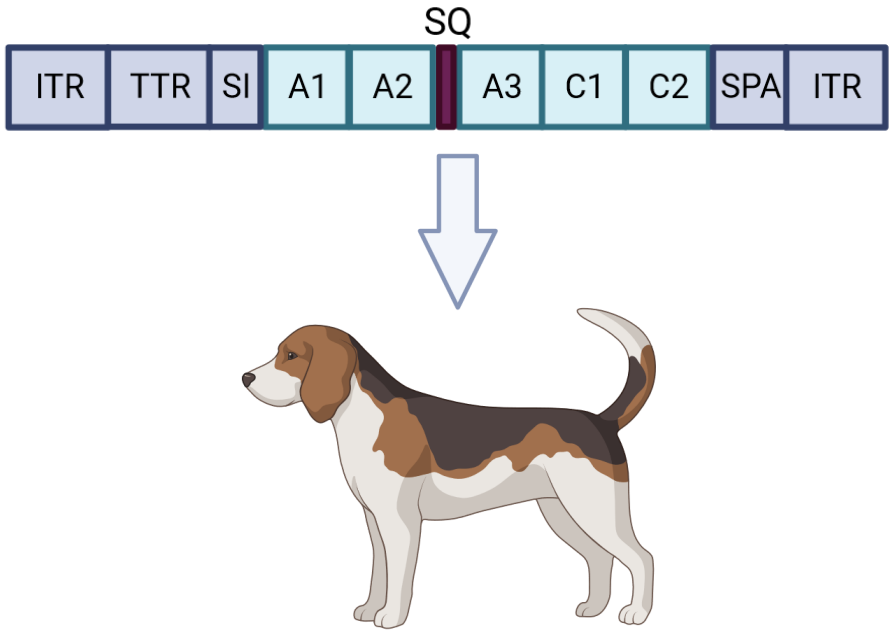
Persistent AAV Capsid nAbs were Observed in AAV-cFVIIITreated Dogs Multi-Years Post Gene Therapy



- 8 severe hemophilia A dogs treated with AAV-cFVIII
  - Median follow up: 10.7 years
  - AAV2 (n=4), AAV6 (n=3), AAV8 (n=1)
- Peak anti-AAV capsid nAbs observed at earliest time point
- Gradual reduction in anti-AAV capsid nAbs observed over 8.2-12.0 year follow up
- Cross-reactivity towards other AAV capsids observed at earlier time points

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

Study Outline



- **Peripheral infusion of AAV5-cFVIII**
  - **Treatment age:** neonatal, infant, adult
  - **Vector:** wild-type (WT) and codon-optimized (CO)
  - **Target dose:** 6e13 – 2e14 vg/kg
  - **Follow up:** 18 – 24 months

Neonatal	Infant	Adult WT	Adult CO (6e13)	Adult CO (2e14)
2 weeks 2e14 AAV5-CO-cFVIII N=2	2 months 2e14 AAV5-CO-cFVIII N=3	6.0 years 6e13 - 2e14 AAV5-WT-cFVIII N=3	4.3 years 6e13 AAV5-CO-cFVIII N=4	3.9 years 2e14 AAV5-CO-cFVIII N=3

Study Aims

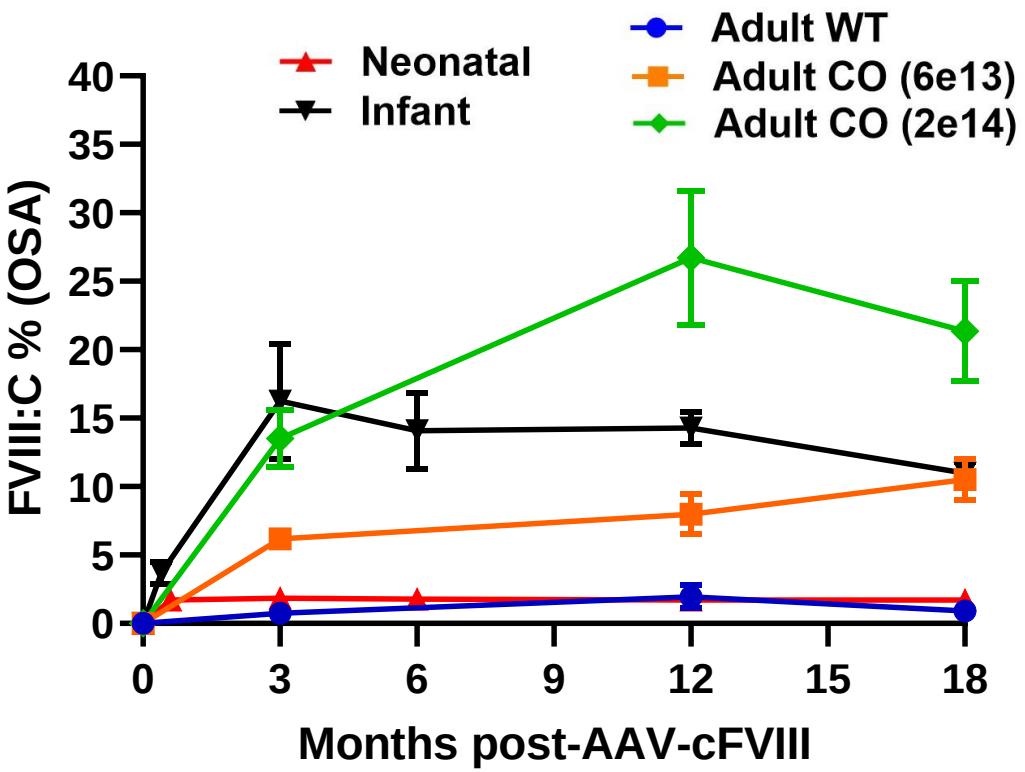
1. Provide an update on the long-term FVIII expression and vector processing kinetics

2. Describe the kinetic profiles of anti-AAV5 total antibody generation

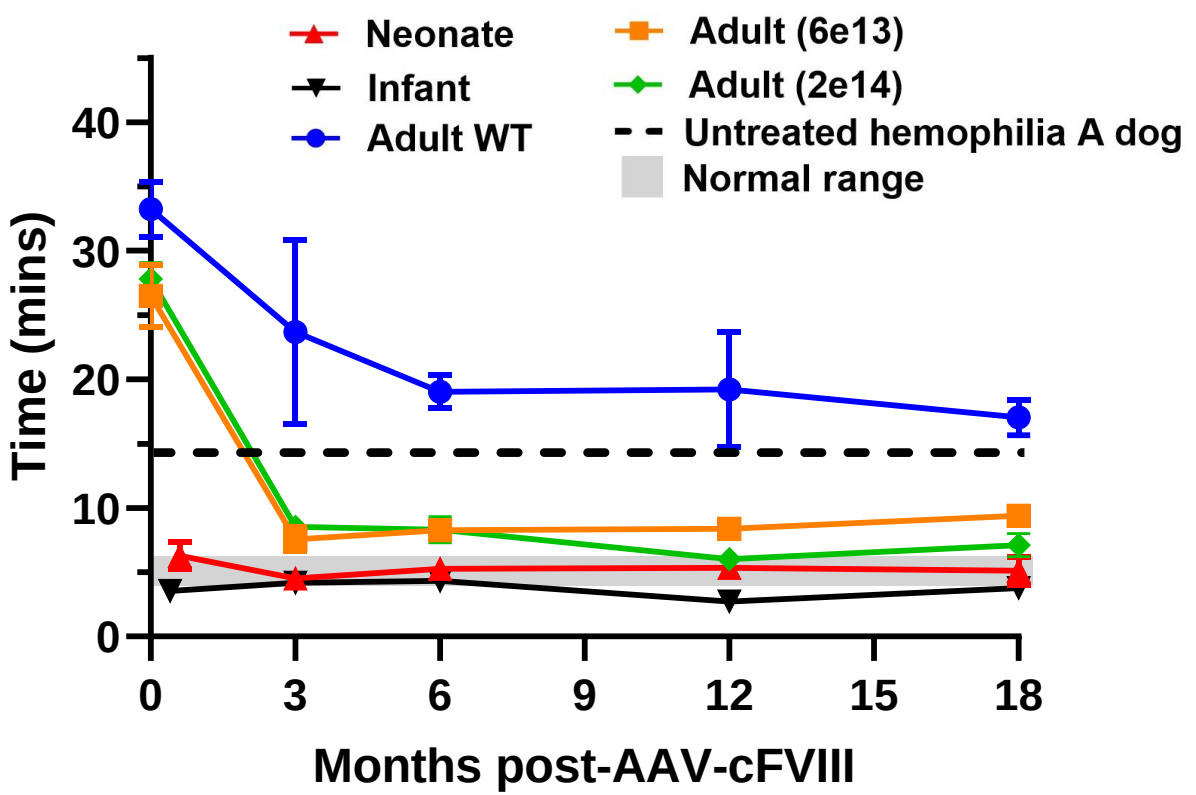
3. Determine the clearance of anti-AAV5 total antibodies in 2 neonates born to an AAV5-cFVIII-treated mother

Adult and Infant Dogs Expressed Persistent and Stable Therapeutic Levels of Transgenic cFVIII

FVIII:C % (One-Stage Assay)



Whole-Blood Clot Time



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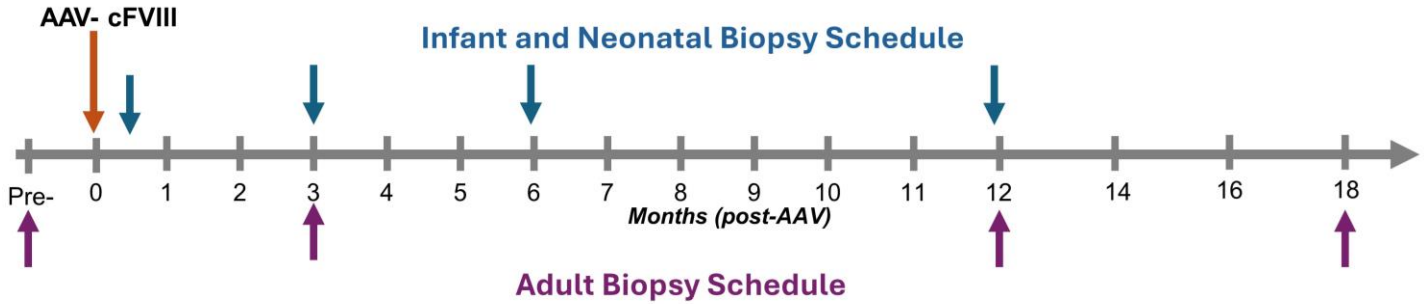
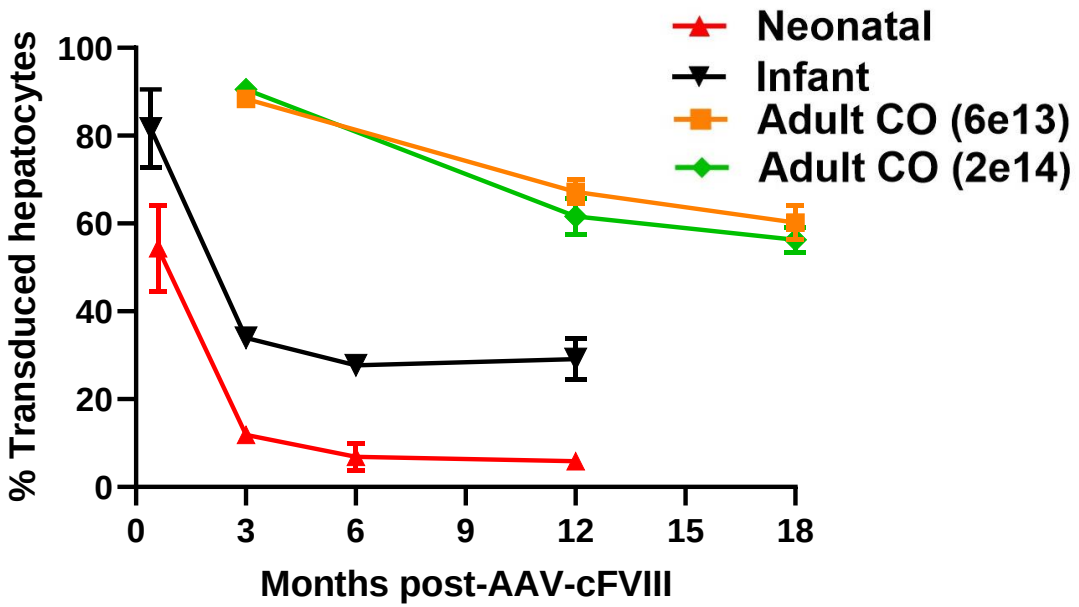
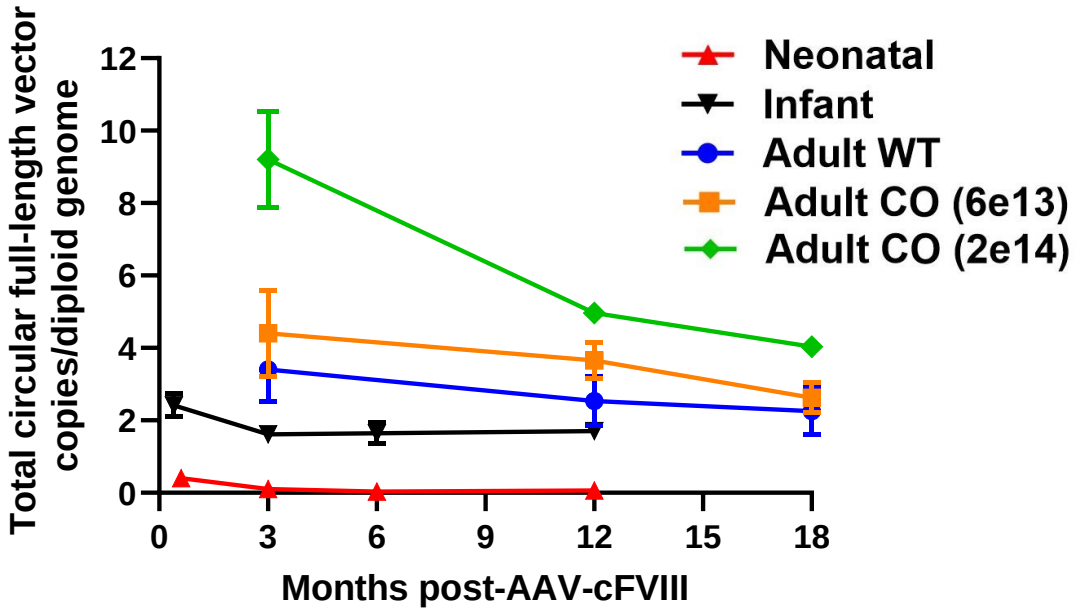
Hepatocyte Transduction was Efficient, Declined Initially, and Stabilized



% Transduced Hepatocytes



Circular Full-Length Vector Genomes



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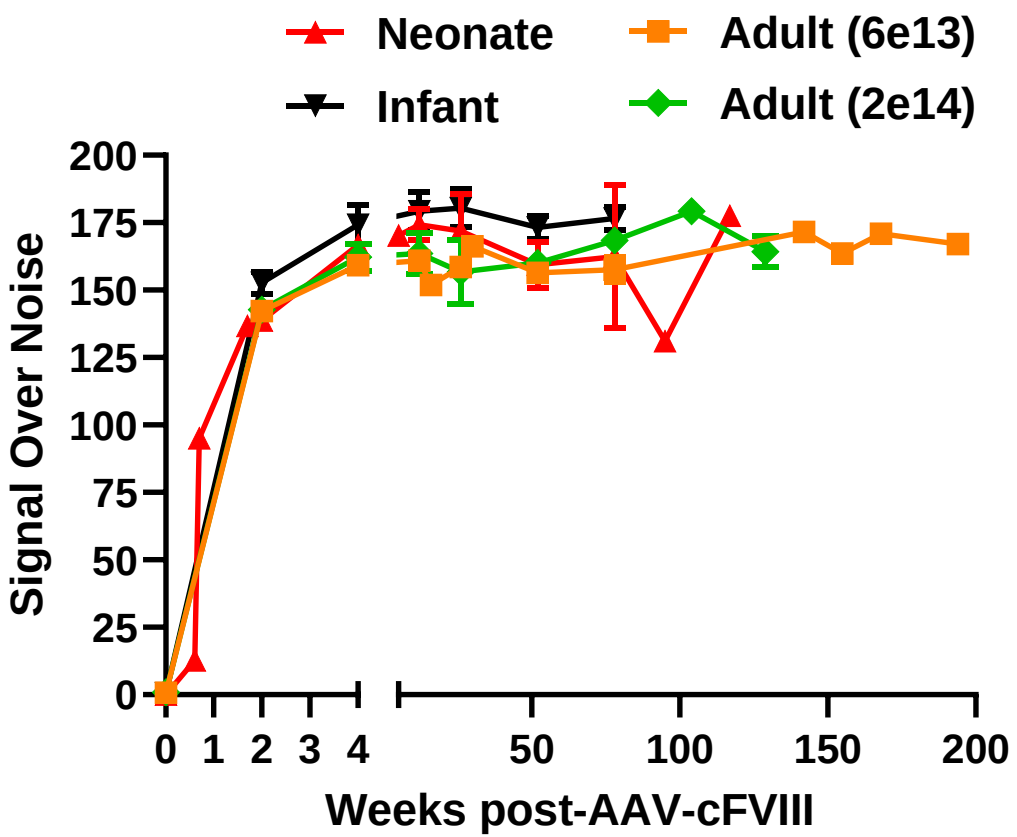
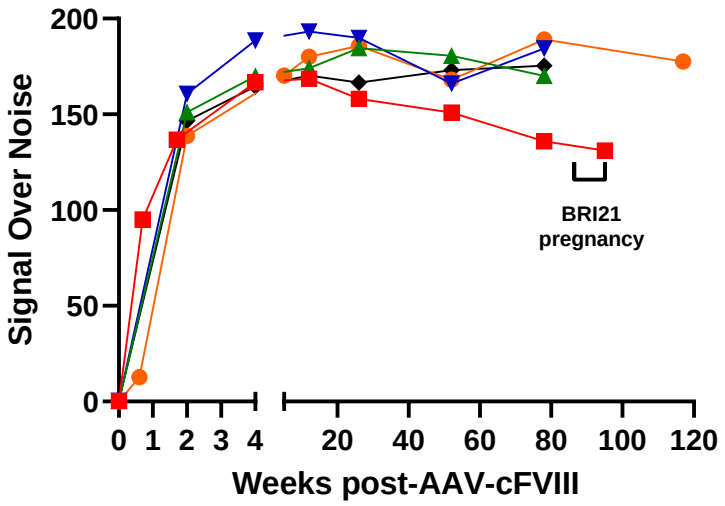
Age-at-Treatment and Vector Dose Did Not Influence anti-AAV5 Antibody Generation



- Total anti-AAV5 antibodies were quantified by ELISA
- Plasma samples tested at 1 – 25 dilution
- S/N positive >1.3
- All pre-treatment samples were negative

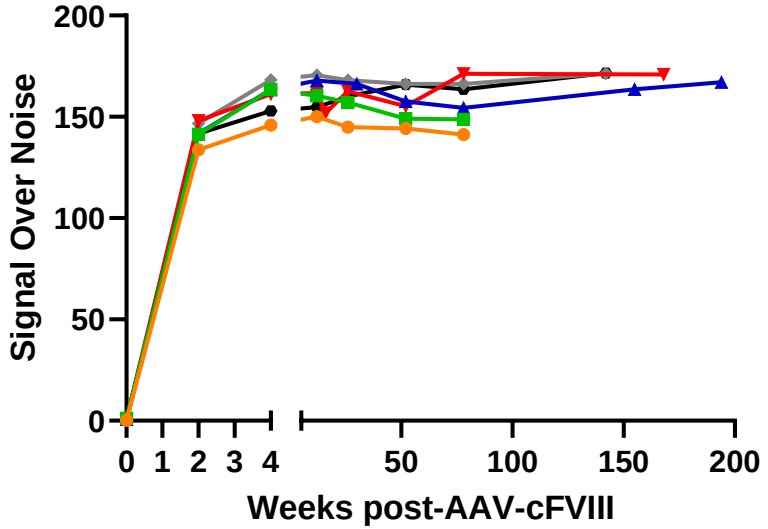
Neonatal Dog (BRI21) Experienced a 20% Decline in anti-AAV5 Antibodies by 95 Weeks post-AAV5-cFVIII

Neonates/Infant



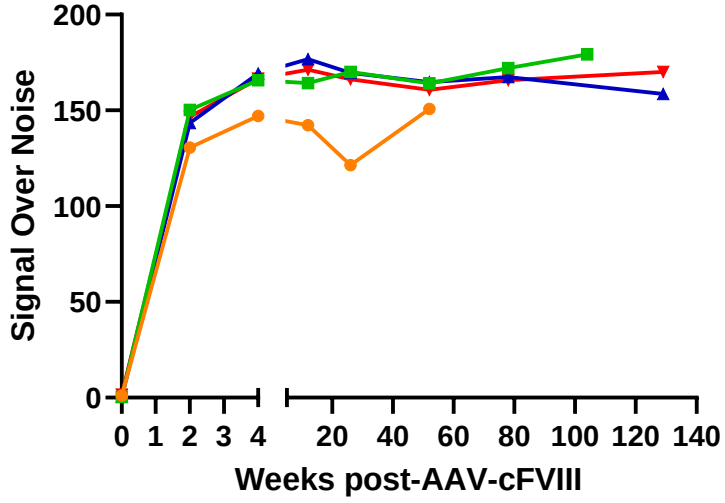
N=5

Adult (6e13)



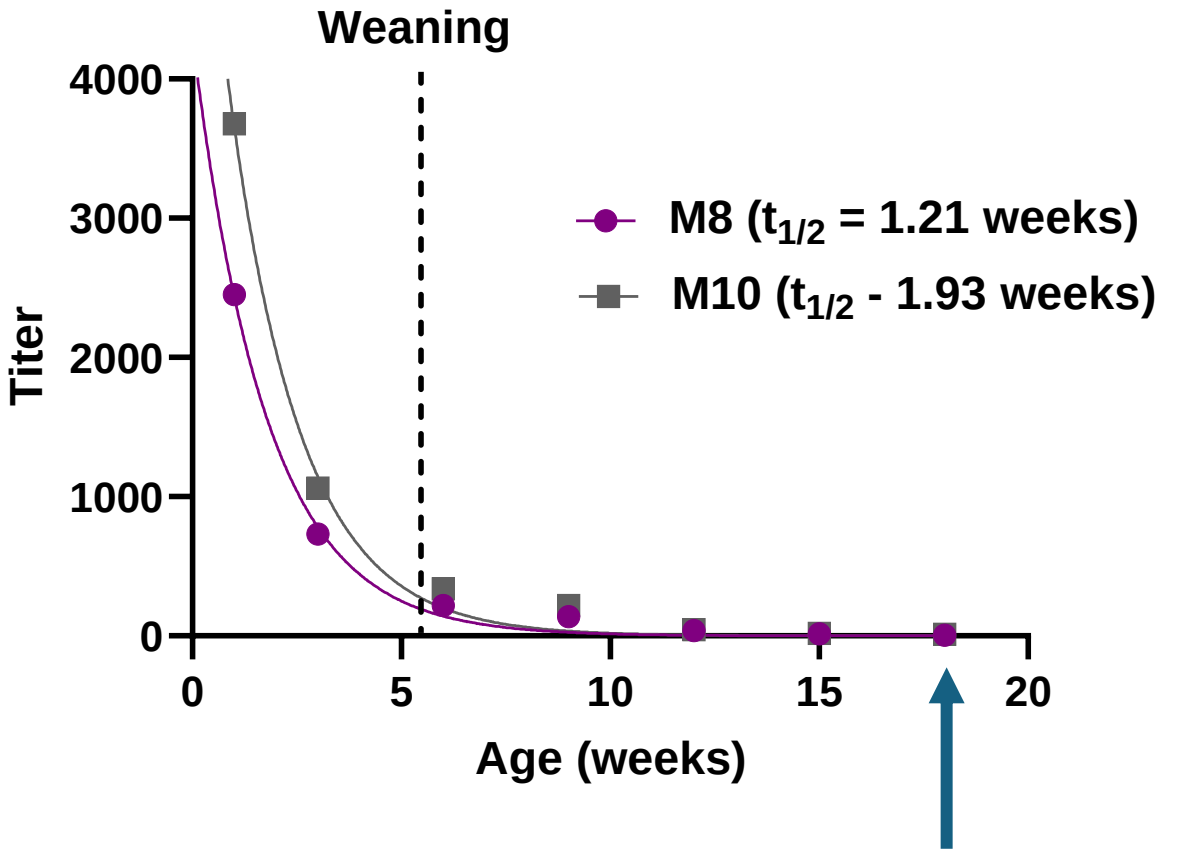
N=6

Adult (2e14)



N=4

Passively Acquired anti-AAV5 Antibodies in Newborn Dogs Show Rapid Clearance Kinetics



Final titer:
M8=5.4, M10=10.9

- Dam: BRI21 (FVIII:C <3%)
  - AAV5-CO-cFVIII, 2e14 vg/kg
  - Treatment age: 2 weeks
- Sire: DOW16 (FVIII:C ~ 25.7 – 28.1%)
  - AAV5-CO-cFVIII, 2e14 vg/kg
- Whelping:
  - 22 months post-AAV-cFVIII (S/N = 131)
  - Litter size: 10
  - Post-partum hemorrhage
- No apparent toxicity in the litter
- $t_{1/2}$ by 1-phase exponential decay

Conclusions

- AAV-cFVIII-treatment of adult-and infant-treated hemophilia A dogs:
  - Persistent and stable therapeutic levels of FVIII
  - Efficient, dose-dependent hepatocyte transduction
  - Transduced hepatocytes and circular full-length vector genome levels are highest early post-treatment and maintained for 12-18 months
- Anti-AAV5 antibody responses in hemophilia dogs are robust and long-lasting
- Passively acquired antibodies in newborn dogs show rapid clearance kinetics

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Poster: Persistent and stable therapeutic levels of transgenic FVIII expression following AAV delivery to adult and infant hemophilic dogs

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