

Recombinant DNA Advisory Committee

**Rockville, Maryland
December 8, 2010**

An Open Label Dose Escalation Study of a Self Complementary Adeno-Associated Viral Vector (scAAV2/8LP1hPPCA) for Gene Transfer in Subjects with Galactosialidosis

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- ***Murine Model of Galactosialidosis***
- Additional Studies Requested by FDA
- Ongoing Trial for Hemophilia B
- Vector Titering
- Questions Raised by RAC Reviewers

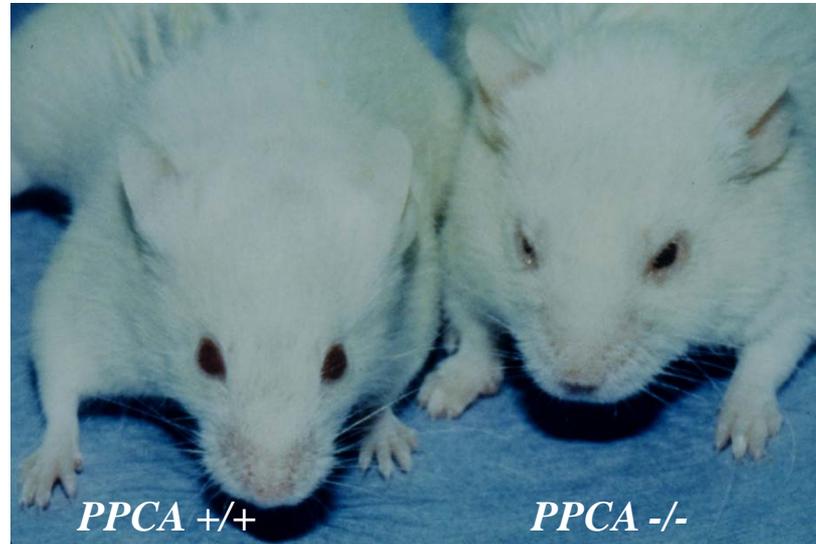
GALACTOSIALIDOSIS

Clinical Phenotypes

Primary deficiency :	PPCA
Secondary deficiency :	NEU1 and β -gal
Accumulated products:	glycoproteins and oligosaccharides

- **Early infantile:** fetal hydrops, edema, ascites, visceromegaly, skeletal dysplasia, early death
- **Late infantile:** hepatosplenomegaly, growth retardation, cardiac and kidney involvement, absence of neurological signs
 - * common allelic mutations -- residual PPCA activity
- **Juvenile/adult:** myoclonus, ataxia, angiokeratoma, mental retardation, neurologic deterioration, long survival

Mouse Model of Galactosialidosis



- Phenocopy of the human disease
- Similar systemic disease affecting most of the visceral organs
- Reduced lifespan (~7-8 months)
- CNS pathology restricted to limbic system and cerebellum

Zhou *Genes & Dev*, 1995; De Geest *HMG* 2001

Preliminary Dose Response Study in Murine PPCA^{-/-} Model

Doses: 10^9 10^{10} or 10^{11} vg/mouse

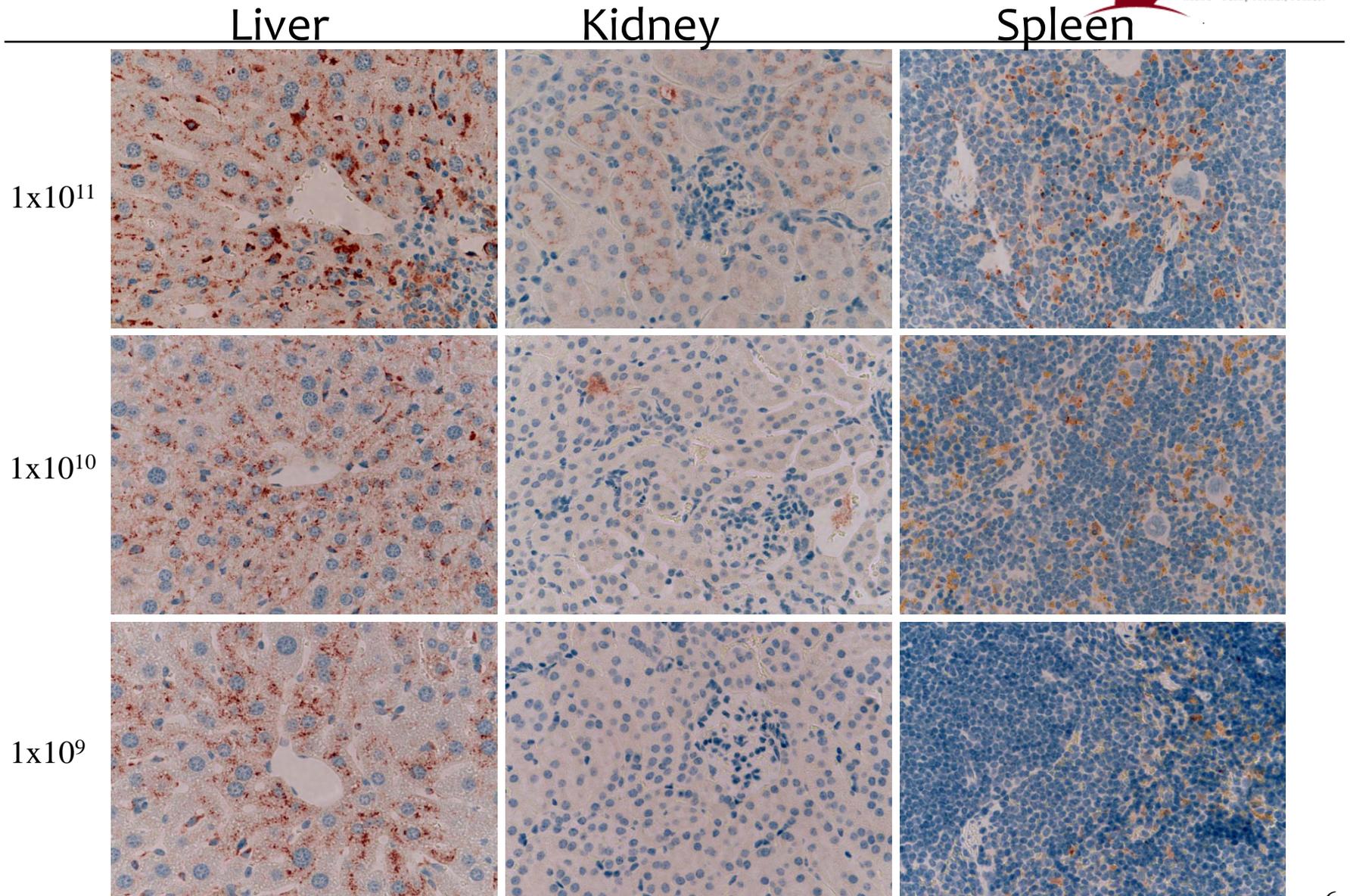
Numbers : 10 males and 10 females/group

Age of injection: 30 ± 5 days old

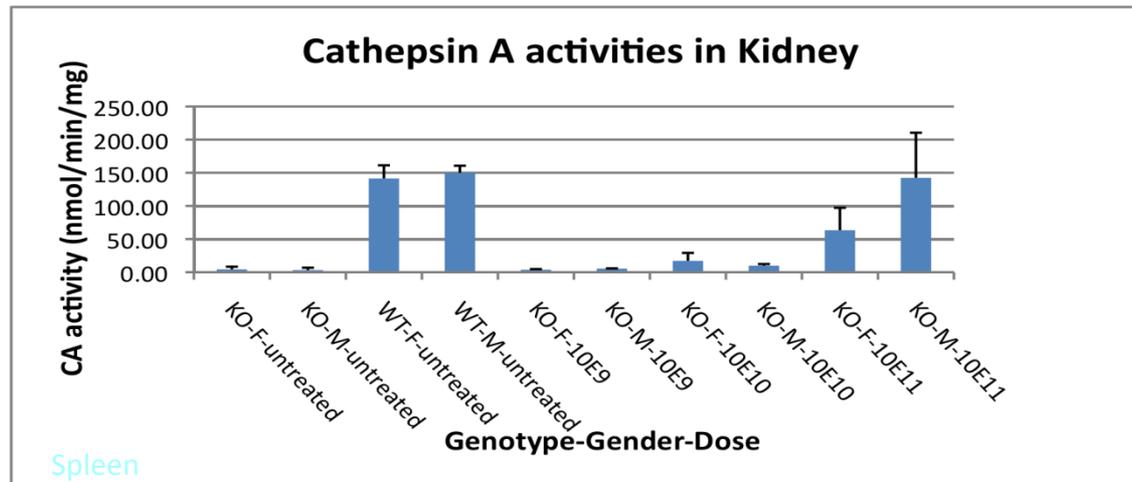
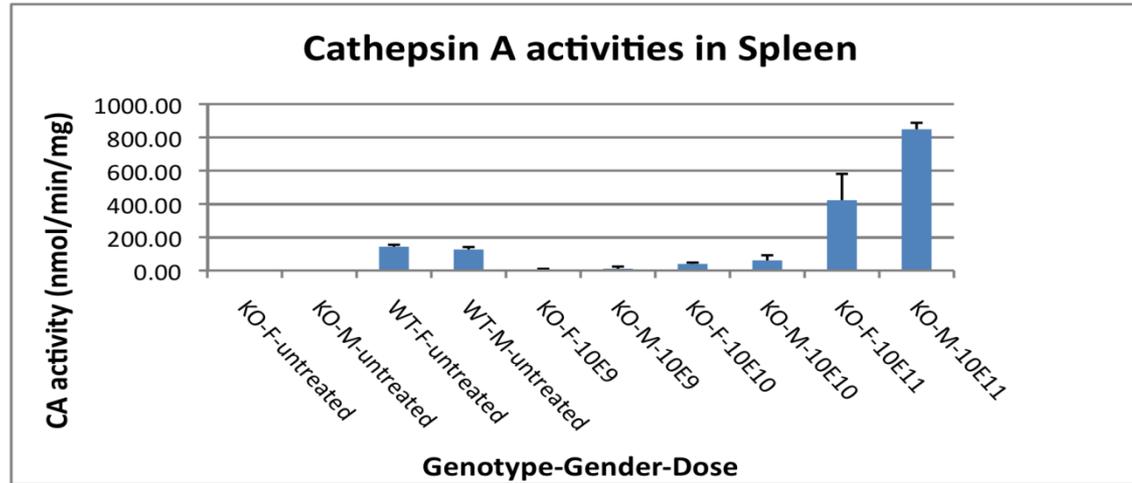
Analysis time point: 16 weeks after injection

Results: Histopathology corrected at all vector doses

Human PPCA Expression in Treated Mice



Cathepsin A Activity in Organs of Treated Mice

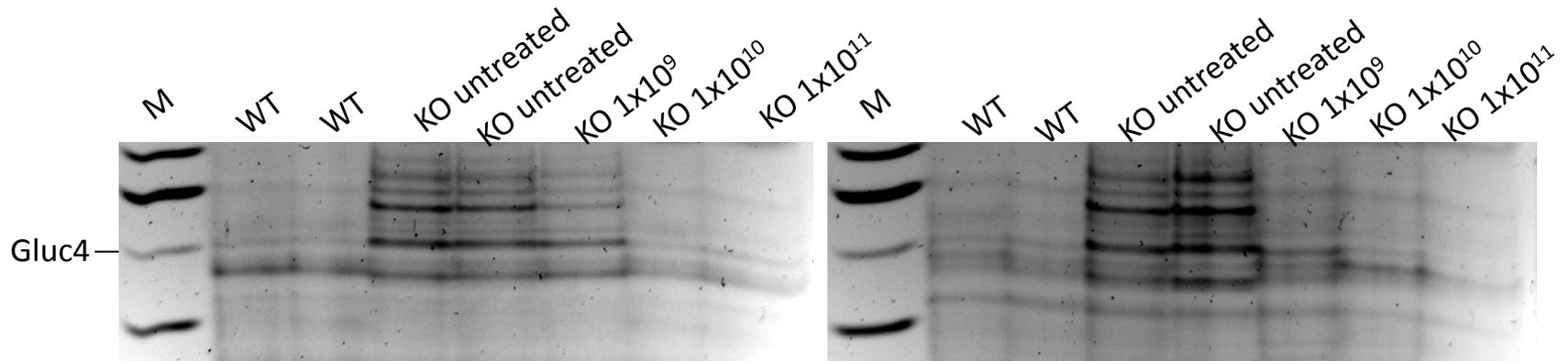


Spleen

Kidney



Urinary Oligosaccharide Analyses



Male

Female

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Additional Studies Requested by FDA To Be Performed In PPCA +/- Mice



- **Timing of Injection Comparison**
- **Determination of Minimal Effective Dose**
- **Toxicity Study at a Dose at Least 10 Fold Higher Than Highest Dose Equivalent in Clinical Trial**

Dose Finding Comparison with FIX Vector

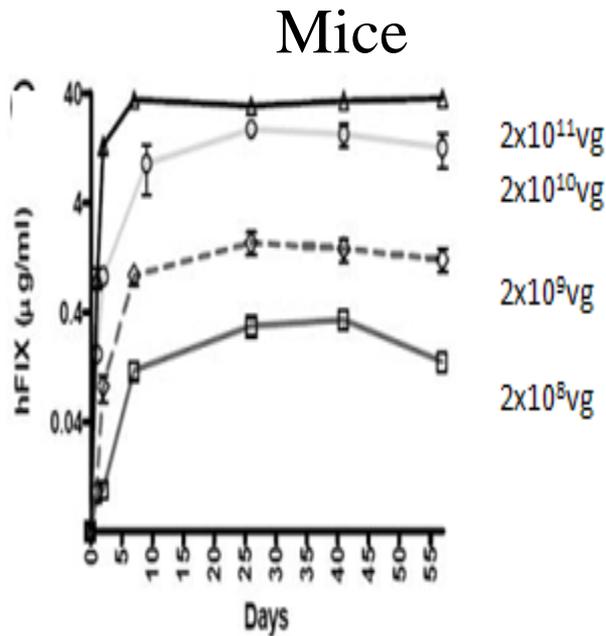


Figure 4A: Dose response curves in mice. Each cohort included 5-8 animals given the vector by tail vein at 6-8 weeks of age. Note that expression is reported using a log scale. Also shown are the standard errors for expression at each time point.

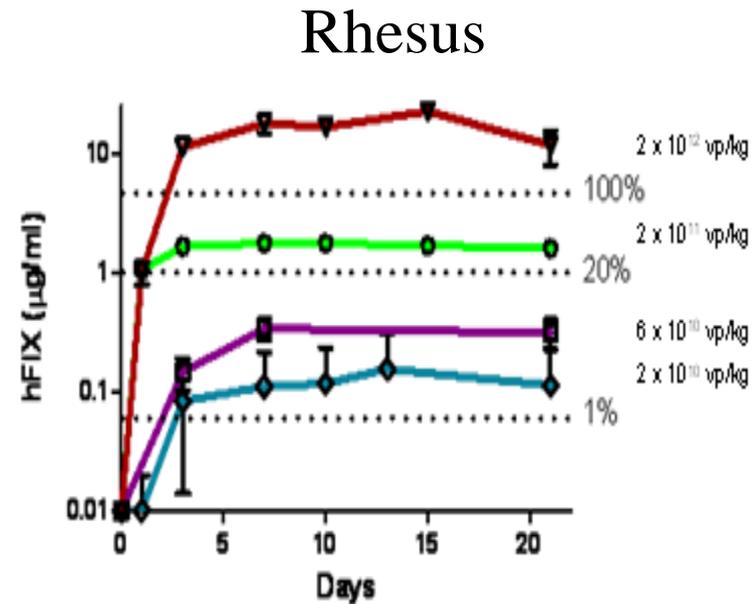


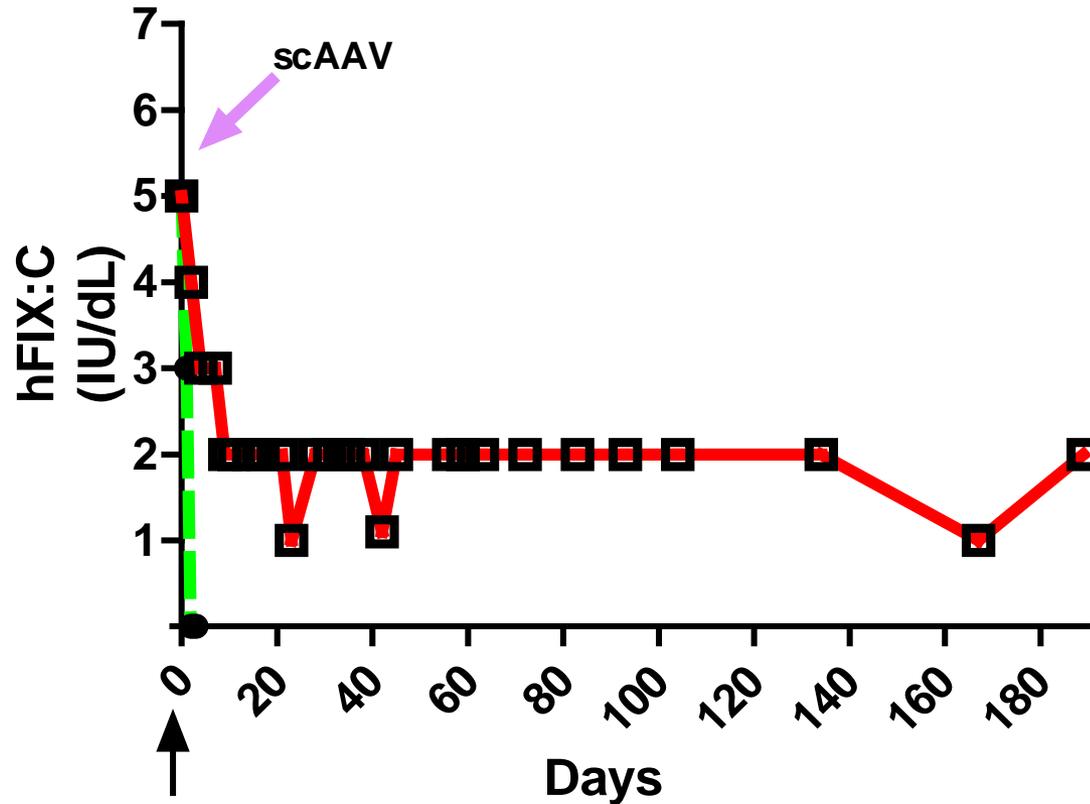
Figure 4B: Dose finding study in rhesus. At least three macaques were treated in each dose level. Note that expression is reported using a log scale. Also shown are the standard errors for expression at each time point.

Initial Clinical Dose = 400 x Minimal effective Dose in $PPCA^{-/-}$ mice

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First “Low-dose” Patient

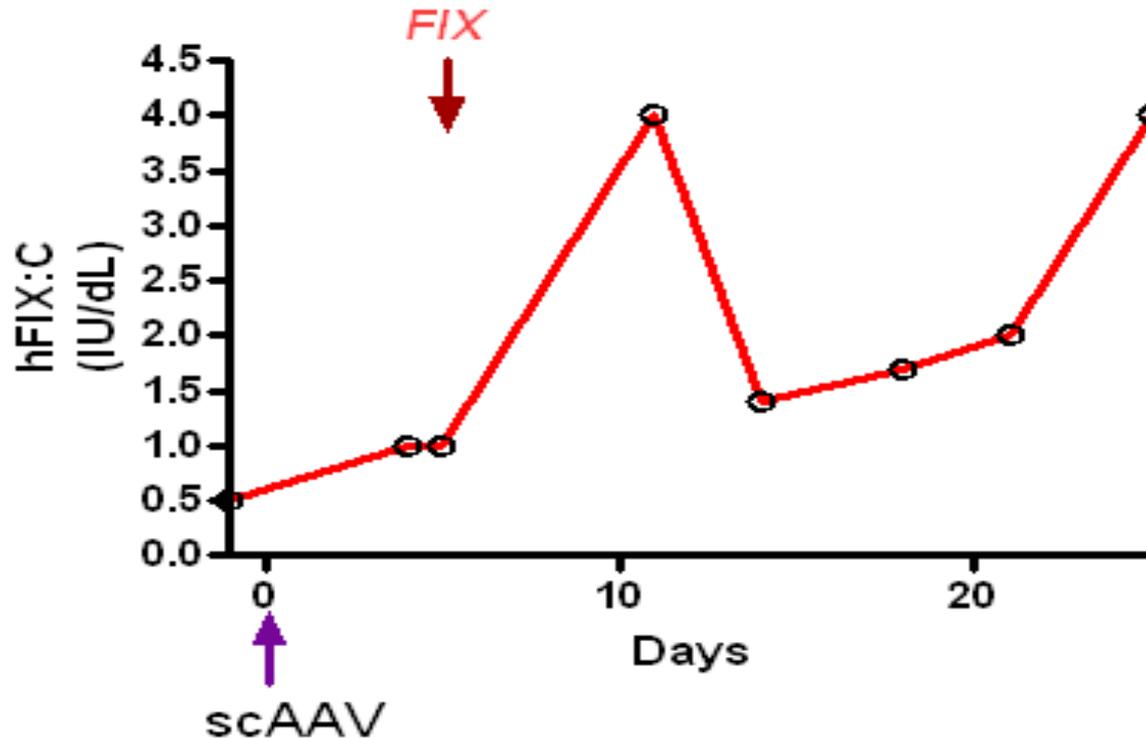
2×10^{10} vg/kg



- Predicted decay of rhFIX
- Actual plasma hFIX level

Second “Intermediate-dose” Participant

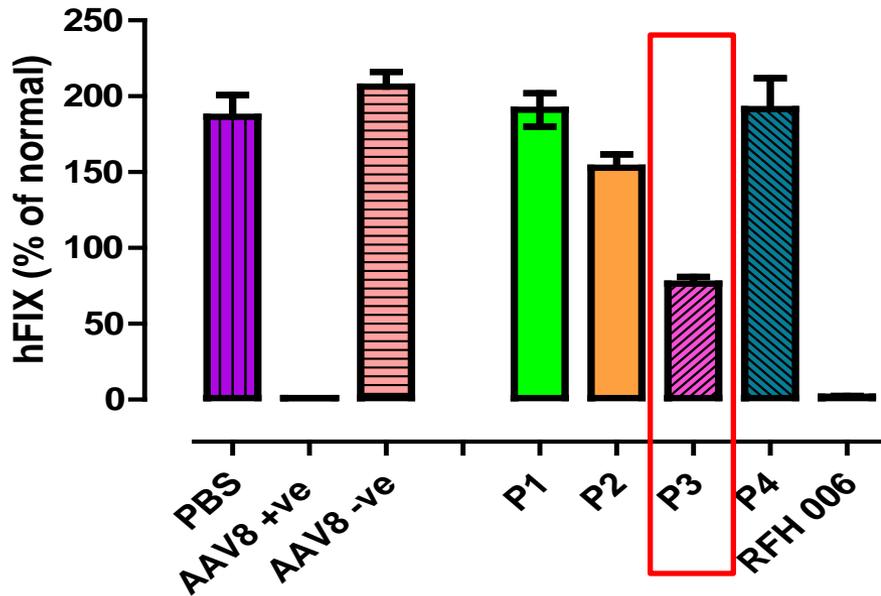
6×10^{10} vg/kg



Reason for Failure in Patient 3

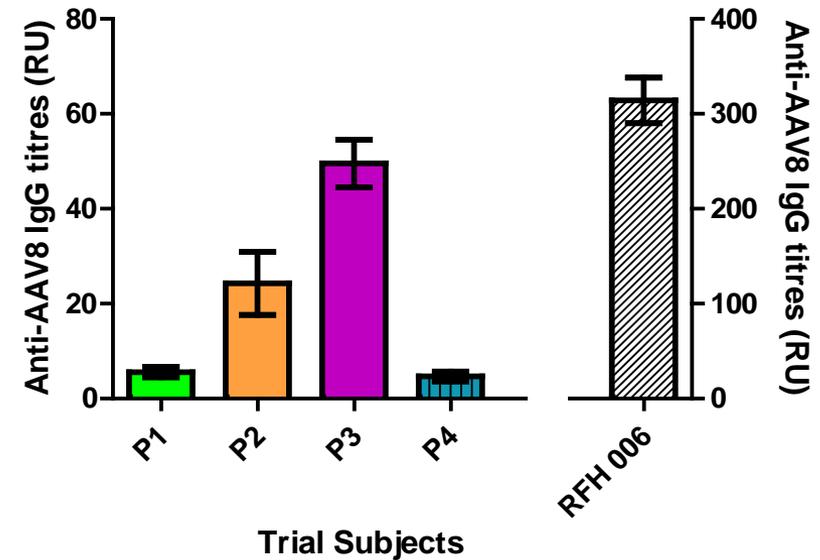
Several potential causes considered

In-vivo transduction inhibition assay



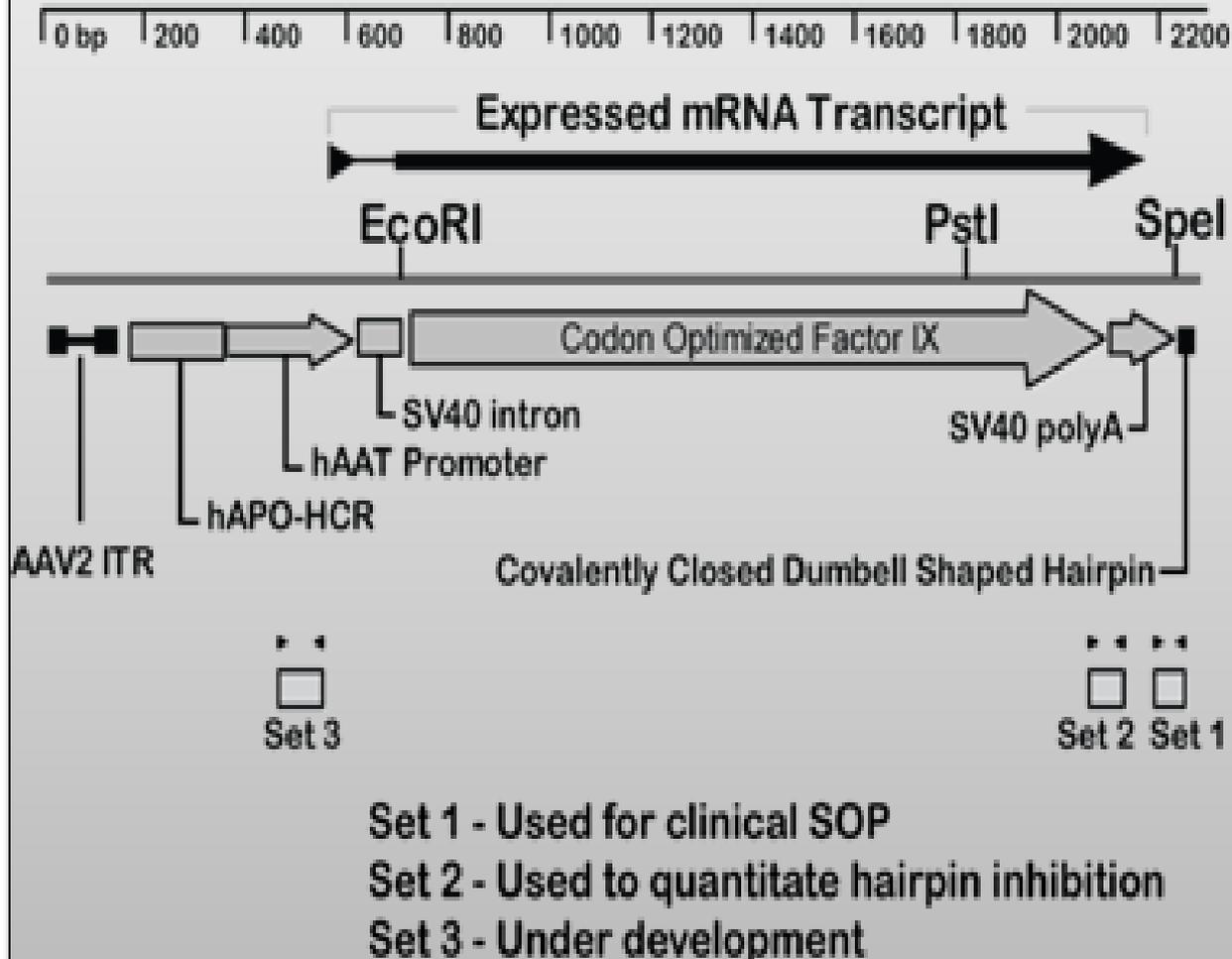
?P3 had relatively higher level of antibodies to AAV8 pre-treatment

More sensitive immunocapture assay using AAV8 specific peptide



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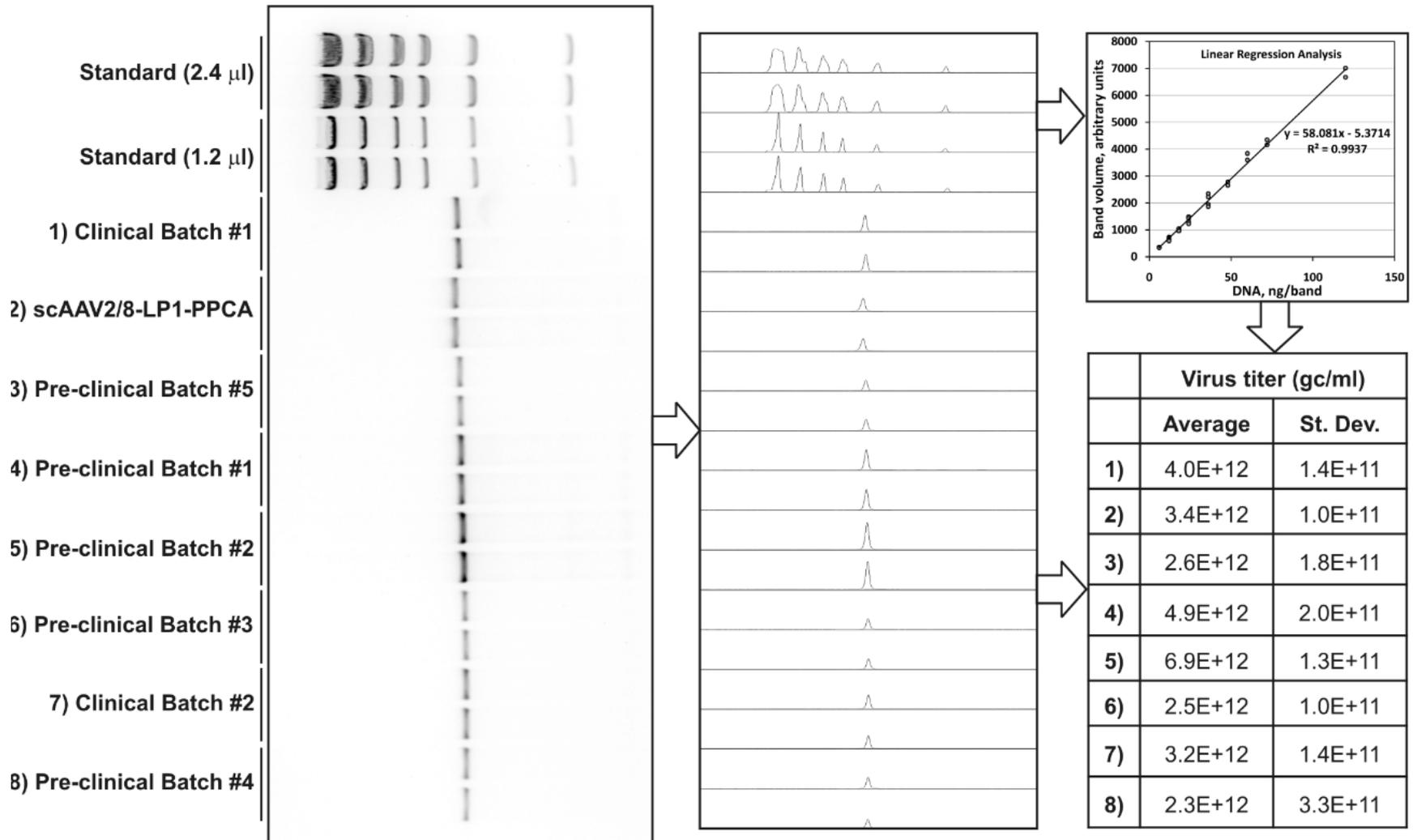
scAAV-LP1-hFIXco Genome with Primer Sets



Gel analysis of Standard and unknown samples

Analysis of band intensity and peak integration

Linear regression analysis and titer calculation



DOSE EQUIVALENCE BASED ON DIFFERENT TITERING METHODS

	qPCR	Gel Method	Clinical Dose
	Vector Genomes/ml		Vector Genomes/kg
<u>FIX Clinical Lot</u>	4.1×10^{11} (x 9.8)	4.0×10^{12}	2×10^{12} (highest dose)
<u>PPCA Dose Finding Lot</u>	1.3×10^{12} (x 2.6)	3.4×10^{12}	1.0×10^{12} (initial dose)*

* Equivalent to 2.6×10^9 vg/mouse by gel titer

•Age Range of Subjects

- Will do patient survey to better define demographics and disease characteristics
- Will treat older individuals first

•45CFR46, Subpart D “...greater than minimal risk with potential for benefit.”

- Reduction in storage material is potential benefit
- Only other treatment is supportive care

•Comments Regarding Consent Form

- Addressed in content of St. Jude IRB policies

•Toxicity Efficacy and Biodistribution

- FIX vector in rhesus macques
- Further study planned in PPCA^{-/-} mice

Questions Raised by RAC Reviewers

Biodistribution In Rhesus Macaques – 2×10^{13} vg/kg



<u>Organ</u>	<u>Copy Number</u>	<u>Organ</u>	<u>Copy Number</u>
•Liver-left	35.700	•Optic nerve	0.022
•Liver-right*	23.005	•Kidney*	0.023
•Liver-caudate	25.804	•Kidney	0.020
•Adrenal	0.331	•Skeletal Muscle	0.019
•Aorta	0.200	•Thyroid	0.019
•Heart	0.137	•Epididymis	0.017
•Spleen*	0.321	•Trachea	0.015
•Spleen	0.085	•Stomach	0.014
•Inguinal lymph node	0.074	•Rectum	0.014
•Bone Marrow	0.069	•Pituitary	0.009
•Gallbladder	0.064	•Testes*	0.004
•Urinary bladder	0.053	•Testes	0.008
•Skin	0.052	•Ileum	0.008
•Mesenteric lymph node	0.049	•Jejunum	0.008
•Cecum	0.045	•Prostate	0.008
•Sciatic nerve	0.043	•Submandibular salivary gland	0.007
•Lung	0.043	•Brain stem	0.007
•Esophagus	0.037	•Pancreas	0.006
•Tongue	0.035	•Spinal cord	0.006
•Parathyroid	0.032	•Seminal vesicle	0.004
•Thymus	0.031	•Cerebrum	0.003
•Submandibular lymph node	0.029	•Cerebellum	0.001
•Colon	0.026		
•Duodenum	0.022	• * Week 1 biopsy	

•Cross Reactivity of Serotype Specific Neutralizing Antibodies

- Rhesus macaques

- Clinical Trial

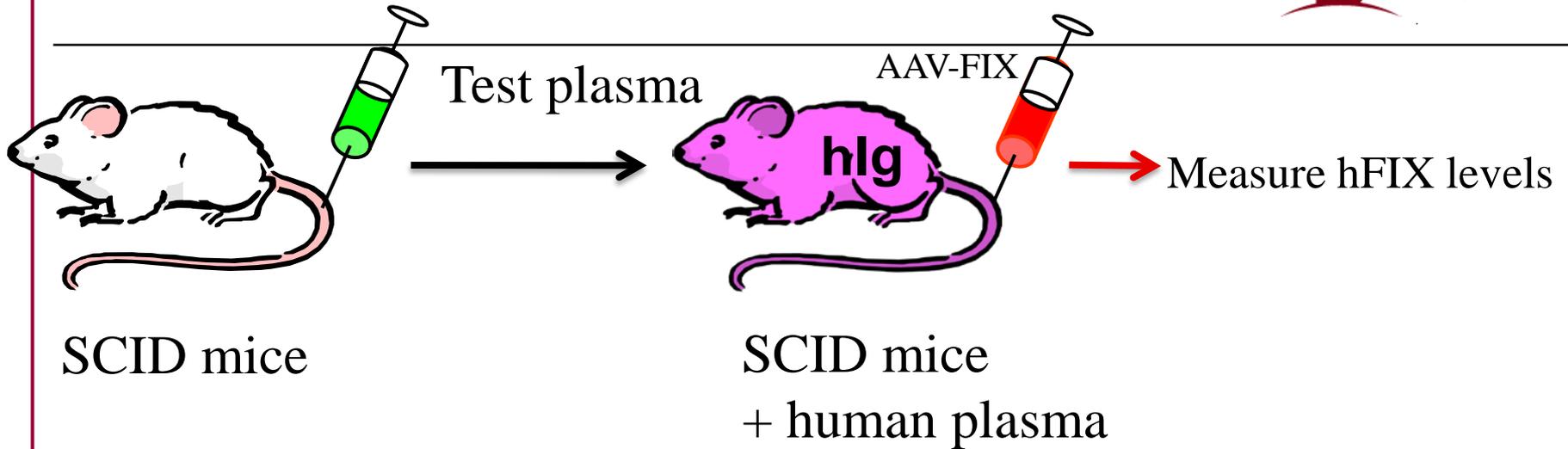
Administration of Alternative Serotypes

Design: 1×10^{12} vg/kg scAAV2/5 → macaques pre-existing immunity to AAV-8

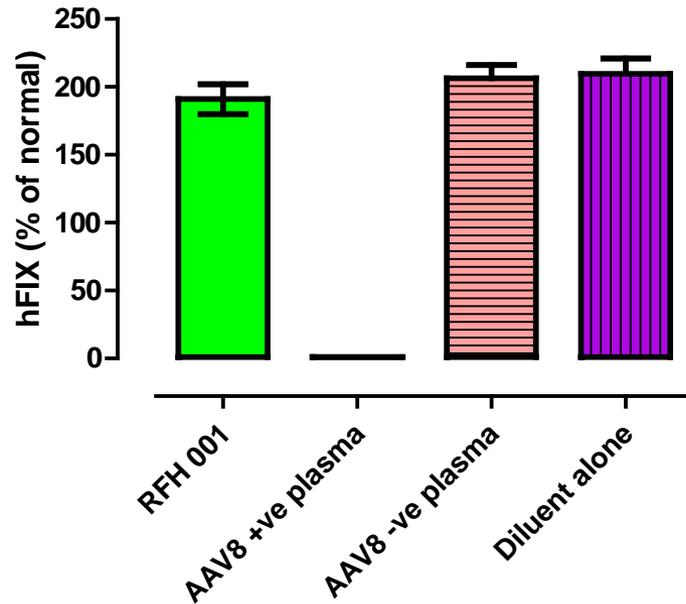
Monkey	Primary Exposure	Anti-AAV8 titres	Secondary vector	Steady state hFIX antigen level (% of normal)	Duration of expression (days)
M8-sc	wt AAV8	1/677	scAAV2/5	11.1	>311
M9-sc	wt AAV8	1/833		12	
M3-sc	rAAV2/8	1/1752		25	
328	wt AAV8	1/1428		9	
M4-sc	wt AAV8	1/714		21	

- Can circumvent natural immunity in nonhuman primates

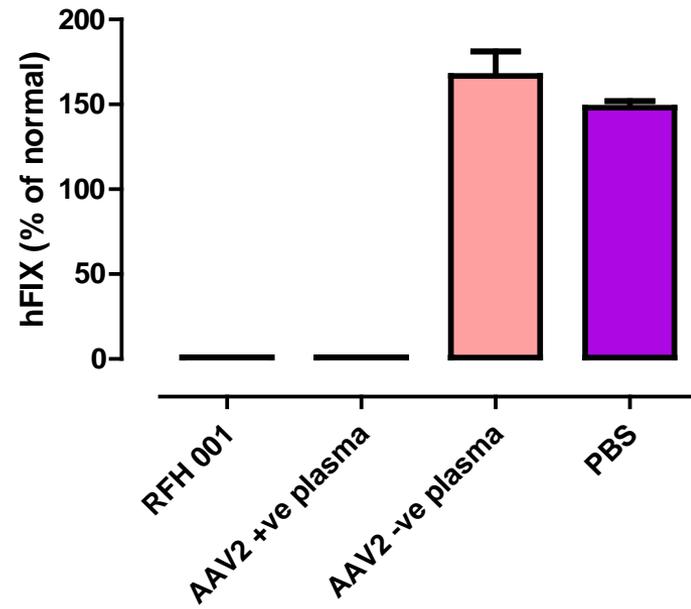
Mouse Screening Assay for AntiAAV AB



Neutralising antibodies to AAV8



Neutralising antibodies to AAV2



•Risk of Germline Transmission

- Not seen in animal models

•Why Liver Specific Expression

- Documented long term expression with liver specific enhancer/promoter
- Avoid expression in antigen presenting cells

• AAV and Liver Tumors In Mice

- Evidence suggesting insertional mutagenesis not reproduced in larger studies.

• Infusion Rate and Early Toxicity

- Well tolerated in adults
- Will monitor pro-inflammatory cytokines

QUESTIONS