

Recombinant DNA Advisory Committee

Bethesda, Maryland

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***An Open Label Dose Escalation Study of a Self
Complementary Adeno-Associated Viral Vector
(scAAV2/8LP1hFIXco) for Gene Transfer in
Subjects with Hemophilia B***

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Hemophilia B

Severe bleeding disorder

- deficiency of clotting factor IX (FIX)
- affects 1 in 30,000 males
 - ~5,000 patients in the US; ~100,000 worldwide
- frequent, spontaneous hemorrhage that can be lethal, especially when occurring in the CNS

Rationale for gene therapy

- long-term expression/delivery of a single protein is required
- moderate increases in FIX levels (>1%) impact symptoms
- tight regulation of FIX expression is not required

Gene therapy for hemophilia B

Adeno-associated virus (AAV)

AAV is a promising vector for FIX gene delivery

- does not cause disease in humans
- requires a helper virus to replicate
- can transduce non-dividing cells
- less likely to stimulate an immune response to transduced cells than other vectors since no viral proteins are expressed
- **facilitates long-term expression of the transgene**

Gene therapy for hemophilia B

Unique aspects of trial design

Vector capsid: serotype 8

- Lower sero-prevalence of wild type AAV8 in humans
- Rapid uncoating of AAV8 capsid
- Lack of transduction of antigen presenting cells

Vector genome: self complementary construct

- Rapid formation of stable, active dsDNA

cDNA: codon optimized FIX

- Improved efficiency of translation

Route of administration: peripheral vein

- Simplify vector delivery
- Avoid invasive procedures

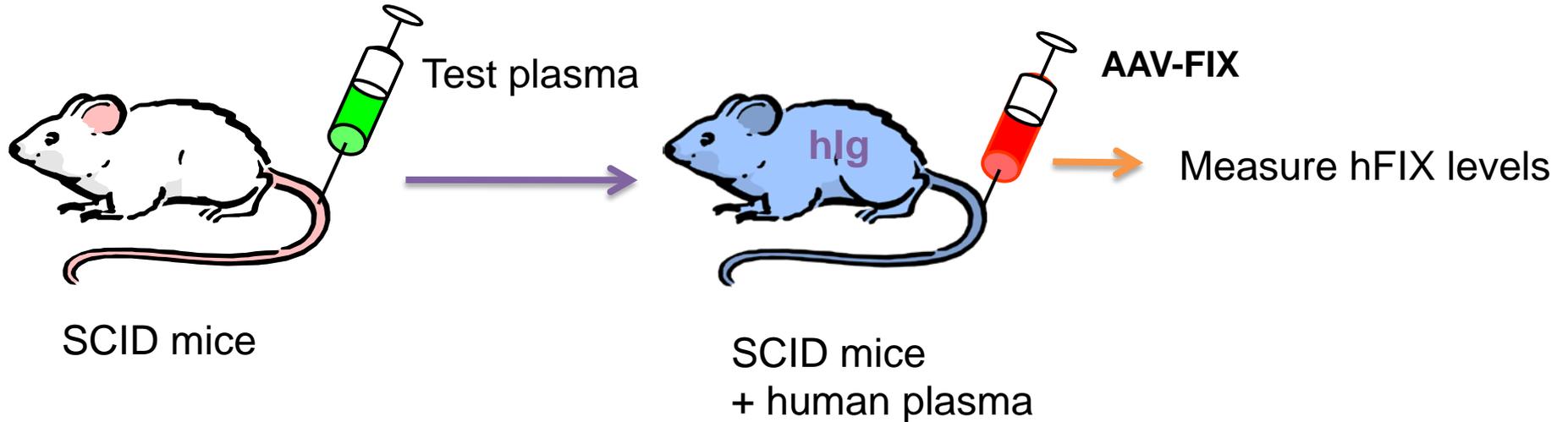
Gene therapy for hemophilia B

Enrollment

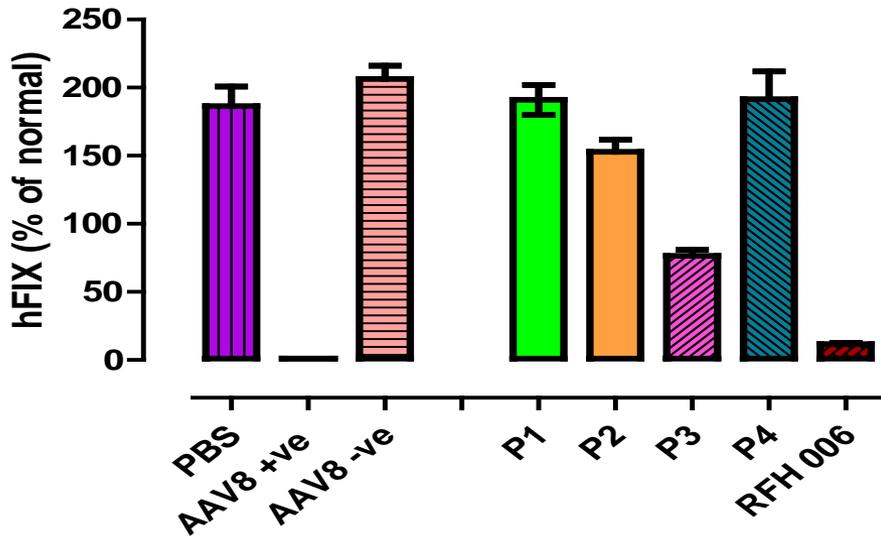
Key entry criteria:

- Severe hemophilia B (<1% of normal) resulting from a missense or null mutation not associated with FIX inhibitory antibodies
- No evidence of active infection with hepatitis B or C virus, or currently on antiviral therapy
- **No evidence of pre-existing immunity to AAV8**

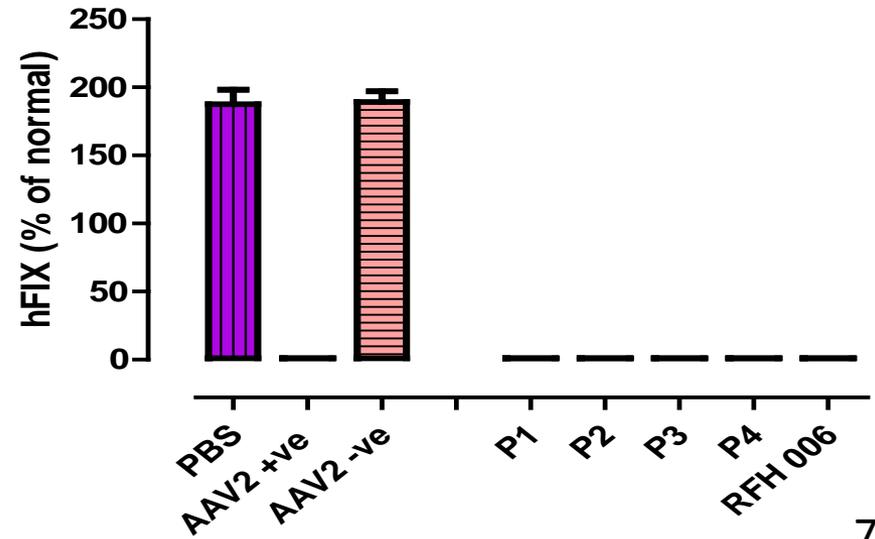
Mouse screening assay for anti-AAV ab



Neutralising antibodies to AAV8



Neutralising antibodies to AAV2 (10 fold higher dose)



Gene therapy for hemophilia B

Enrollment

- Six subjects have received vector since the trial opened last year (2010)
 - Two @ low dose
 - 2×10^{11} vp/kg
 - Two @ intermediate dose
 - 6×10^{11} vp/kg
 - Two @ high dose
 - 2×10^{12} vp/kg

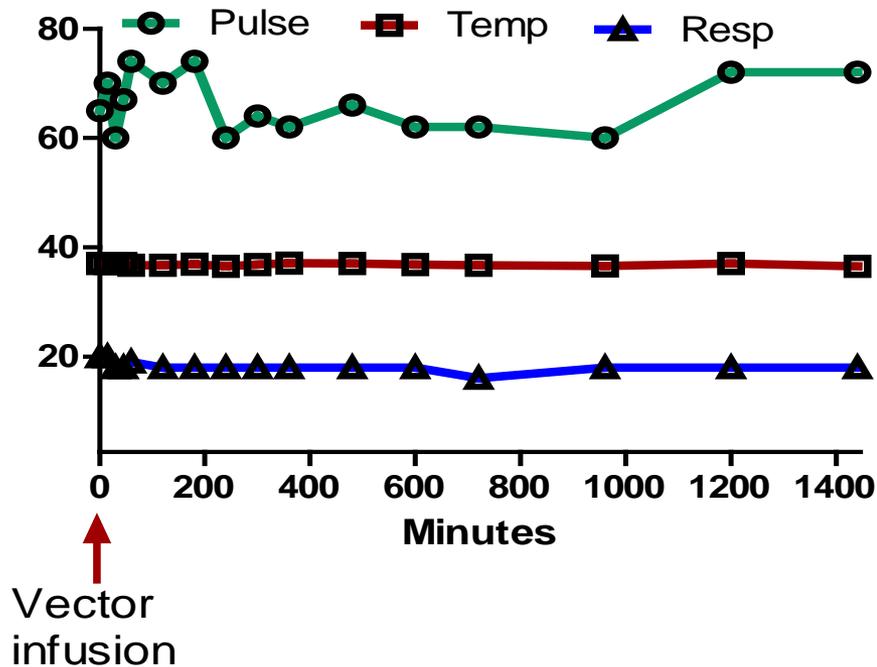
Patient demographics

Patients	1	2	3	4	5	6
Dose category	Low		Intermediate		High dose	
Age	31	64	43	29	32	27
Base line FIX level	<1%	<1%	<1%	<1%	<1%	<1%
Mutation	Missense	Null (frame shift)	Missense	Missense	Missense	Promoter
Prophylaxis	2x/week	3x/week	2x/week	Targeted	2x/week	3x/week
Spontaneous bleeds/year	3-4	6-8	6-8	4-6	3-4	1-2
Target joints	Ankles, Knees	Hips, Ankles, Knees, Shoulders, Elbows	Elbow, Hips, Knees, Ankle	Ankles, Hip	Knees shoulders and wrist	Knees

Peripheral vein infusion of vector

Well-tolerated

Vital signs during and immediately post infusion



AAV shedding in body fluids/excretions

Cleared within 16 days

Low

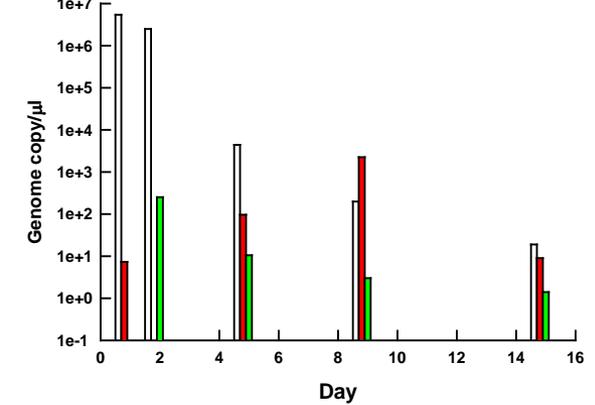
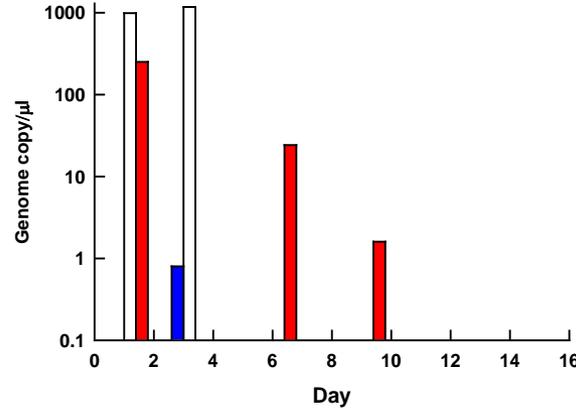
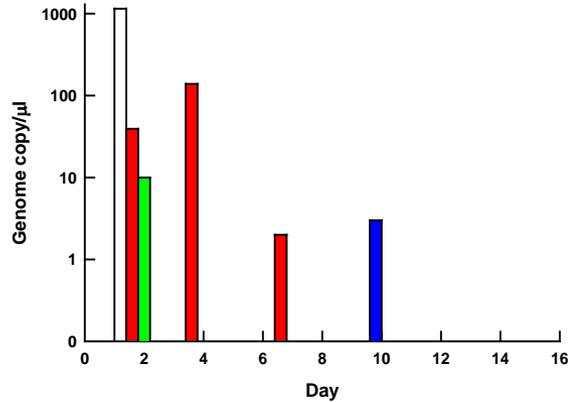
Intermediate

High

P1

P3

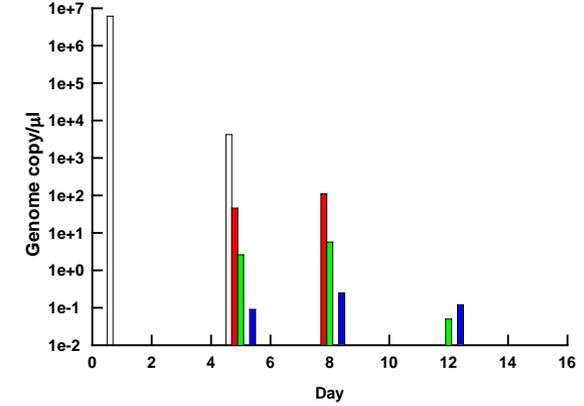
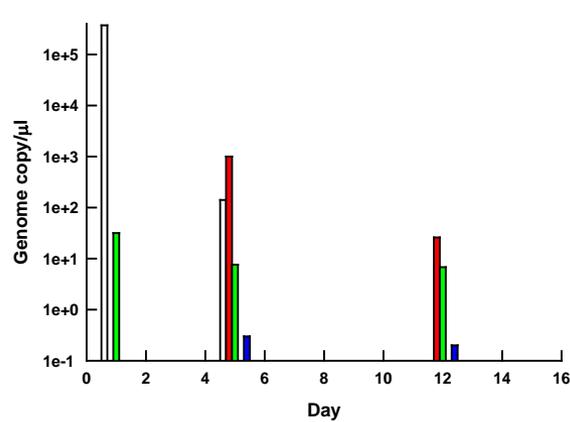
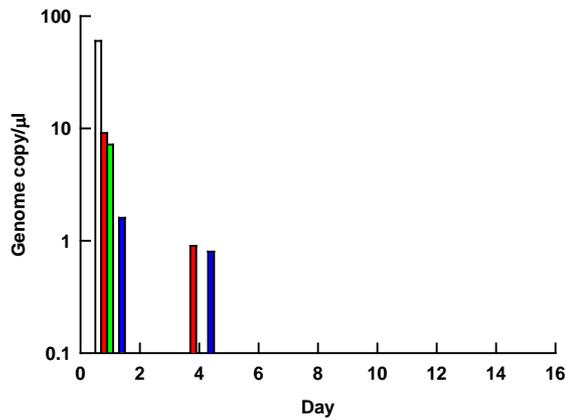
P5



P2

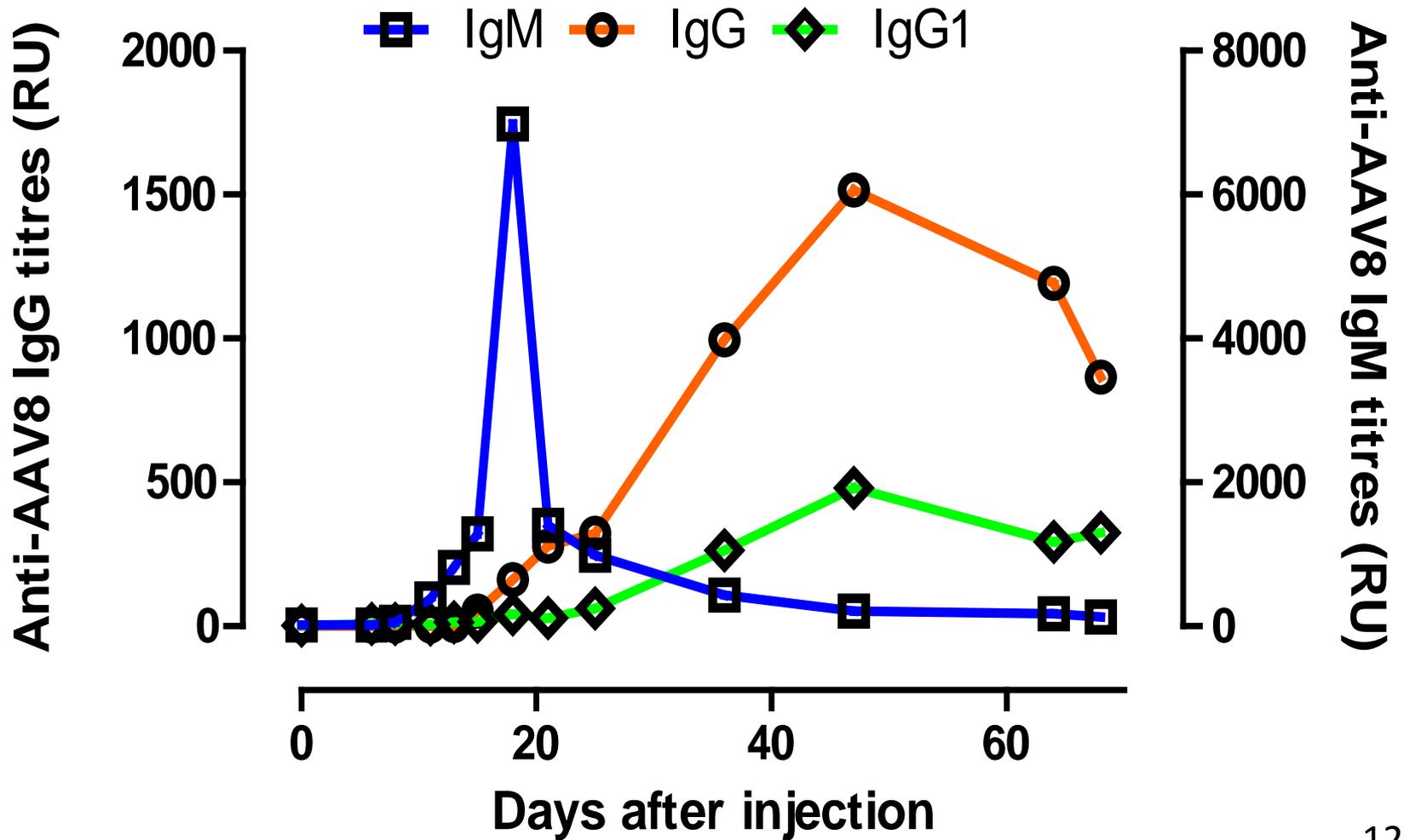
P4

P6



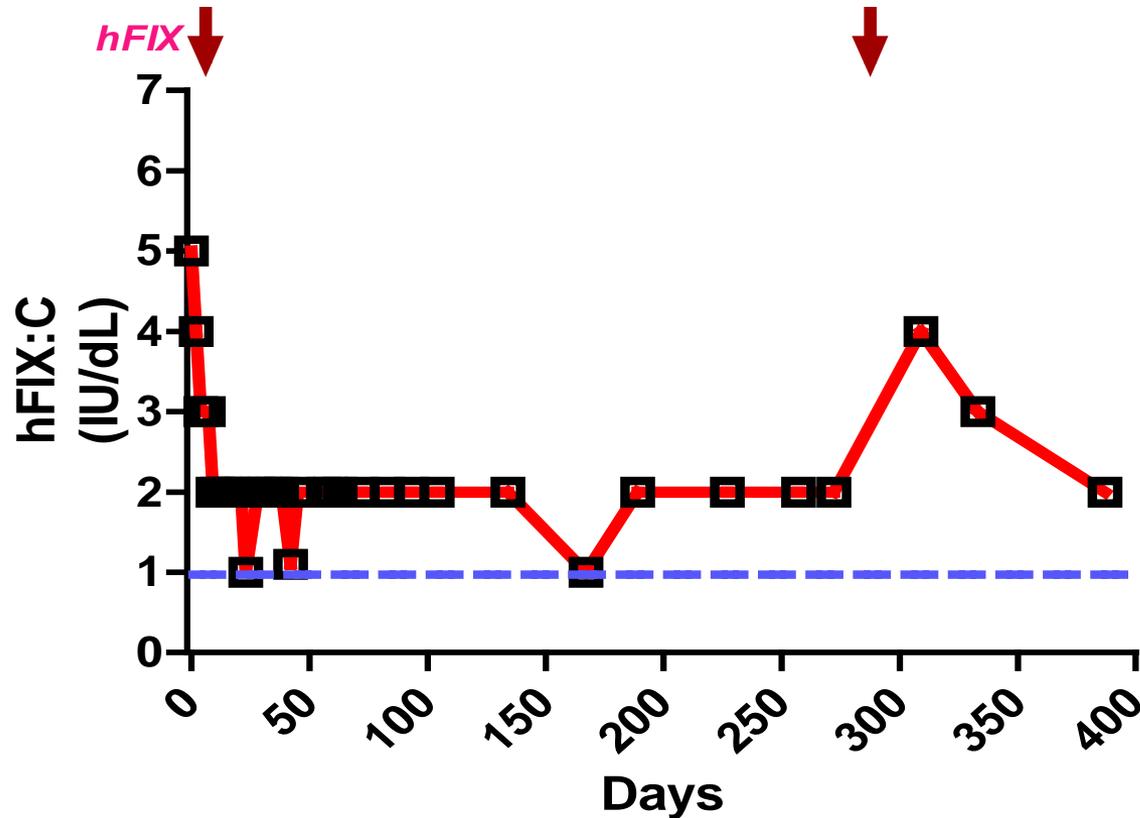
■ Plasma ■ Stools ■ Saliva ■ Urine ■ Semen

Humoral response to AAV8



Plasma FIX levels

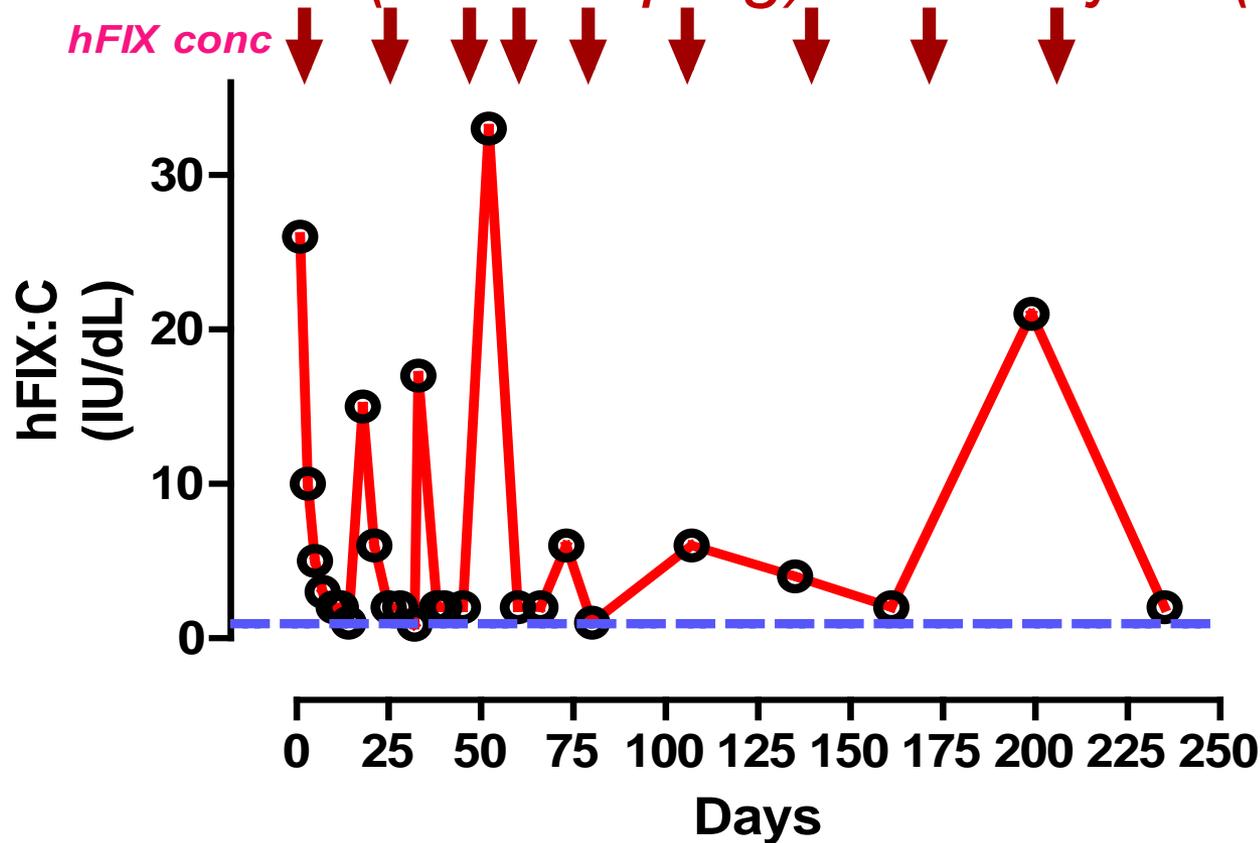
Low vector dose (2×10^{11} vp/kg): 1st subject (P1)



- Off prophylaxis >12 months → FIX level @ 1-2%
 - except prior to a minor surgical procedure
- No spontaneous bleeding episodes
 - received vector 14 months ago

Plasma FIX levels

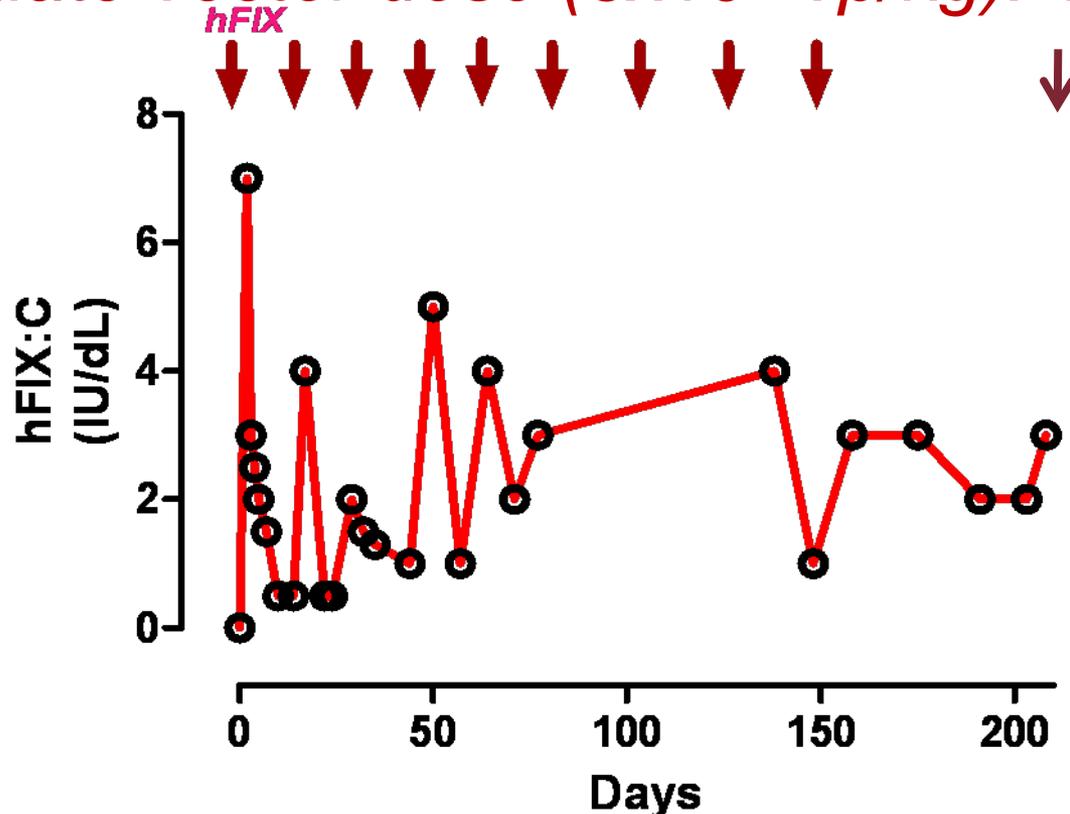
Low vector dose (2×10^{11} vp/kg): 2nd subject (P2)



- Has remained on prophylaxis due to the severity of joint disease
 - frequency has decreased from 2-3x/week to once every 10 days
 - FIX level was 2% recently, 3 weeks off exogenous FIX
 - Received vector 9.5 months ago

Plasma FIX levels

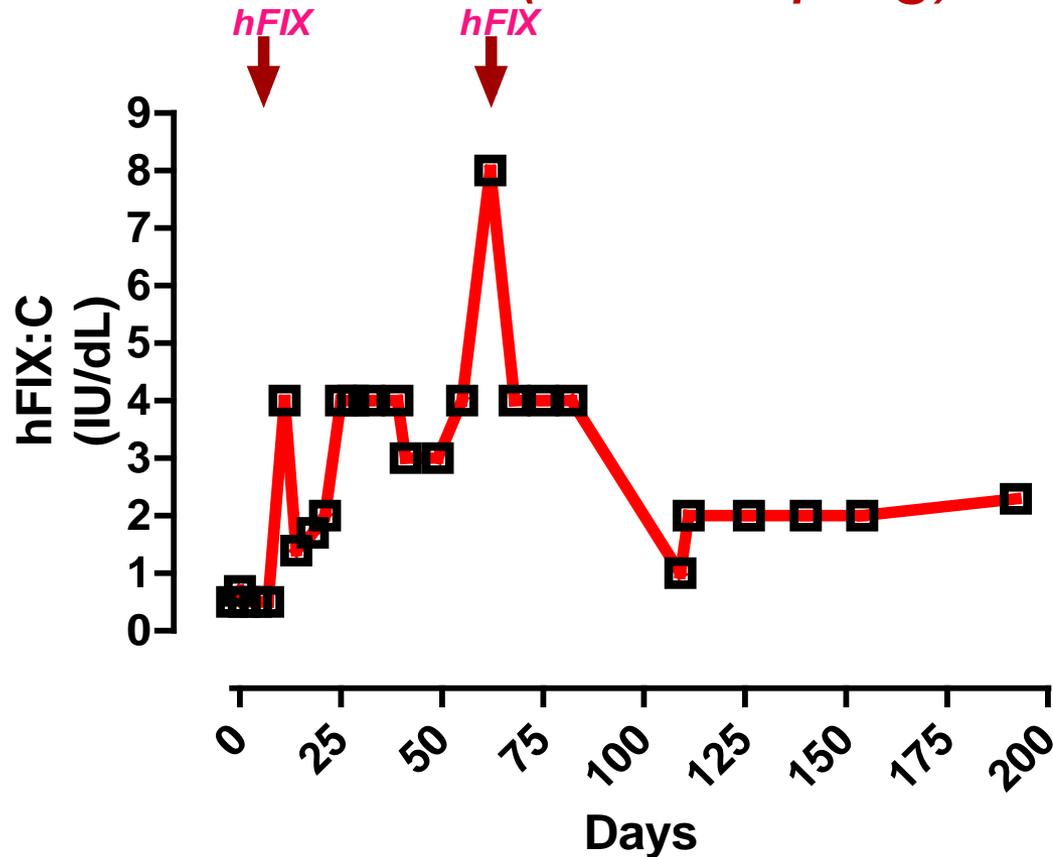
Intermediate vector dose (6×10^{11} vp/kg): 1st subject (P3)



- Off prophylaxis for >2 months → FIX level @ 2-3%
- No spontaneous hemorrhage during that time
 - Resumed prophylaxis recently
 - Received vector 8 months ago

Plasma FIX levels

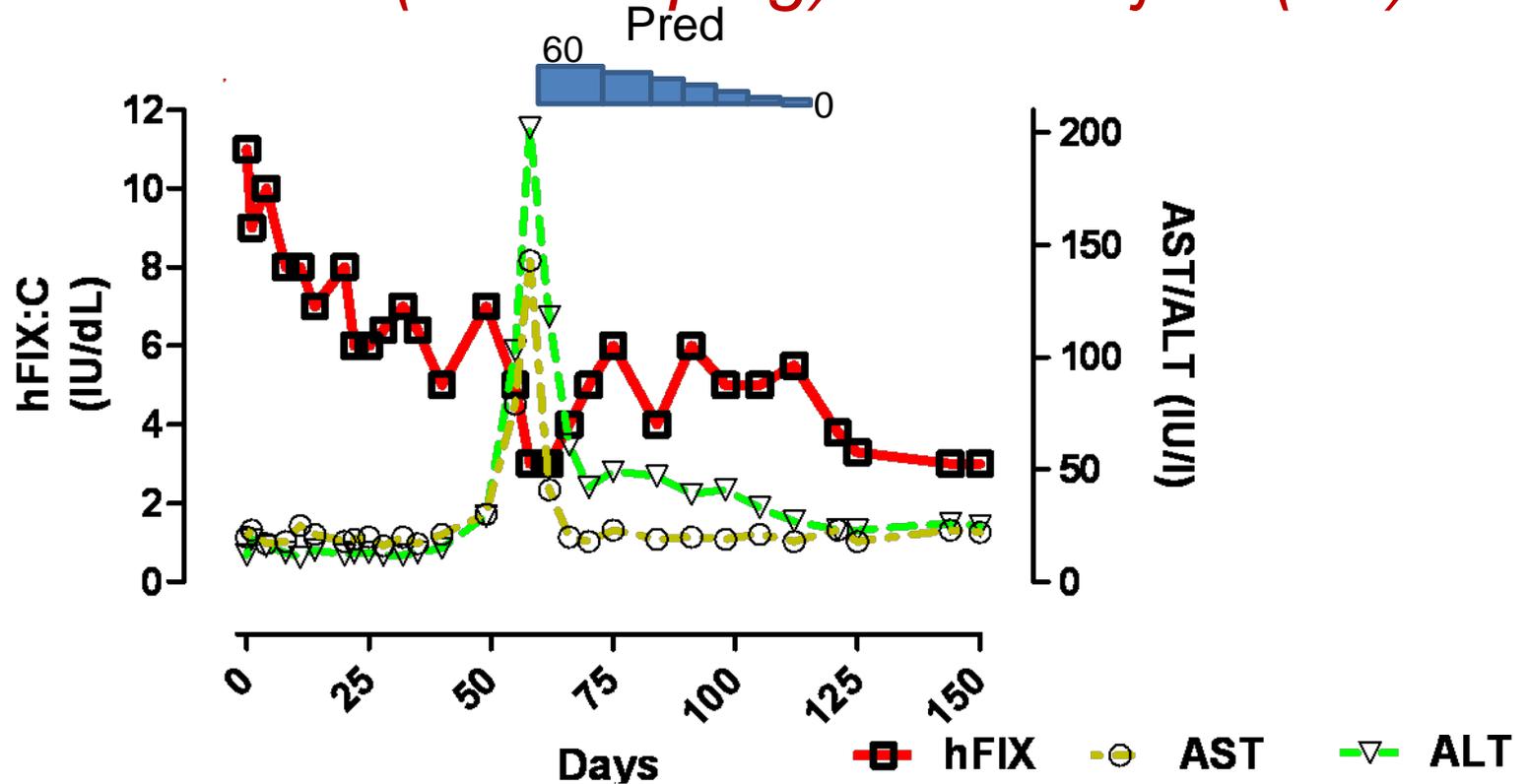
Intermediate vector dose (6×10^{11} vp/kg): 2nd subject (P4)



- Off prophylaxis for >5 months → FIX level @ 2-3%
- No spontaneous hemorrhage during that time
 - Received vector 6.5 months ago

Plasma FIX levels

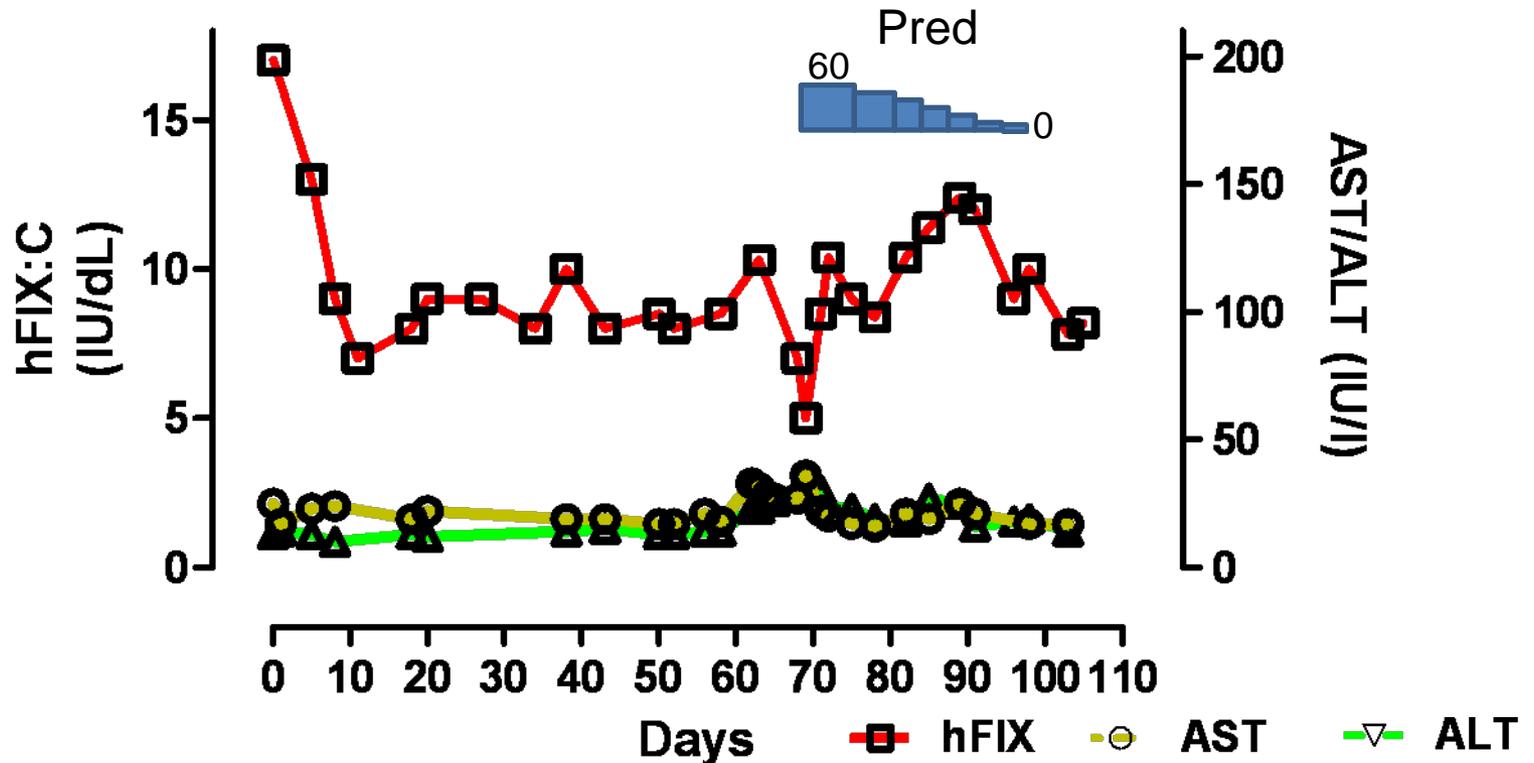
High vector dose (2×10^{12} vp/kg) – 1st subject (P5)



- FIX @ 6-8% at week 8 post-vector infusion
- Asymptomatic transaminitis (10-fold increase), ↓ FIX level
- Prednisolone 60mg/day started and tapered off over 8 weeks
- Currently 4 weeks off steroids
 - LFT's back to baseline, FIX level @ 3%
 - Received vector 5 months ago

Plasma FIX levels

High vector dose (2×10^{12} vp/kg) – 2nd subject (P6)



- FIX @ 8-11% at week 9 post-vector infusion
- Asymptomatic mild transaminitis (1.5-fold increase), ↓ FIX level
- Prednisolone 60mg/day started and tapered off over 4 weeks
- Currently 3 weeks off steroids
 - LFT's back to baseline, FIX level @ 8.5%
 - Received vector 4 months ago

Summary

6 subjects received AAV: 2 at each of 3 dose levels

- Peripheral vein administration of scAAV2/8-LP1-hFIXco was well tolerated without any acute side-effects
- **Persistent, stable FIX expression in all 6 subjects (1-8%)**
 - 4/6 able to stop prophylaxis
 - 2/6 able to extend the interval between prophylaxis
 - P1: 1-2% for >1 year
 - P6: 8% for >4 months
- Vector present in excretions/secretions
 - Cleared by two weeks
- Preliminary Results Are Encouraging
- Protocol Amendment in Progress to Expand High Dose Cohort.

Aim: to monitor T cell responses in the context of an AAV8-scF.IX study

- **Study sponsored by St. Jude Children's Research Hospital and University College London**
- **Uses a more efficient vector that can achieve therapeutic levels of transgene expression at lower vector doses**
 - **Codon-optimized transgene**
 - **Self-complementary vector**
 - **AAV serotype 8 vector, with high liver tropism, which was described as potentially less immunogenic than AAV-2**
(Vandenberghe et al., Nat Med 2006)
- **Vector administered i.v. at the following doses:**
 - **2×10^{11} vg/kg**
 - **6×10^{11} vg/kg**
 - **2×10^{12} vg/kg**

Monitoring of T cell responses in PBMC from AAV8-F.IX infused subjects by IFN-g ELISpot and polyfunctional T cell assay

3 antigens tested:

1. AAV8 capsid VP1 protein

Pool 1		Pool 2		Pool 3		Pool 4		Pool 5		Pool 6	
1	2	3	4	5	6	7	8	9	10	11	12
13	14	15	16	17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32	33	34	35	36
37	38	39	40	41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70	71	72
73	74	75	76	77	78	79	80	81	82	83	84
85	86	87	88	89	90	91	92	93	94	95	96
97	98	99	100	101	102	103	104	105	106	107	108
109	110	111	112	113	114	115	116	117	118	119	120
121	122	123	124	125	126	127	128	129	130	131	132
133	134	135	136	137	138	139	140	141	142	143	144
145	146										

2. hF.IX transgene product

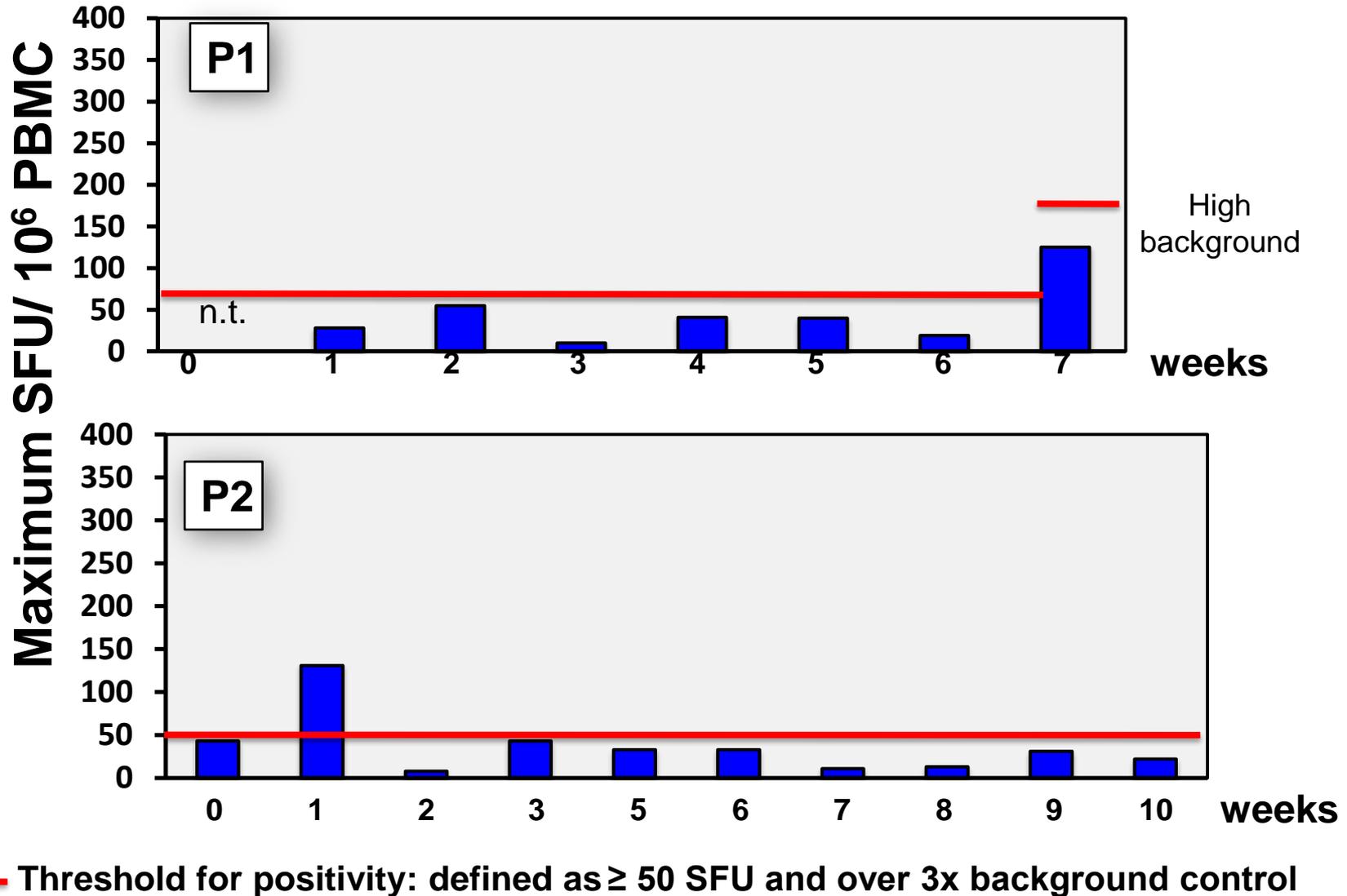
Pool 1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Pool 2	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Pool 3	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
	76	77	78	79	80	81	82	83	84	76	77	78	79	80	81
	82	83	84	85	86	87	88	89	90	91					

3. F.IX transgene Alternative Open Reading Frame (ARF) (Li et al., PNAS 2009;106(26):10770-4)

15mers
overlapping
by 10
amino acids

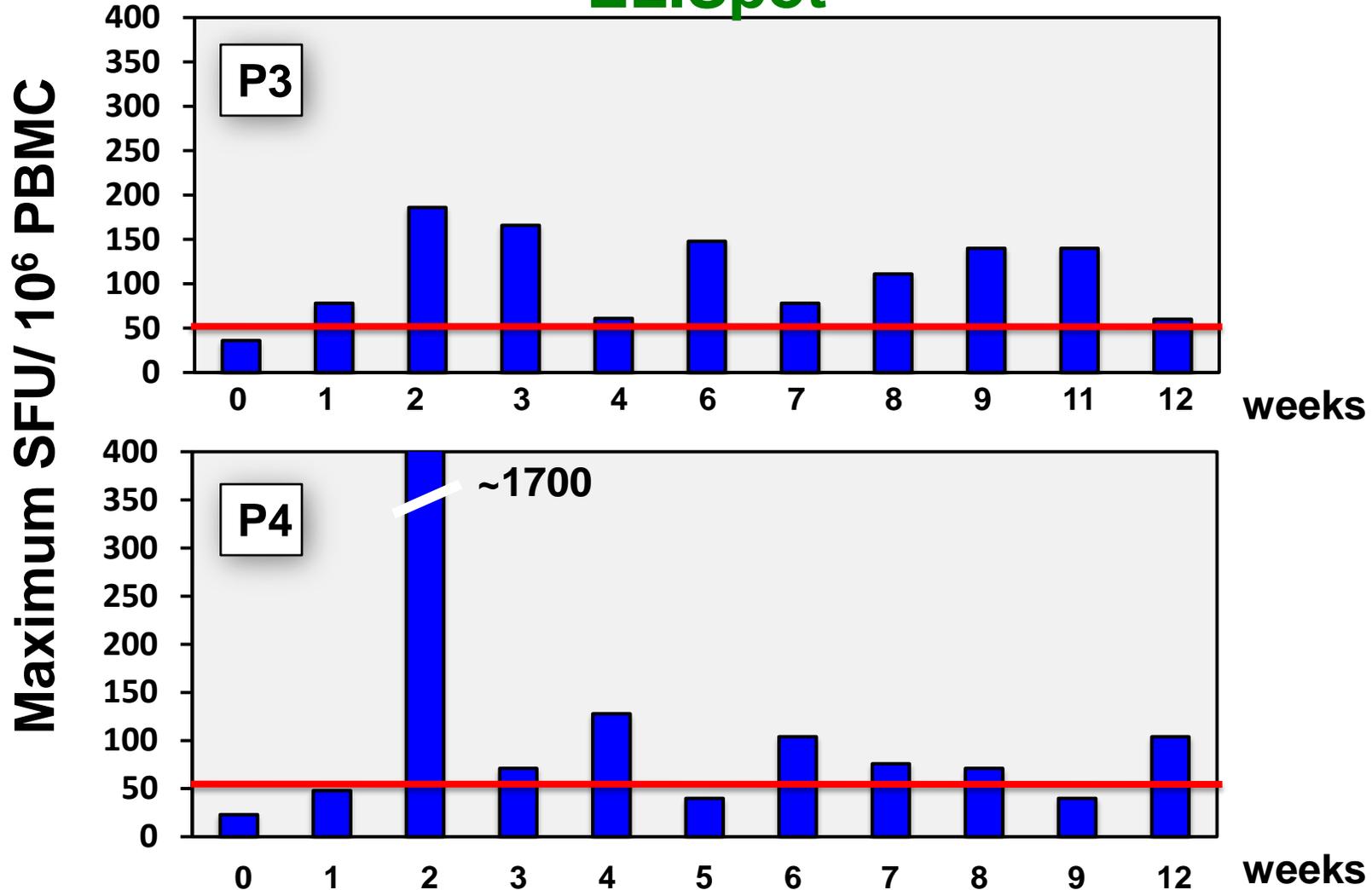
	Name	Sequences
Pool 1	FIXARF_D #2	WWGGRMPSLASSPGKWC
	FIXARF_E #2	STTMTLPCWSWMSPWC
	FIXARF_G #1	MRGAGTAARGTLGAPM
	FIXARF_A#1	MGAAARMTSTAMSAG
	FIXARF_A#2	RMTSTAMSAGAPLAL
Pool 2	FIXARF_A#3	AMSAGAPLALRARTV
	FIXARF_A#4	AGAPLALRARTVSWM
	FIXARF_A#5	AGAPLALRARTVSWM
	FIXARF_B#1	MADVSSSARTLLTTR
	FIXARF_B#2	SSARTLLTRWCAAA
	FIXARF_B#3	LLTRWCAALRATG
Pool 3	FIXARF_B#4	WCAALRATGWLRTR
	FIXARF_B#5	LRATGWLRTRRAVSL
	FIXARF_B#6	WLRTRRAVSLCHSH
	FIXARF_B#7	RAVSLCHSHVAECL
	FIXARF_C#1	MWQSVCEPDQQADQG
	FIXARF_D#1	MTSPGWVGGRMPSLA
	FIXARF_E#1	MLPSTTMTLPCWS
FIXARF_F#1	MCLAGAGCSTRAGLP	
FIXARF_F#2	AGCSTRAGLPWCCST	

AAV8-F.IX vector doses of 2×10^{11} vg/kg do not result in detectable capsid T cell responses in PBMC



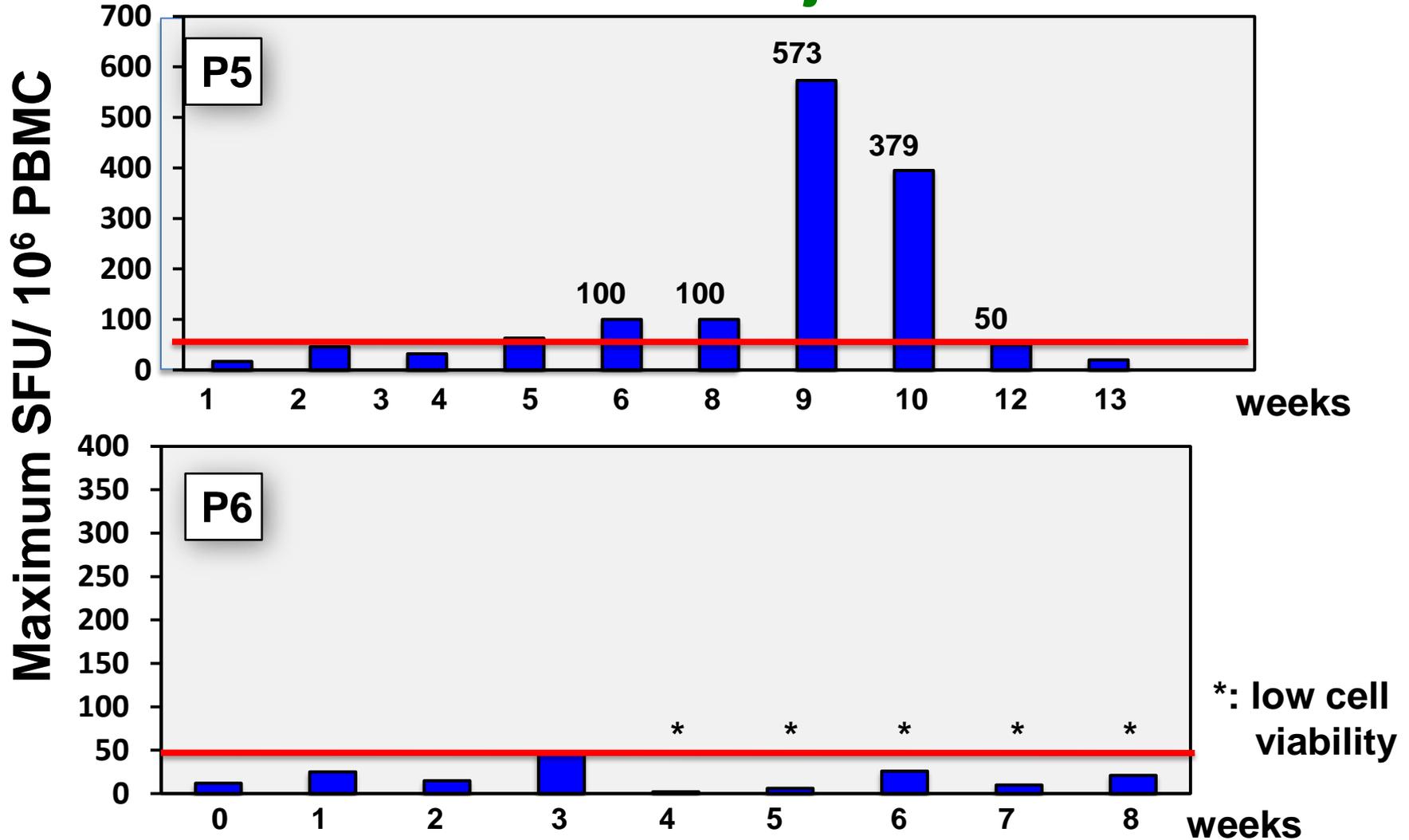
Dose escalation to 6×10^{11} vg/kg triggers capsid T cell activation/expansion in PBMC detected by IFN- γ ELISpot

ELISpot



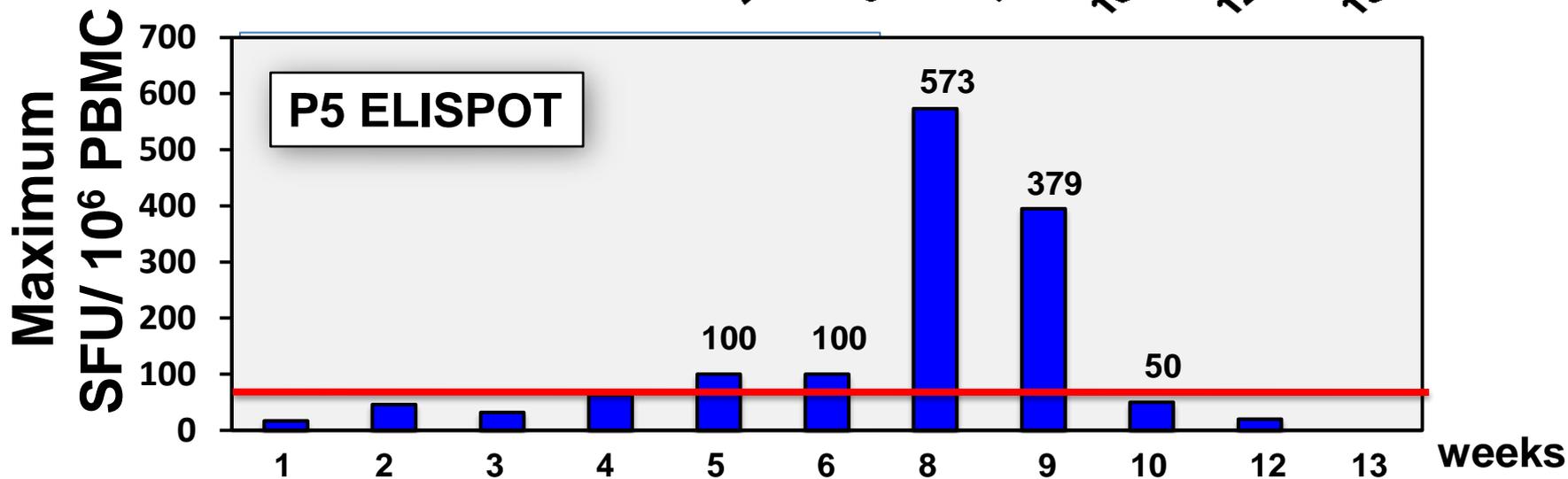
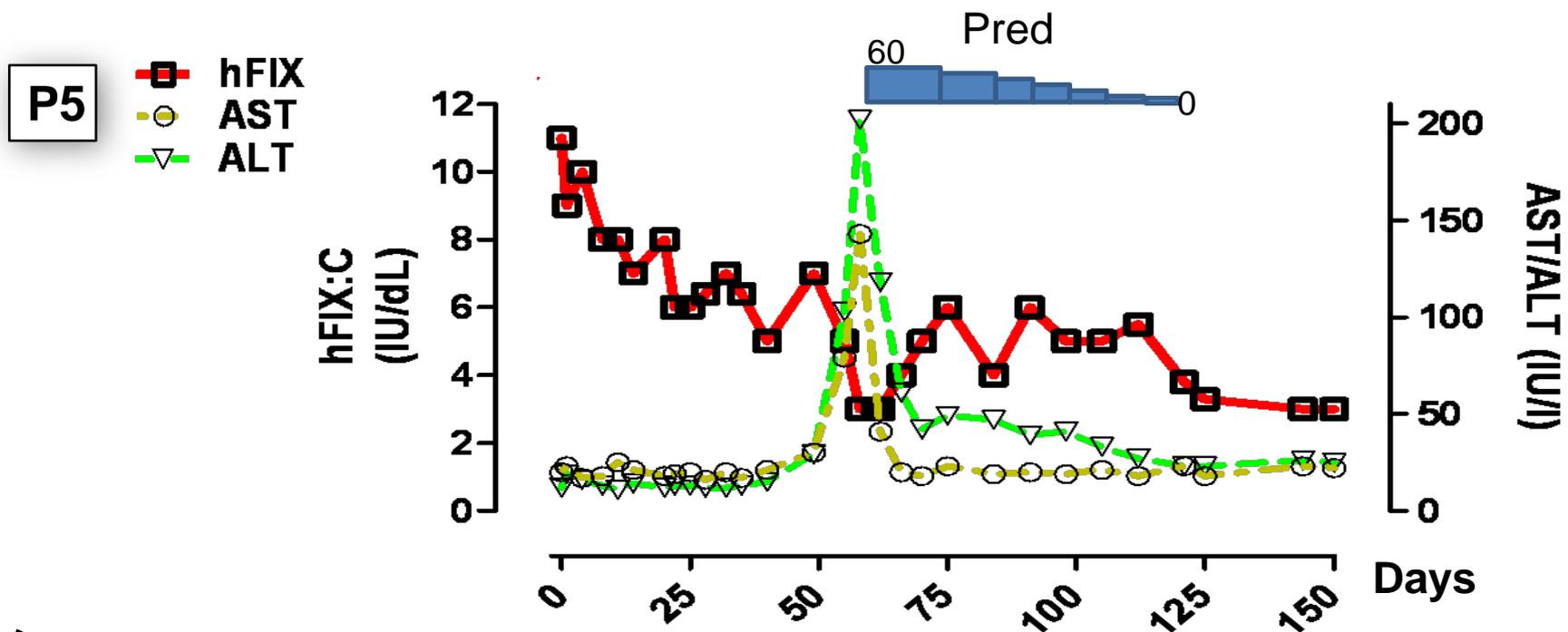
— Threshold for positivity: defined as ≥ 50 SFU and over 3x background control

Further dose escalation to 2×10^{12} vg/kg results in capsid T cell expansion and liver enzyme elevation in 1 of 2 subjects



— Threshold for positivity: defined as ≥ 50 SFU and over 3x background control

Capsid T cell activation was synchronous with liver enzyme elevation



Summary of results

AAV8-scF.IX trial

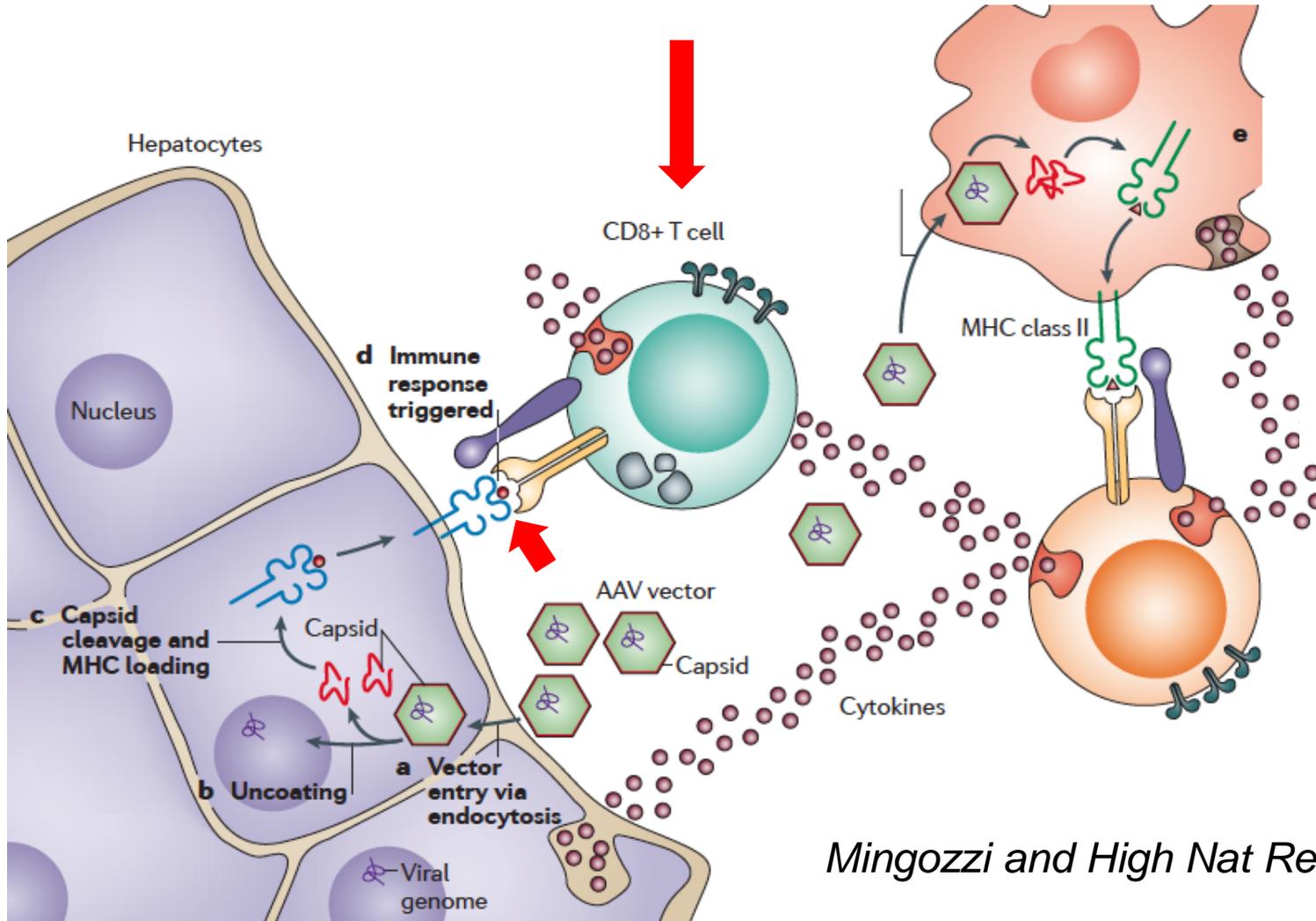
Vector dose	T cell response	Clinical sequelae (□AST/ALT)
2×10^{11} vg/kg	No	0/2
6×10^{11} vg/kg	Yes	0/2
2×10^{12} vg/kg	Yes	1/2

AAV2-ssF.IX trial

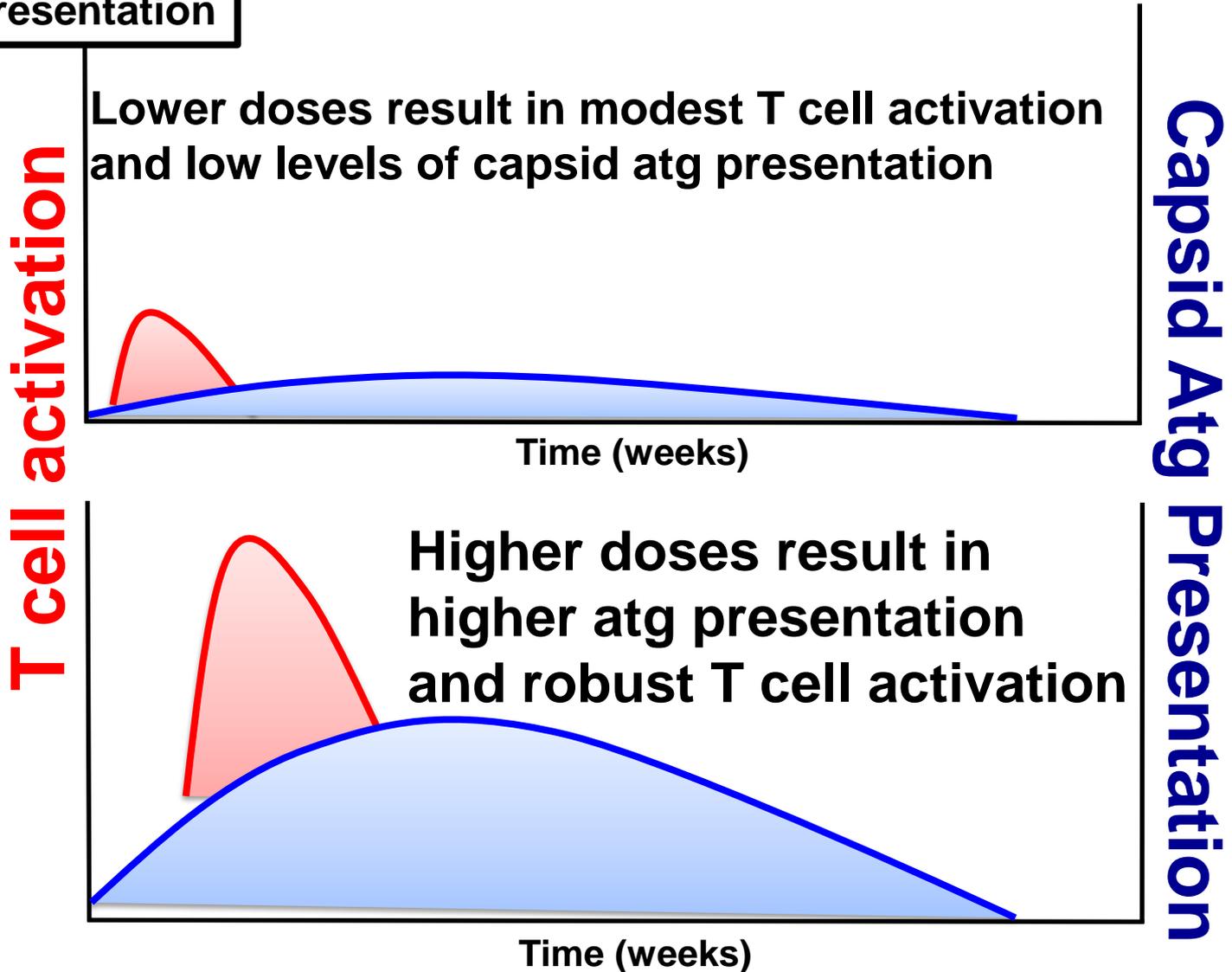
Vector dose	T cell response	Clinical sequelae (□AST/ALT)
8×10^{10} vg/kg	Incomplete dataset	0/2
4×10^{11} vg/kg		1/3
2×10^{12} vg/kg		1/2

Clinically detectable response depends upon two elements

- T cell activation
- Capsid antigen presentation on the surface of transduced cells



Proposed model: clinically detectable response is a function of BOTH T cell activation and capsid antigen presentation



Conclusions

- **Lowest doses of vector tested were not associated with detectable T cell responses to capsid in PBMCs**
- **CD4⁺ and CD8⁺ T cell responses to capsid were detected following infusion of an AAV8 vector at doses $\geq 6 \times 10^{11}$ vg/kg.**
- **Dose escalation to 2×10^{12} vg/kg was associated with elevation of liver enzymes concomitant with the detection of capsid-specific T cells in PBMC in one subject.**
- **No response to the F.IX transgene product or products of alternate reading frame translation could be detected.**
- **These immunological findings are in agreement with previous results of capsid-specific T cell responses in a phase I/II study of AAV2-hF.IX gene transfer.**
- **These data suggest that, for liver-directed gene transfer, there may be a threshold vector dose at which detection of capsid T cell responses are clinically relevant to the outcome of gene transfer.**
- **Clinical data suggest response, if it occurs, can be blunted by a course of prednisolone initiated when AST/ALT begin to rise.**