Gene Therapy for Haemophilia

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Declaration of financial interests by Edward Tuddenham

- I am a named inventor of patents in the field of gene therapy for haemophilia held by UCL
- I am a consultant to Biomarin Inc.
- I am a consultant to Freeline Therapeutic Ltd.

AAV Transduction Pathway

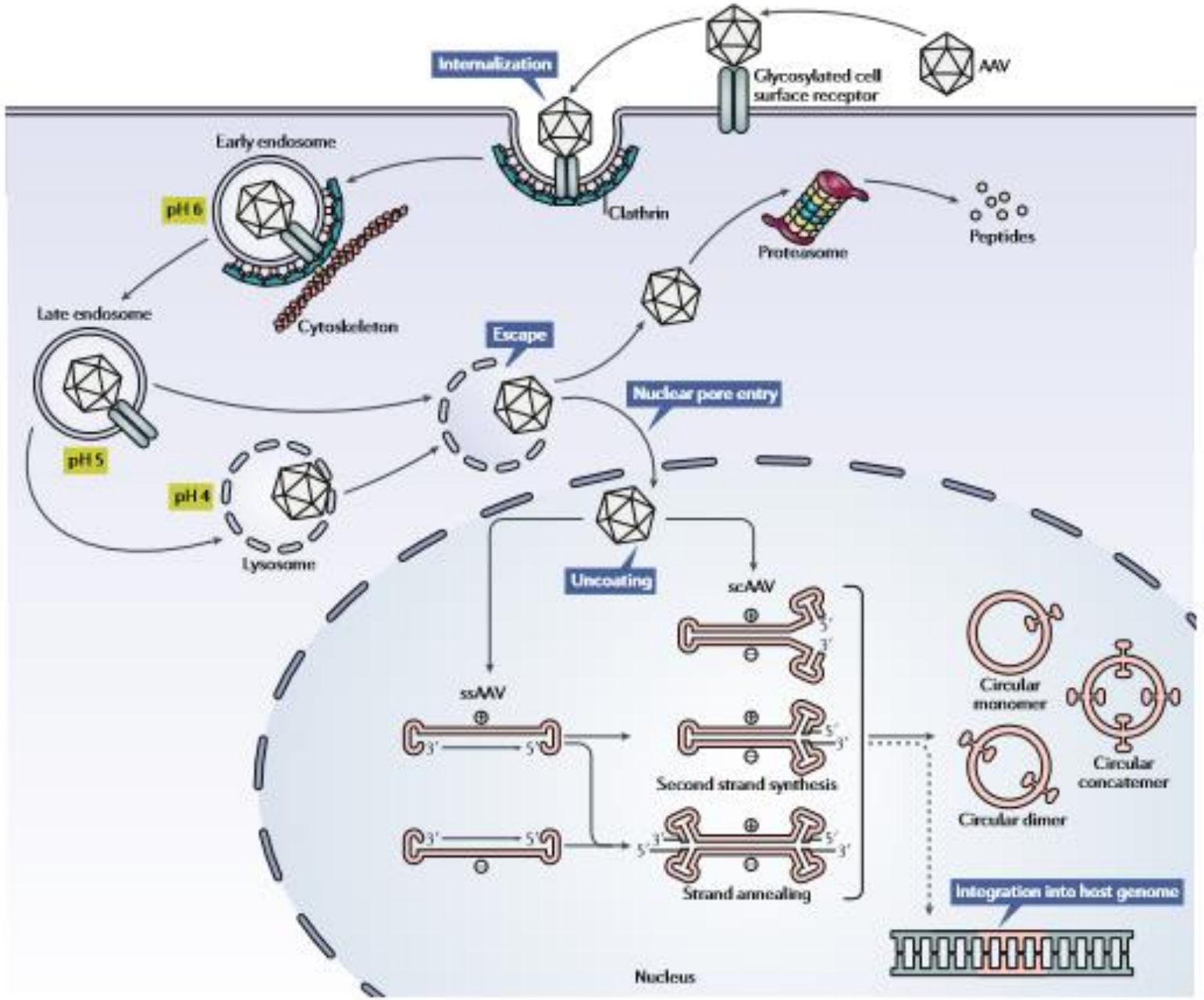
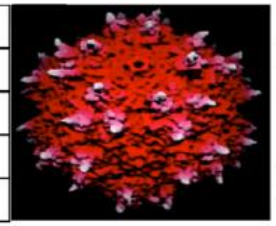
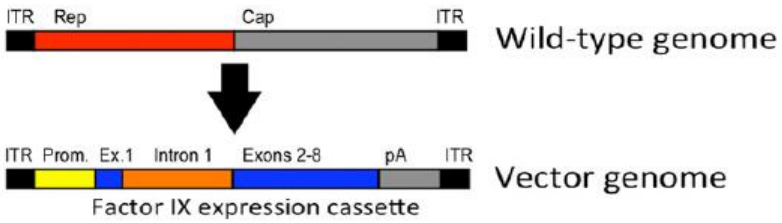


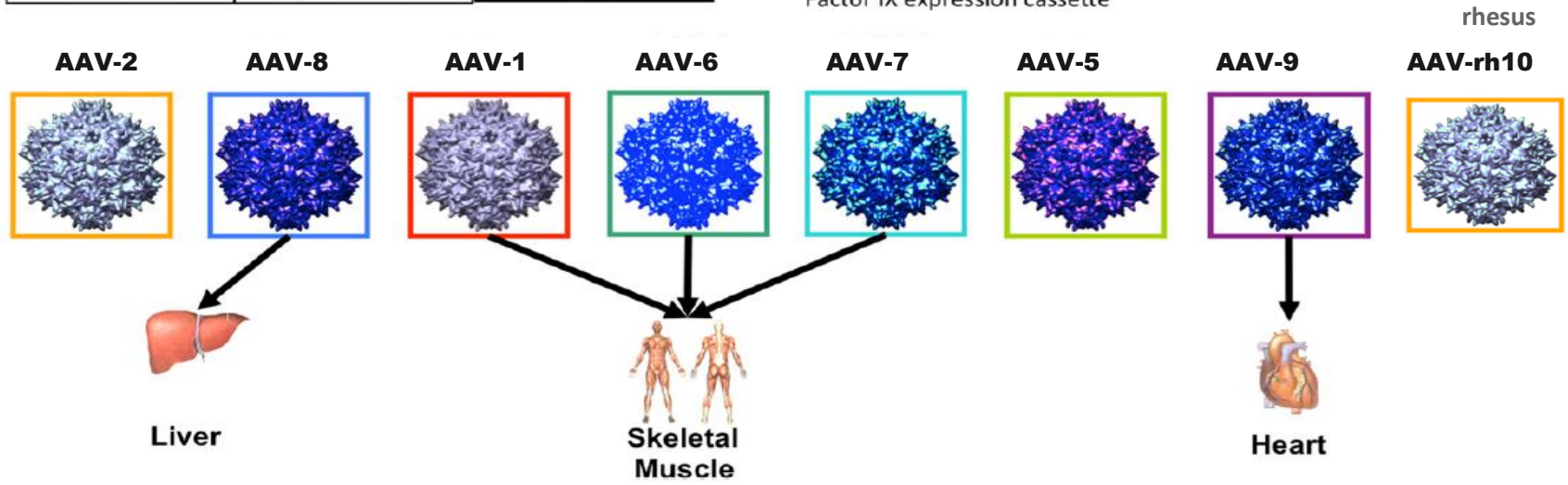
Fig. 2 | **Diagram of rAAV transduction pathway.** Adeno-associated virus (AAV) is recognized by glycosylated cell surface

AAV Vectors: Gene Transfer

Particle radius	25nm
Molecular weight	
protein (74%)	M_r ~ 3750 kDa
DNA (26%)	M_r ~ 1350 kDa
total virus	M_r ~ 5100 kDa







Spark SPK 8011
AAVspk FVIII

Sangamo SB-525
AAV6 FVIII

BioMarin BMN 270
AAV5-FVIIISQ

Dimension DTX201
AAV rh10 FVIII

UCL/St Jude
AAV8-FVIIIv3

Shire SHP654
AAV8 FVIII

Adapted from Arruda VR & Xiao W, JTH 2007

Rationale: Gene Therapy for Hemophilia

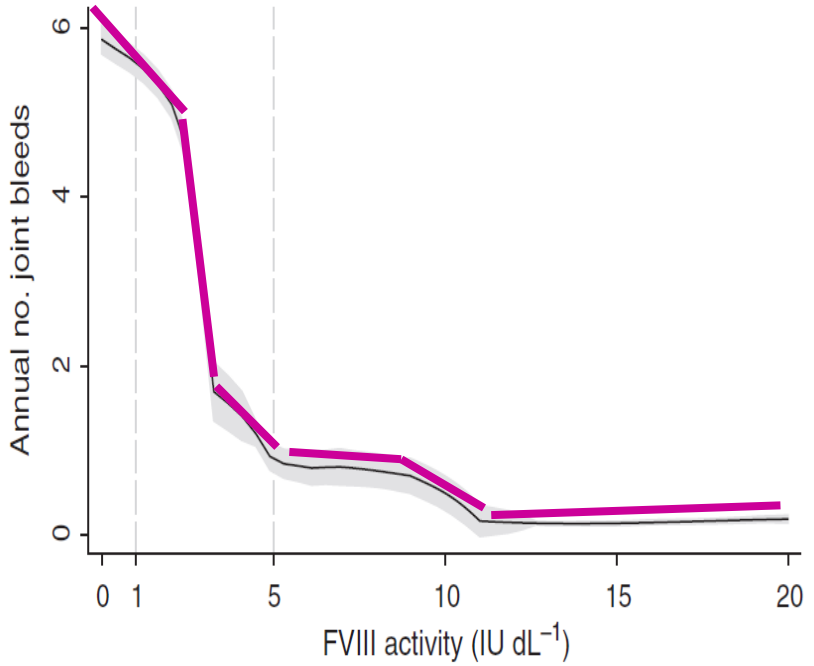
- Hemophilia is monogenic
- Wide range of FVIII, IX effective
- Tissue-specific expression not required
- Well-characterized animal models exist
- One-time treatment required for gene transfer
- Avoids cost, morbidity, complications of current therapy
- Potential global treatment for all affected

Aim: Hemophilia Gene Transfer

- **Sustain:** long-term expression
- **Achieve:**

  Past: FVIII, IX ≥ 15%

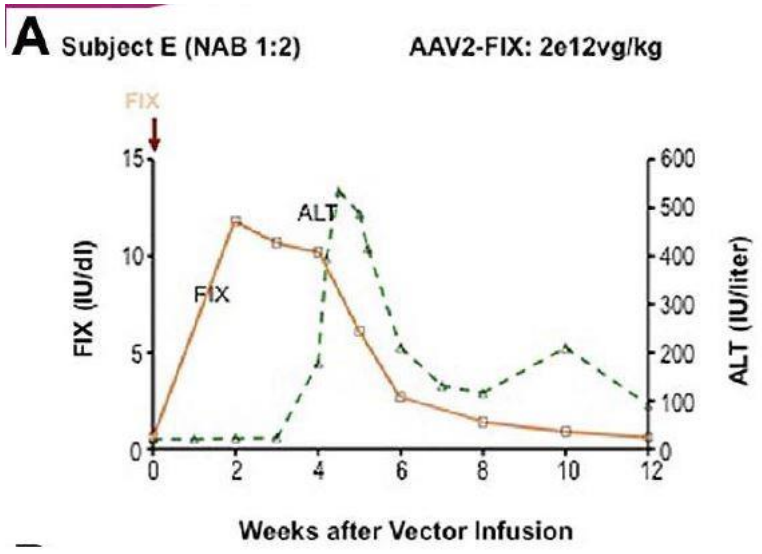
  Current: FVIII, IX > 50%
- **Convert:** severe to normal
- **Avoid:** all bleeds

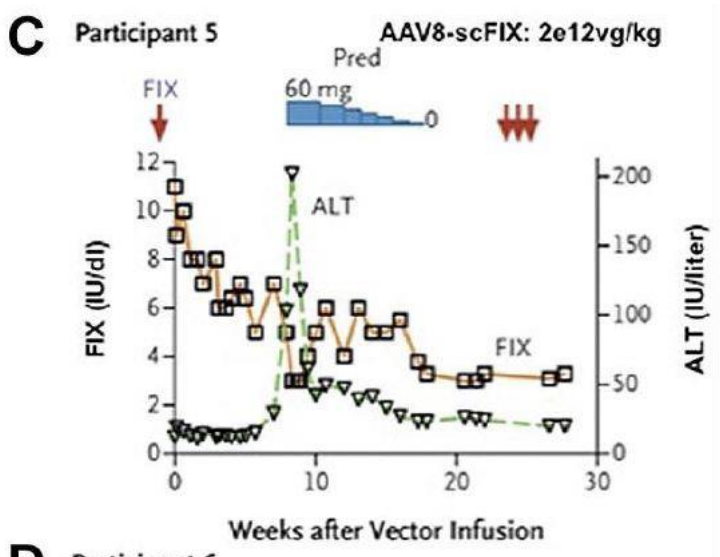


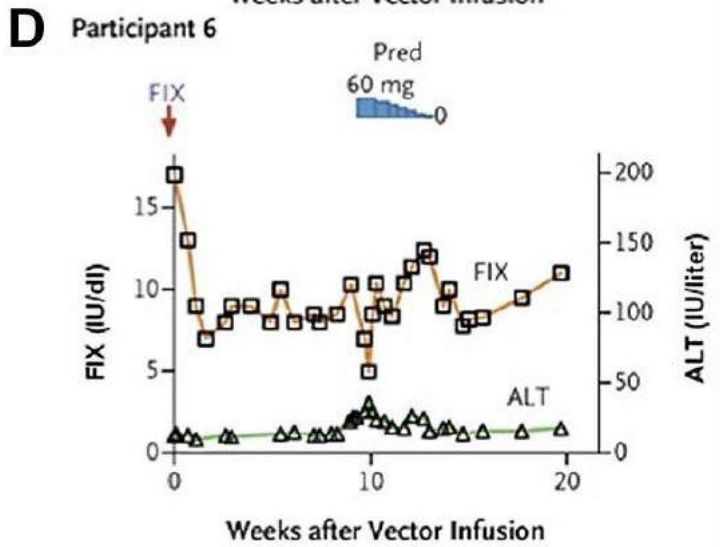
den Uijl et al, Haemophilia 2011

Two trials that established gene therapy for haemophilia B

Manno et al 2006; Nathwani et al 2010



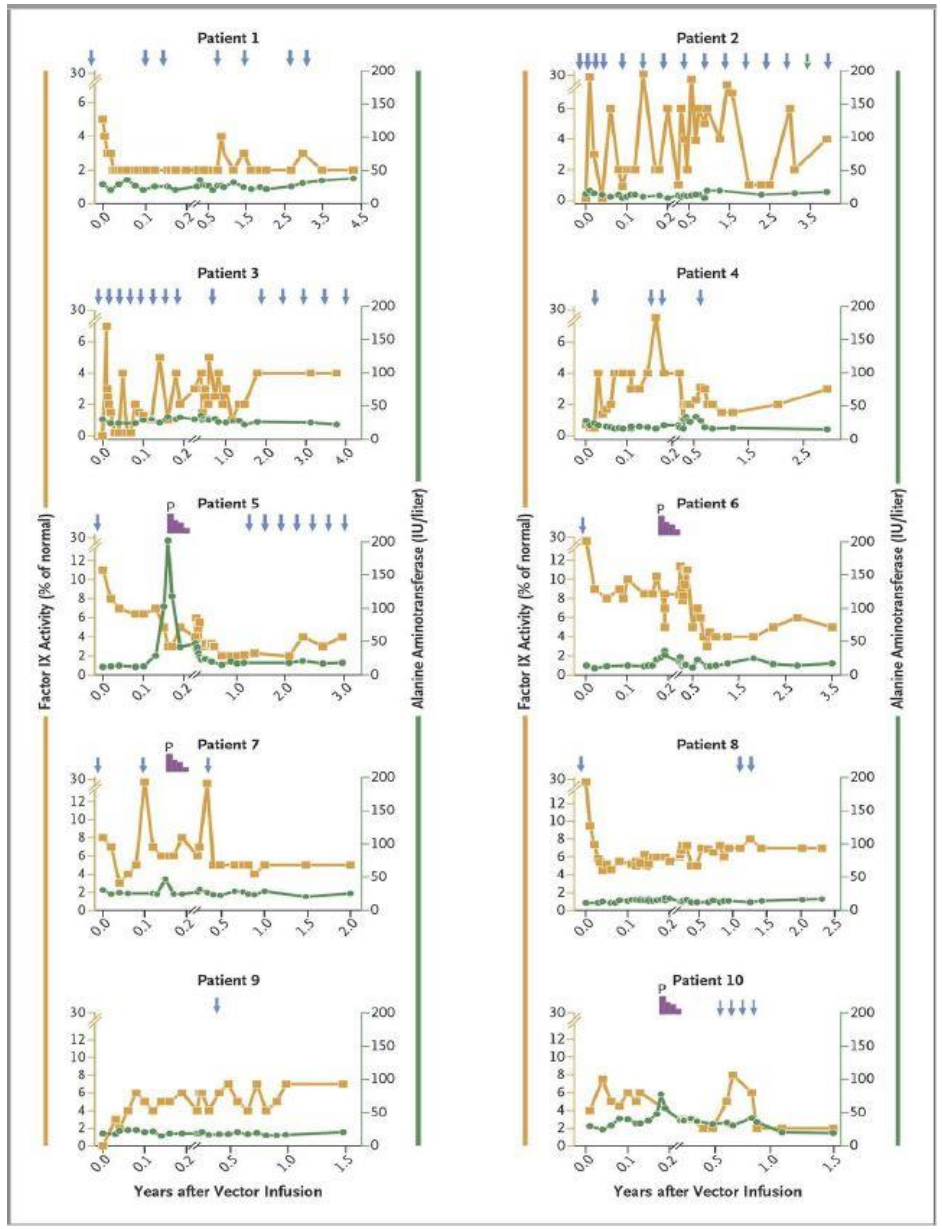




. Success- ful transduction of liver in hemophilia by AAV-Factor IX and limitations
e. *Nat Med.* 2006;12(3): 342-347.

ajan S, et al. Adenovirus-associated virus vector-mediated gene transfer in
(25):2357-2365.

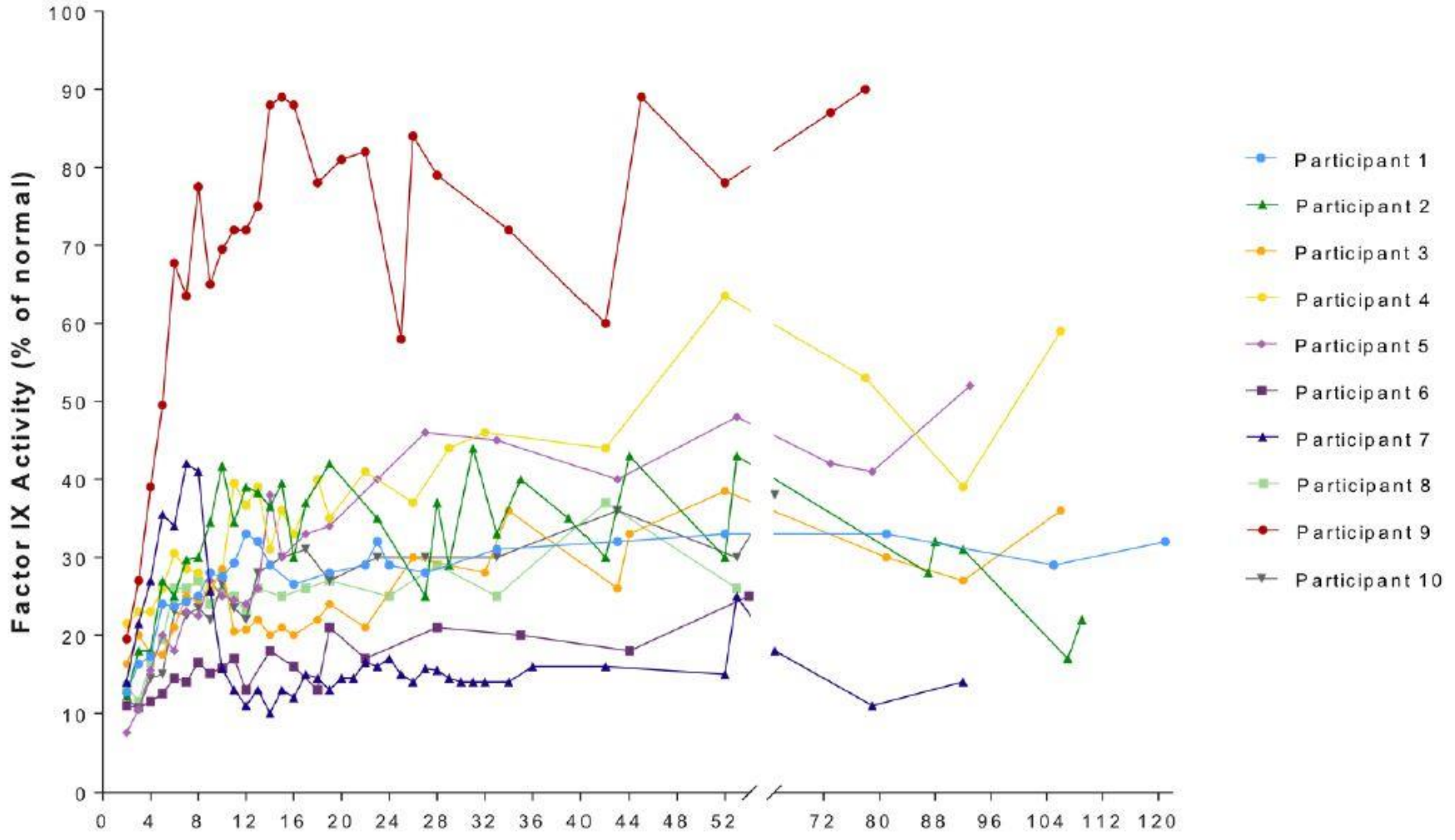
Longer term follow up published in 2014 is considered a ‘landmark study’



1. Data for up to 4 years
2. Consistent and durable response
3. No lasting adverse effects
4. Liver enzyme elevation controlled by steroid treatment
5. Bleeding rate reduced substantially
6. Factor usage reduced to near zero in 6 of 10 patients.

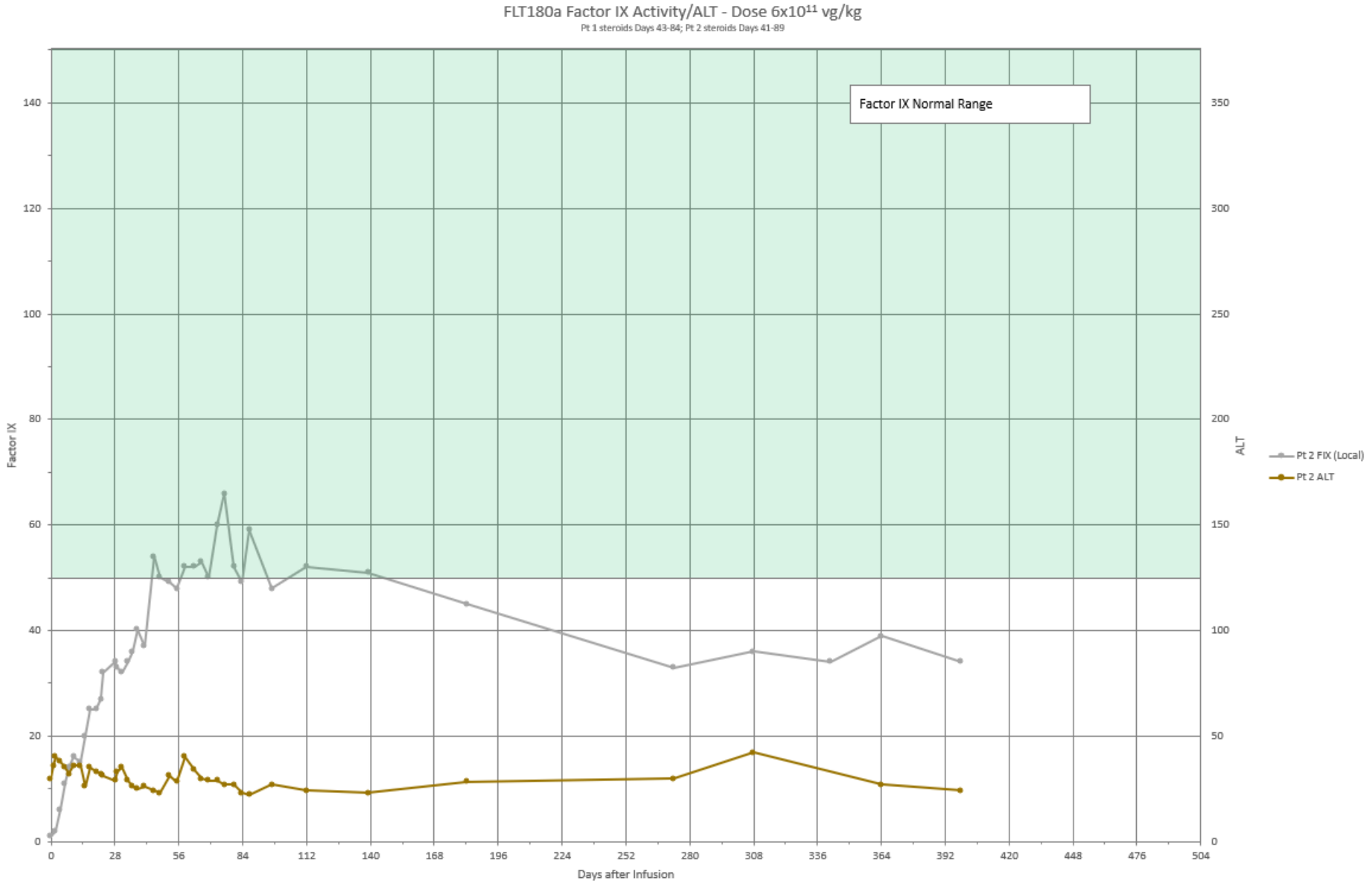
New study in haemophilia B using high activity factor IX Padua variant

- Pioneered successful trial with R338L FIX
- Range of mean steady-state FIX activity: 14.3-76.8%
- 2 transient transaminase elevations
- 3 received steroids
- Evidence of anti-capsid response
- No inhibitors
- Profound reduction in bleeding rate and factor utilization through 2 years



07-May-18 data cutoff
FIX activity levels obtained from local labs

Freeline study with Padua Factor IX in rAAVS3 capsid dose 6x10^{11} vg/kg



A possible cause of “transaminitis”

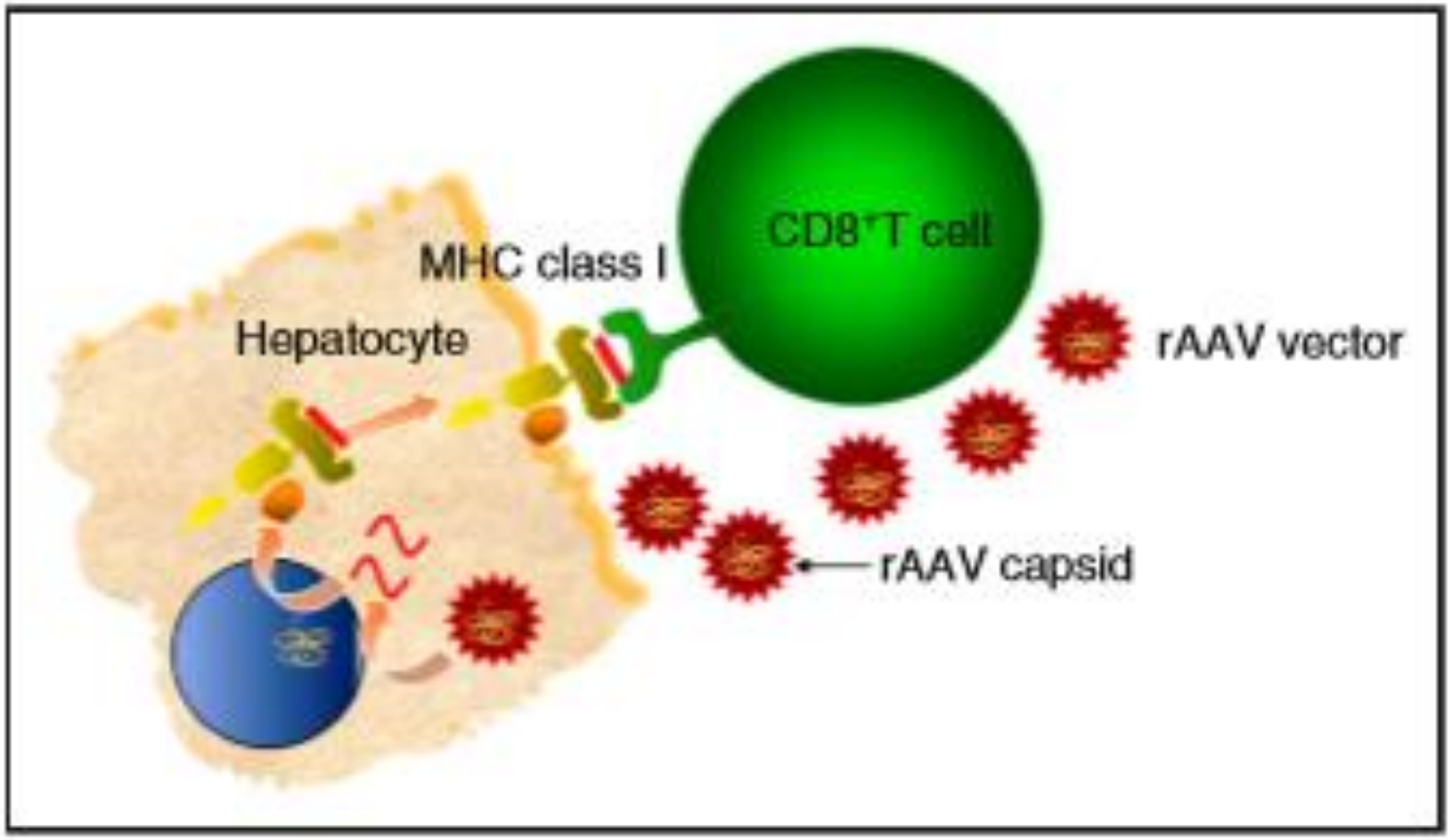


Figure 1. Hypothesis of the cellular immune response to hepatocytes after rAAV transduction. rAAV enters the hepatocyte by receptor-mediated endocytosis,

Immune barriers to successful transduction by AAV vectors in humans

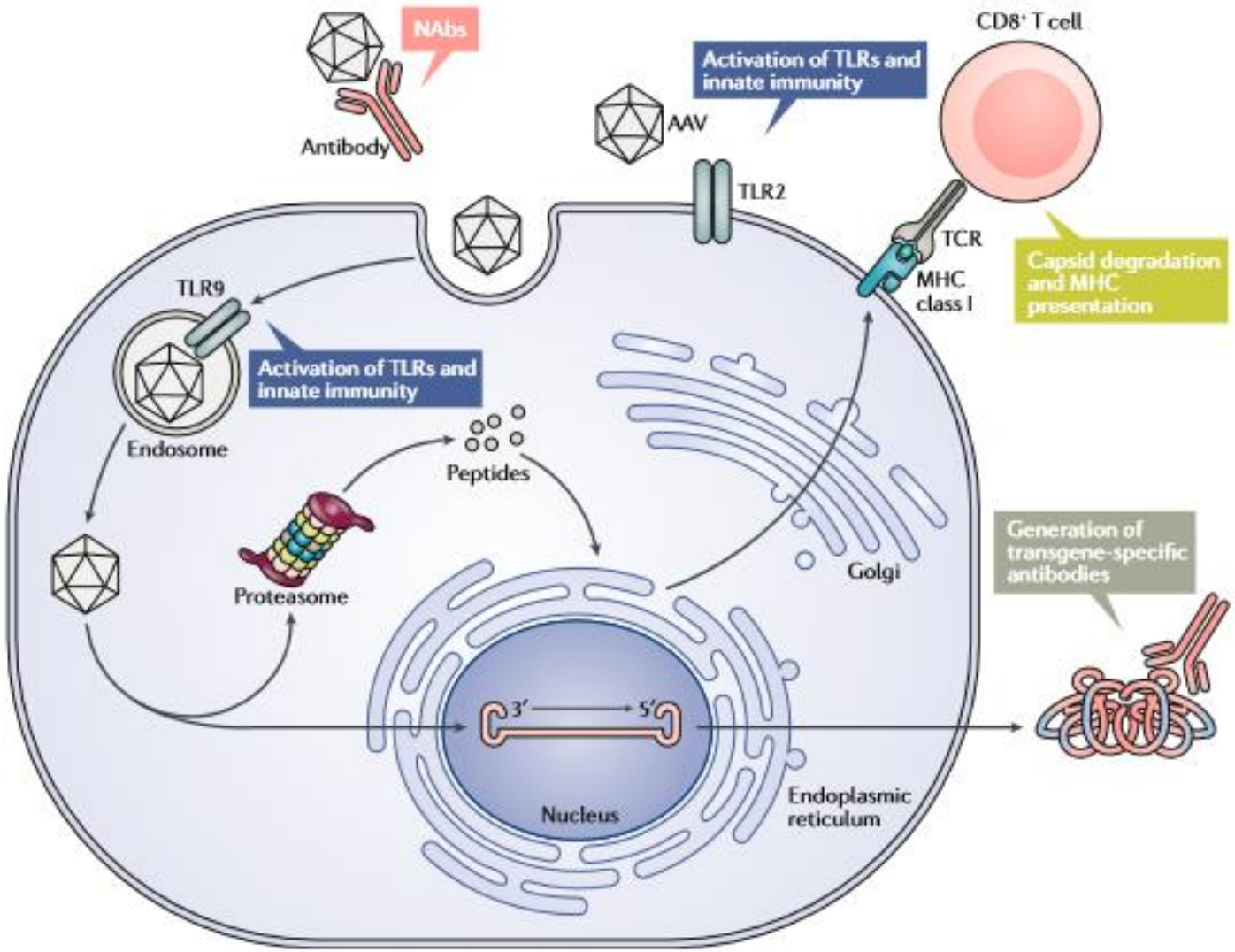


Fig. 5 | **Immunological barriers to successful rAAV gene delivery.** The recombinant adeno-associated virus (rAAV) may

Rapid increase of investment and new trials using AAV 2014-2019

Table 1 | A selection of ongoing rAAV interventional clinical trials

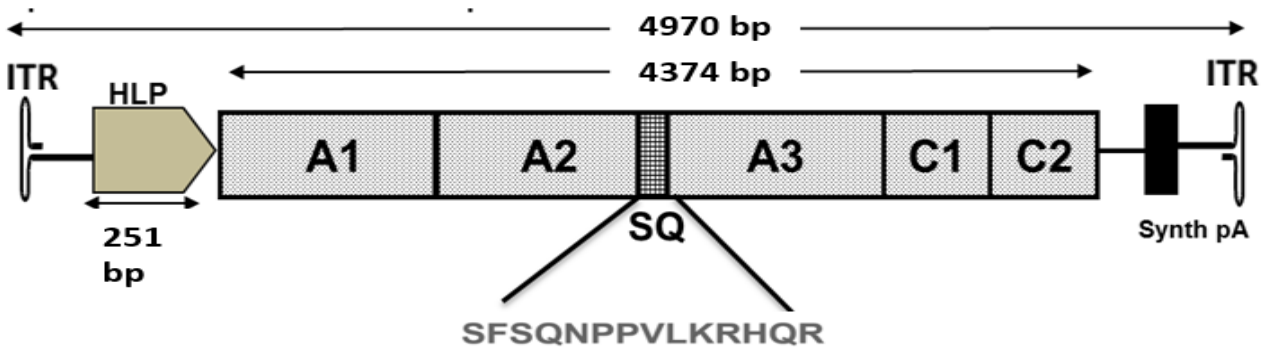
Primary gene delivery target	Condition	AAV capsid	Transgene product	Strategy	Sponsor	Phase	ClinicalTrials.gov identifier
Brain	AADC deficiency	AAV2	AADC	Replacement	Krystof Bankiewicz, UCSF	Phase I	NCT00852213
		AAV2	AADC	Replacement	National Taiwan University Hospital	Phase II	NCT02926066
	Batten disease (CLN2)	AAVrh.10	CLN2	Replacement	Weill Cornell	Phase I/II	NCT01414985
	Batten disease (CLN6)	AAV9	CLN6	Replacement	Nationwide Children's Hospital	Phase I/II	NCT02725580
	MPS-IIIB	AAV5	NAGLU	Replacement	uniQure	Phase I/II	NCT03300453
	Parkinson disease	AAV2	AADC	Addition	Jichi Medical University	Phase I/II	NCT02418598
		AAV2	GDNF	Addition	MNDS	Phase I	NCT01621581
		AAV2	Neurturin	Addition	Sangamo	Phase I/II	NCT00985517
		AAV2	AADC	Addition	Voyager	Phase I	NCT03065192
Spinal cord	SMA	AAV9	SMN	Replacement	AveXis	Phase III	NCT03461289
	Giant axonal neuropathy	AAV9	GAN	Replacement	MNDS	Phase I	NCT02362438
Eye	Achromatopsia	AAV2	CNGB3	Replacement	AGTC	Phase I/II	NCT02599922
		AAV8	CNGB3	Replacement	MeiraGTx	Phase I/II	NCT03001310
	Choroideremia	AAV2	REP1	Replacement	Nightstar	Phase III	NCT03496012
		AAV2	REP1	Replacement	Spark	Phase I/II	NCT02341807
		AAV2	REP1	Replacement	STZ eyetrial	Phase II	NCT02671539
		AAV2	REP1	Replacement	University of Oxford	Phase II	NCT02407678
	LCA	AAV2	RPE65	Replacement	Spark	Phase III	NCT00999609
		AAV5	RPE65	Replacement	MeiraGTx	Phase I/II	NCT02781480
	LHON	AAV2	ND4	Replacement	GenSight	Phase III	NCT03293524
		AAV2	ND4	Replacement	John Guy, University of Miami	Phase I	NCT02161380
	RP (RLBP1)	AAV8	RLBP1	Replacement	Novartis	Phase I/II	NCT03374657
	Wet AMD	AAV8	Anti-VEGF antibody	Silencing (mAb)	Regeneron	Phase I	NCT03066258
	X-linked RP	AAV2	RPGR	Replacement	AGTC	Phase I/II	NCT03316560
		AAV2	RPGR	Replacement	MeiraGTx	Phase I/II	NCT03252847
		ND	RPGR	Replacement	Nightstar	Phase I/II	NCT03116113
	X-linked retinoschisis	AAV2	RS1	Replacement	AGTC	Phase I/II	NCT03419637
		AAV8	RS1	Replacement	NEI	Phase I/II	NCT03371887
Liver	Crigler–Najjar syndrome	AAV8	UGT1A1	Replacement	Audentes	Phase I/II	NCT03223194
		ND	UGT1A1	Replacement	Genethon	Phase I/II	NCT03466665
	FH (homozygous)	AAV8	LDLR	Replacement	University of Pennsylvania	Phase I/II	NCT02651675
	GSD1a	AAV8	G6PC	Replacement	Ultragenyx	Phase I/II	NCT03517085
	Haemophilia A	AAV8	FVIII	Replacement	Shire	Phase I/II	NCT03370172
		AAVhu.37	FVIII	Replacement	Bayer	Phase I/II	NCT03588299
		AAV5	FVIII	Replacement	BioMarin	Phase III	NCT03370974
		AAV5	FVIII	Replacement	Sangamo	Phase I/II	NCT03061201
		ND	FVIII	Replacement	Spark	Phase I/II	NCT03003533
		AAV8	FVIII	Replacement	UCL	Phase I	NCT03001830

Table 1 (cont.) | A selection of ongoing rAAV interventional clinical trials

Primary gene delivery target	Condition	AAV capsid	Transgene product	Strategy	Sponsor	Phase	ClinicalTrials.gov identifier
Liver	Haemophilia B	AAV8	FIX	Replacement	Shire	Phase I/II	NCT01687608
		ND	FIX	Replacement	Pfizer	Phase II	NCT02484092
		ND	FIX	Replacement	Pfizer	Phase III	NCT03587116
		AAV6	FIX	Replacement	Sangamo	Phase I	NCT02695160
		AAV8	FIX	Replacement	St. Jude Children's Research Hospital	Phase I	NCT00979238
		AAV5	FIX	Replacement	uniQure	Phase III	NCT03569891
		ND	FIX	Replacement	UCL	Phase I	NCT03369444
	MPS-I	AAV6	ZFN1, ZFN2 and IDUA donor	Editing	Sangamo	Phase I	NCT02702115
	MPS-II	AAV6	ZFN1, ZFN2 and IDS donor	Editing	Sangamo	Phase I	NCT03041324
	MPS-IIIA	AAVrh.10	SGSH	Replacement	LYSOGENE	Phase II/III	NCT03612869
	MPS-VI	AAV8	ARSB	Replacement	Fondazione Telethon	Phase I/II	NCT03173521
	OTC deficiency	AAV8	OTC	Replacement	Ultragenyx	Phase I/II	NCT02991144
Muscle	A1AT deficiency	AAV2	A1AT	Replacement	UMMS	Phase I	NCT00377416
	CMT1A	AAV1	NTF3	Addition	Nationwide Children's Hospital	Phase I/II	NCT03520751
	DMD	AAVrh.74	Micro-dystrophin	Replacement	Nationwide Children's Hospital	Phase I/II	NCT03375164
		AAV9	Mini-dystrophin	Replacement	Pfizer	Phase I	NCT03362502
		AAV9	Micro-dystrophin	Replacement	Solid Biosciences	Phase I/II	NCT03368742
	Dysferlinopathy	AAVrh.74	DYSF	Replacement	Nationwide Children's Hospital	Phase I	NCT02710500
	HIV infections	AAV1	PG9 antibody	Addition	International AIDS Vaccine Initiative	Phase I	NCT01937455
		AAV8	VRC07 antibody	Addition	NIAID	Phase I	NCT03374202
	Pompe disease	AAV8	GAA	Replacement	Actus Therapeutics	Phase I/II	NCT03533673
		AAV9	GAA	Replacement	University of Florida	Phase I	NCT02240407
	X-linked MTM	AAV8	MTM1	Replacement	Audentes	Phase I/II	NCT03199469

A1AT, α1-antitrypsin; AADC, aromatic L-amino acid decarboxylase; AGTC, Applied Genetic Technologies Corporation; AMD, age-related macular degeneration; ARSB, arylsulfatase B; CLN2, neuronal ceroid lipofuscinosis type 2; CMT1A, Charcot–Marie–Tooth disease type 1A; CNGB3, cyclic nucleotide-gated channel β3;

BMN 270: AAV vector construct

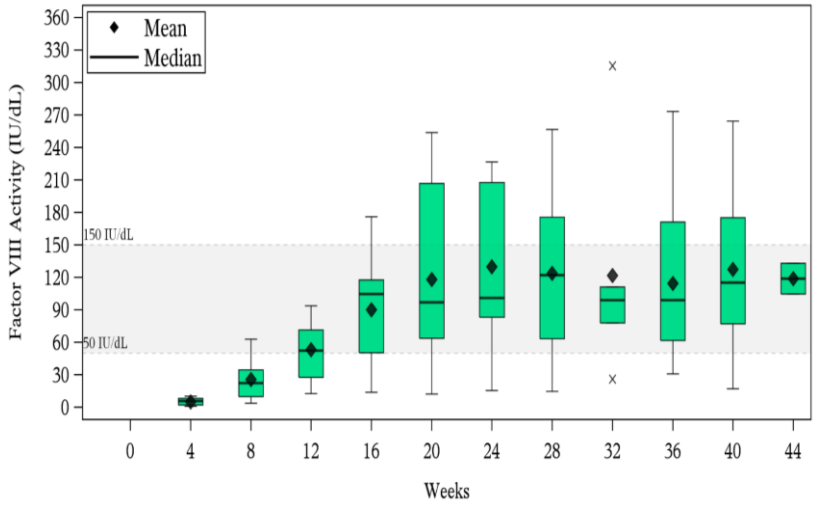


- Due to packaging constraints, size of vector components minimized
- Efficient transduction of the liver by AAV5
- Liver specific promoter
- Active Factor VIII with B domain deletion of Refacto™

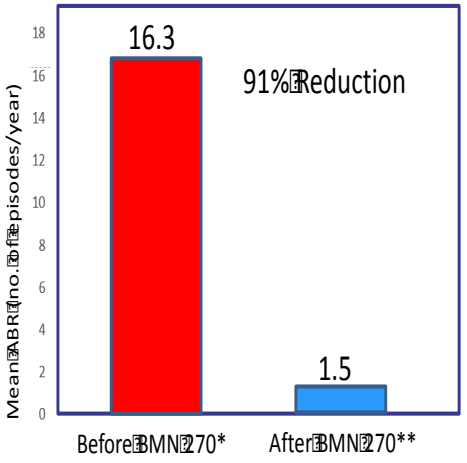
AAV5-hFVIII

Biomarin: BMN270 – AAV5-BDD-FVIII

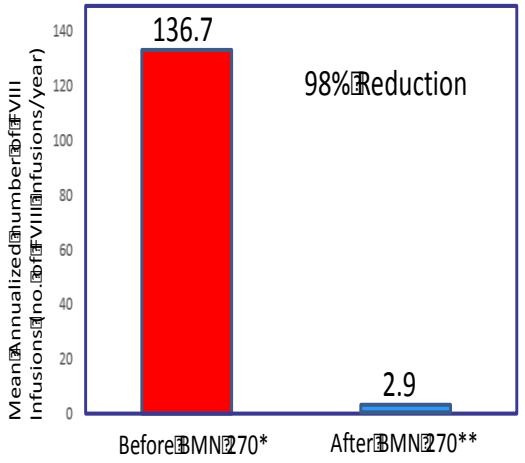
Factor VIII Activity



Annualized Bleed Rate



Factor VIII Use



Clinical Findings:

- **Factor VIII Activity:** Mean peak 115-130%
- **Durability:** Maintained to > 44 weeks
- **ABR:** 91% reduction (No bleeds in 5/6)
- **FVIII Use:** 98% reduction (No FVIII in 5/6)
- **Immune response:** High-dose cohort - steroid responsive

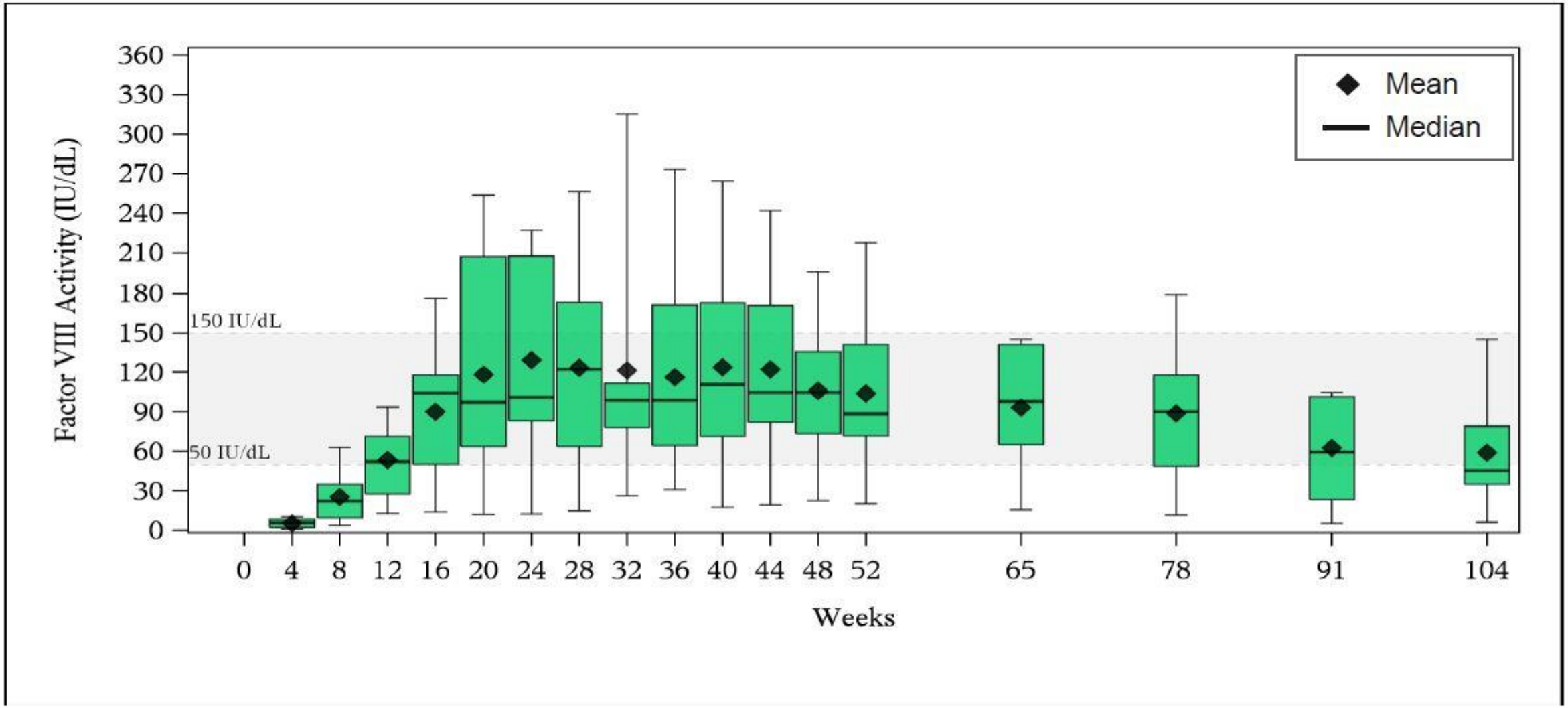
Conclusion: Safe, effective, durable > 44 weeks
Steroid-responsive immune response

Liver Function Tests

	Peak ALT	Last ALT	Status
1	60	15	Normal
2	95	16	Normal
3	82	42	Normal
4	87	33	Normal
5	43	38	Normal
6	81	45	<1.1 ULN
7	66	27	Normal

Biomarin, 2016, 2017

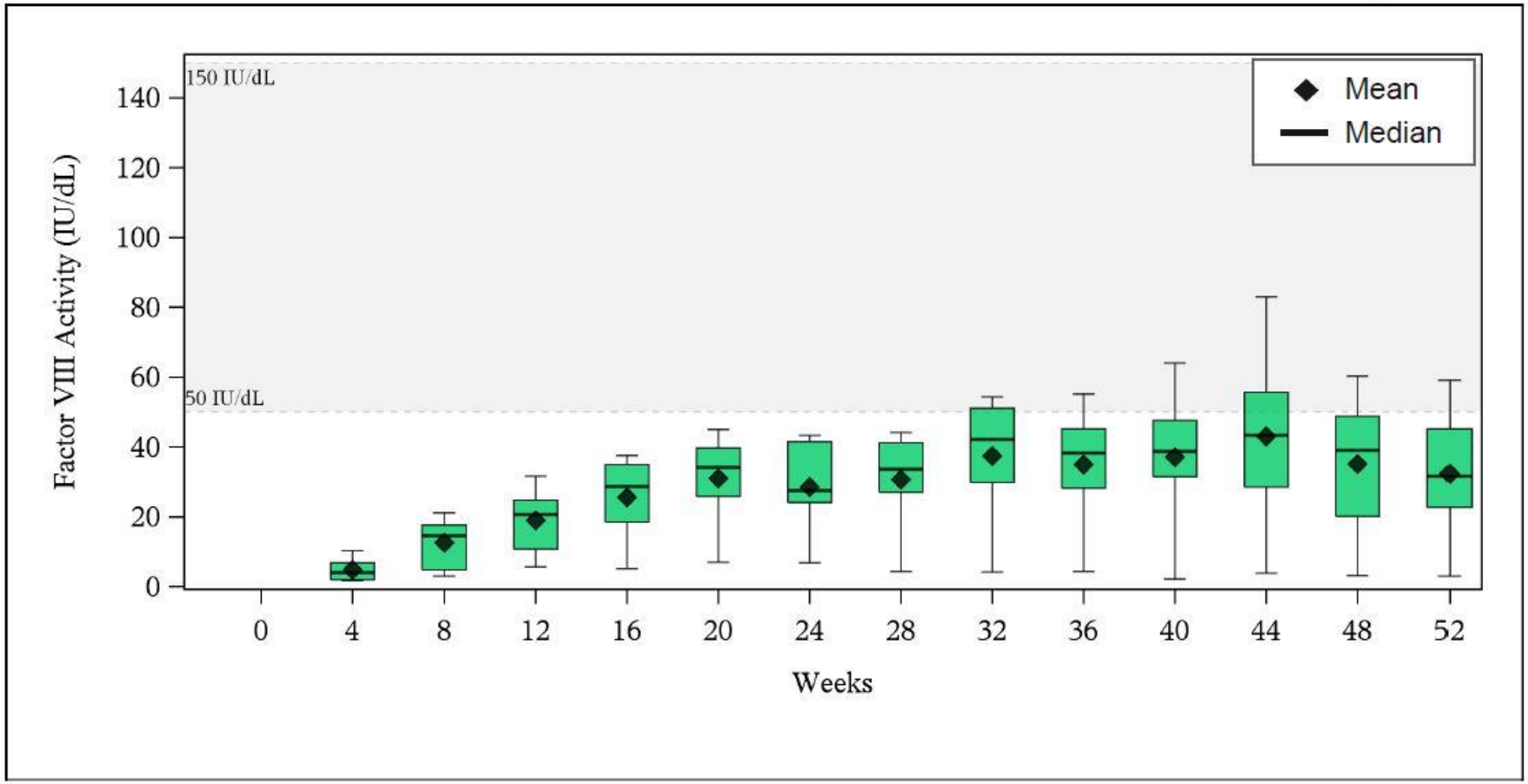
MEAN FVIII ACTIVITY LEVELS SETTLING IN NORMAL RANGE (6e13 VG/KG



- **No FVIII activity above upper limit of normal at year 2**

The upper and lower box bounds represent 25th and 75th percentiles. The whisker lines represent the minimum and maximum values

MEAN FVIII ACTIVITY LEVELS AT HIGH END OF MILD RANGE (4e13 VG/KG)



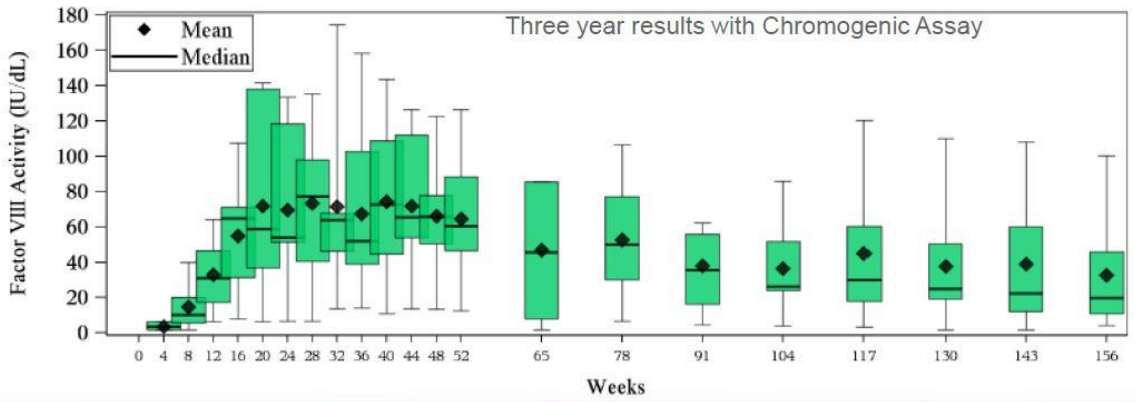
- **No FVIII activity above normal**

3 year data from BioMarin study

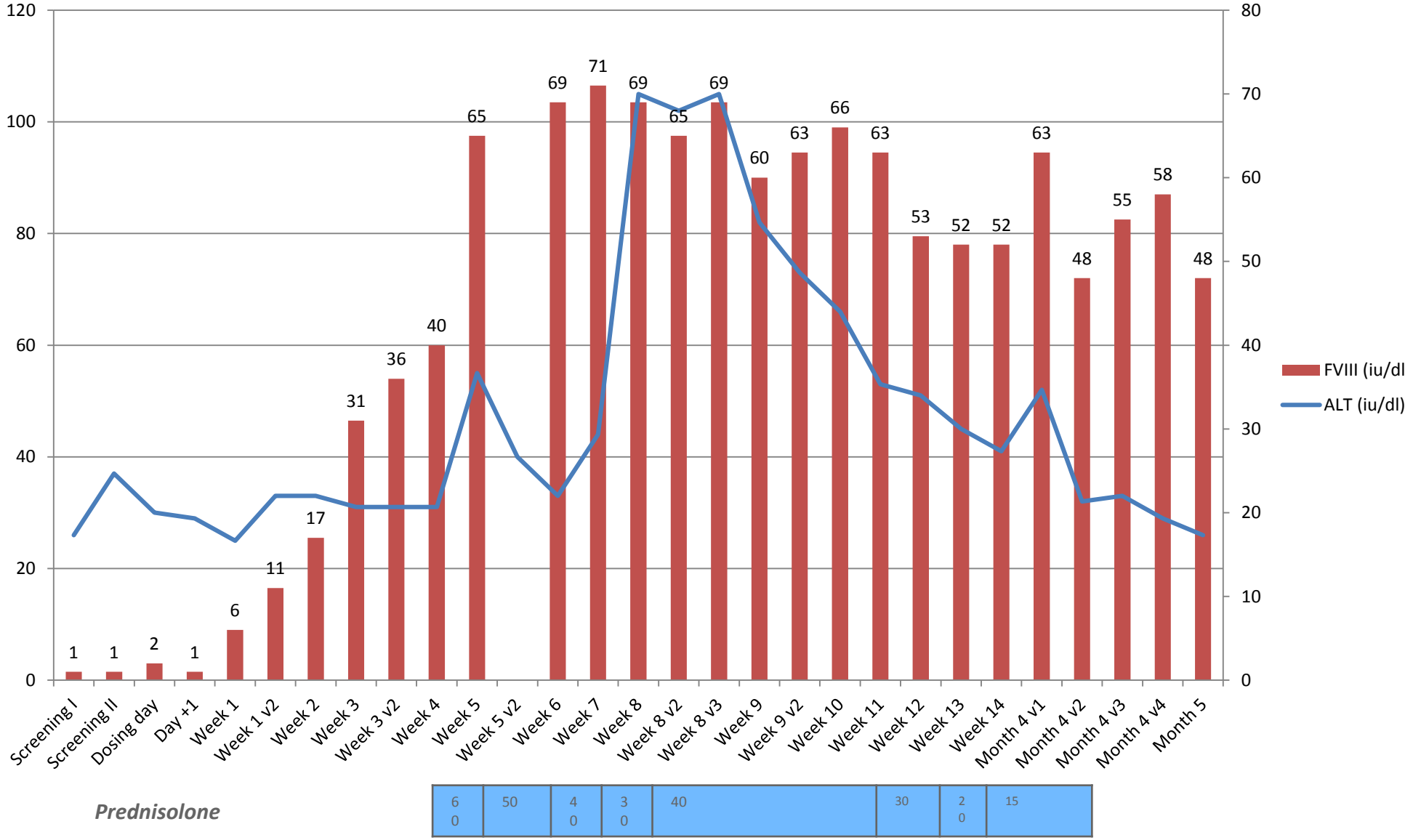
3 Year Phase 2 6e13 vg/kg Data Demonstrates Durable Factor VIII Expression

Rate of FVIII decline continued to be expression level dependent, slowed in year 3, and appears to be approaching plateau

FVIII activity level (IU/dL) time point	Mean (Chromogenic)	Median (Chromogenic)	Mean (One Stage)	Median (One Stage)
Weeks 23-26	68	57	127	100
Week 52	64	60	104	89
Week 104	36	26	59	46
Week 156	33	20	52	30



GO8-4R


120
100
80
60
40
20
0
80
70
60
50
40
30
20
10
0
1
1
2
1
6
11
17
31
36
40
65
69
71
69
65
69
60
63
66
63
53
52
52
63
48
55
58
48
Screening I
Screening II
Dosing day
Day +1
Week 1
Week 1 v2
Week 2
Week 3
Week 3 v2
Week 4
Week 5
Week 5 v2
Week 6
Week 7
Week 8
Week 8 v2
Week 8 v3
Week 9
Week 9 v2
Week 10
Week 11
Week 12
Week 13
Week 14
Month 4 v1
Month 4 v2
Month 4 v3
Month 4 v4
Month 5
FVIII (iu/dl
ALT (iu/dl)
Prednisolone
60
50
40
30
40
30
20
15

Durability: Hemophilia Gene Clinical Trials

Factor IX	NCT	Vector/Gene	AAV-IR	Expression	Durability
▪ **UCL/St. Jude**	NCT00979238	**AAV2/8**-LP1-hFIX$_{co}$	+ 5/10	2-8%	up to 8 years
▪ **Shire**	NCT01687608	**AAV8-**scIX	+ 2/7	0-25%	-
▪ **Spark**	NCT02484092	**AAV8**$_{(spk100)}$-hFIX	+	29-35%	up to 2 years
▪ **UniQure**	NCT02396342	**AAV5**-hFIX	+ 1/5	5.2-6.9%	up to 2 years
▪ **Dimension**	NCT02618915	**AAVrh10**-FIX		10-20%	up to 52 week s
▪ **Sangamo**	NCT02695160	**AAV2/6**-ZFN			
Factor VIII					
▪ **BioMarin**	NCT02576795	**AAV5**-hFVIII	+	4-300%	up to 2 years - falling
▪ **Spark**	NCT03003533	**AAV8**$_{(spk200)}$-hFVIII			
▪ **UCL/St. Jude**	NCT03001830	**AAV2/8**-HLP-FVIII-V3			
▪ **Sangamo**	NCT03061201	**AAV2/6**-hFVIII			

Known Unknowns in Gene Therapy

- Consequences of integrating DNA into chromosomal genome?
- How to manage hepatocyte toxicity?
- Where else apart from liver does vector genome go?
- How to overcome existing immunity to AAV?
- How long will transgenic synthesis of factor last?
- Can we repeat treatment with another AAV serotype or viral type?
- Should we treat children despite their growing livers?
- What will it cost?

Where are we now?

We are learning how to do gene therapy for haemophilia one patient at a time

Questions.