Gene Therapy for Hemophilia A: Are we really getting better?

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Abano Terme

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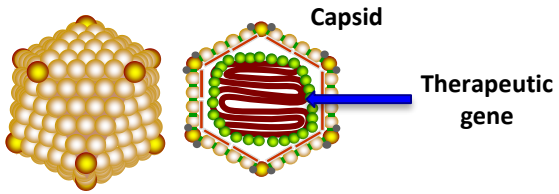
Hemophilia

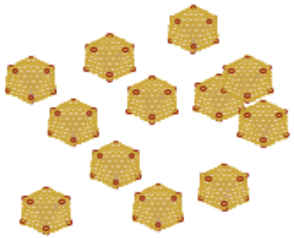
- **An X-linked bleeding disorder caused by a mutation in the gene for Factor VIII (hemophilia A) or Factor IX (hemophilia B)**
- **Affects 1:5000 (HA) to 1:30000 (HB) males worldwide, 30% are *de novo* cases**
- **Severe disease (< 1%), clinically very similar diseases**
- **Therapeutic levels of the protein are well established**
- **Gene therapy should be ONCE and DONE**

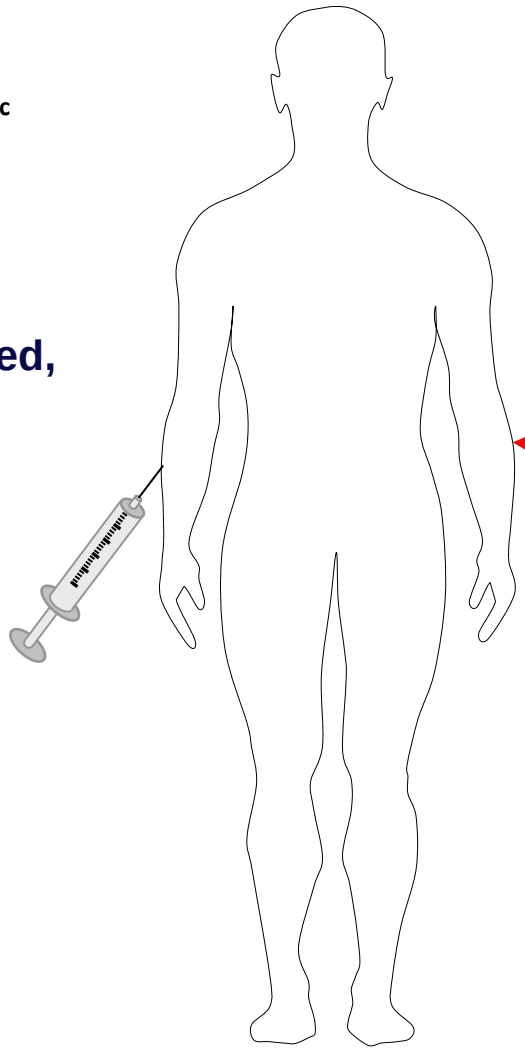
Two basic strategies to achieve long-term expression

Viral Vector

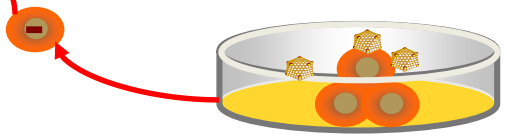


In vivo
Transduction of a long-lived, post-mitotic cell type, in which case integration not needed



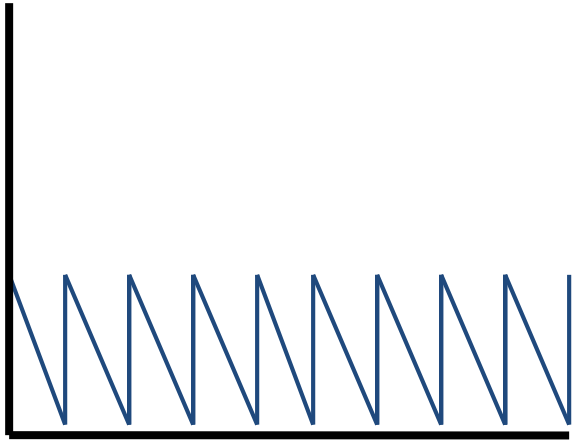


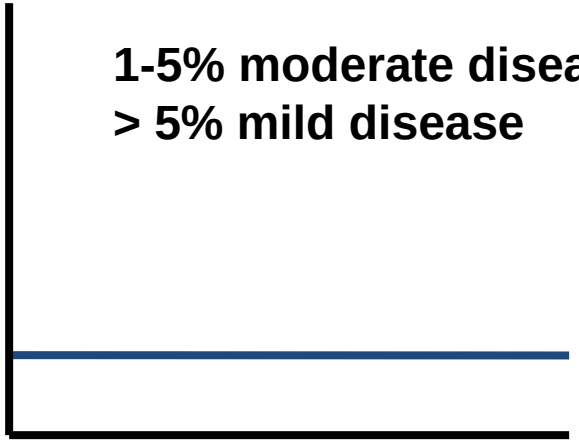
Integration into a stem cell



Modest elevation in the factor level is clinically beneficial

- **Successful continuous maintenance of clotting factor levels adequate to prevent, rather than stop an ongoing bleeding**
- **No need of frequent intravenous of clotting factors**
- **Risks of blood-borne infectious disease are avoided**
- **Pediatric: no need of central vein access**





Hemophilia A and B: Gene therapy perspective

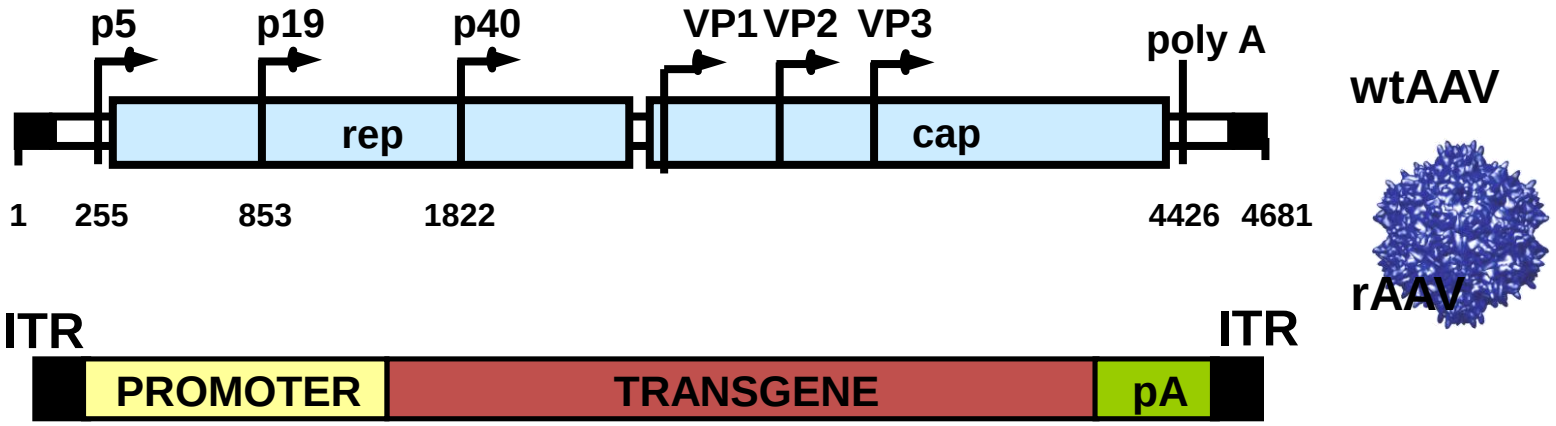
- **FVIII cDNA 3-fold larger than FIX**
- **Synthesis and secretion of FVIII is notoriously ineffective in vitro and in vivo (small and large animal models in gene therapy strategies)**
- **High risk of antibody formation (inhibitor) to FVIII protein (20%) compared to FIX (3%)**
- **Therapeutic levels of FVIII are 50-fold lower than FIX. However, preclinical studies suggest that therapeutic vector doses will be higher for HA**

Ongoing and planned clinical trial

Coalescence around a single vector class strategy is usually a positive sign

Adeno-associated viral (AAV) vector: not an adenoviral vector

Wild-type and recombinant AAV



wtAAV :

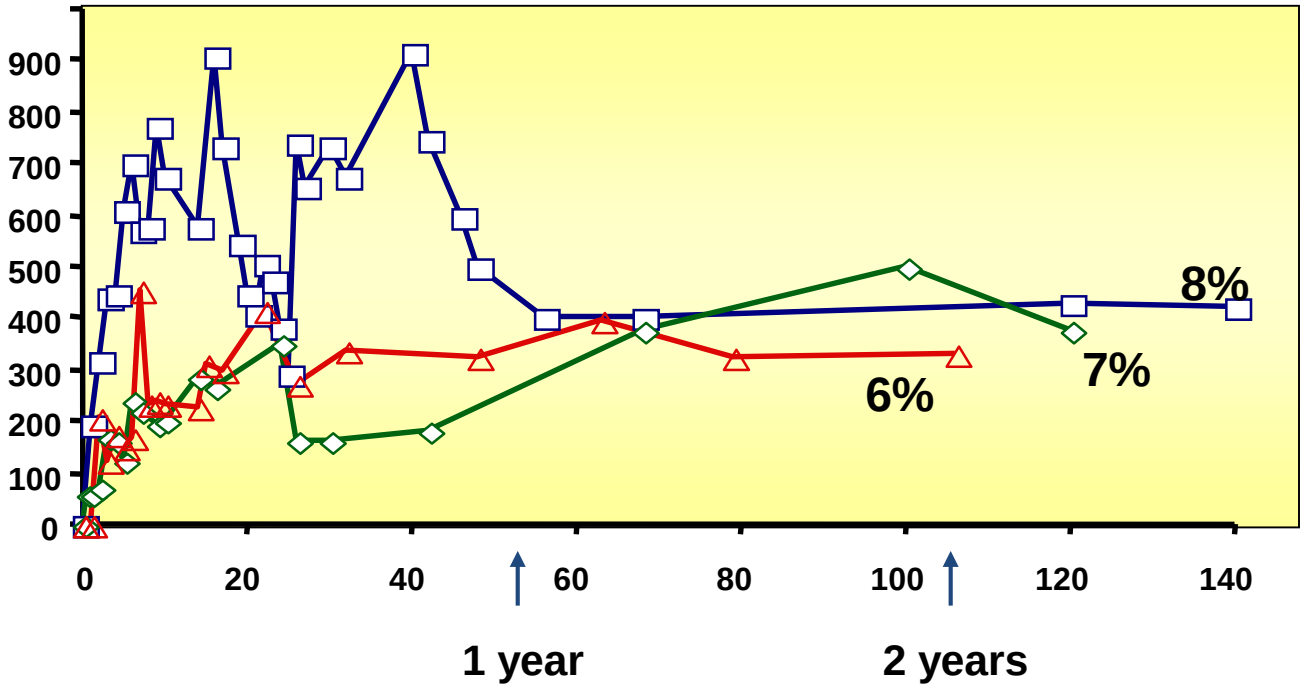
- parvovirus family
- ~4.7-kb ss DNA genome
- replication-defective
- non-pathogenic

rAAV vectors:

- no viral coding sequences
- tropism for long-lived post-mitotic cells, e.g. muscle, liver, CNS
- predominantly non-integrating
- long-term expression in immunocompetent animals

Xiao et al., J. Virol. 1996
Kessler et al., PNAS 1996

Liver-Derived Expression of cFIX in Hemophilia B Dogs
AAV-2 ~ $1x10^{12}$ vg/kg

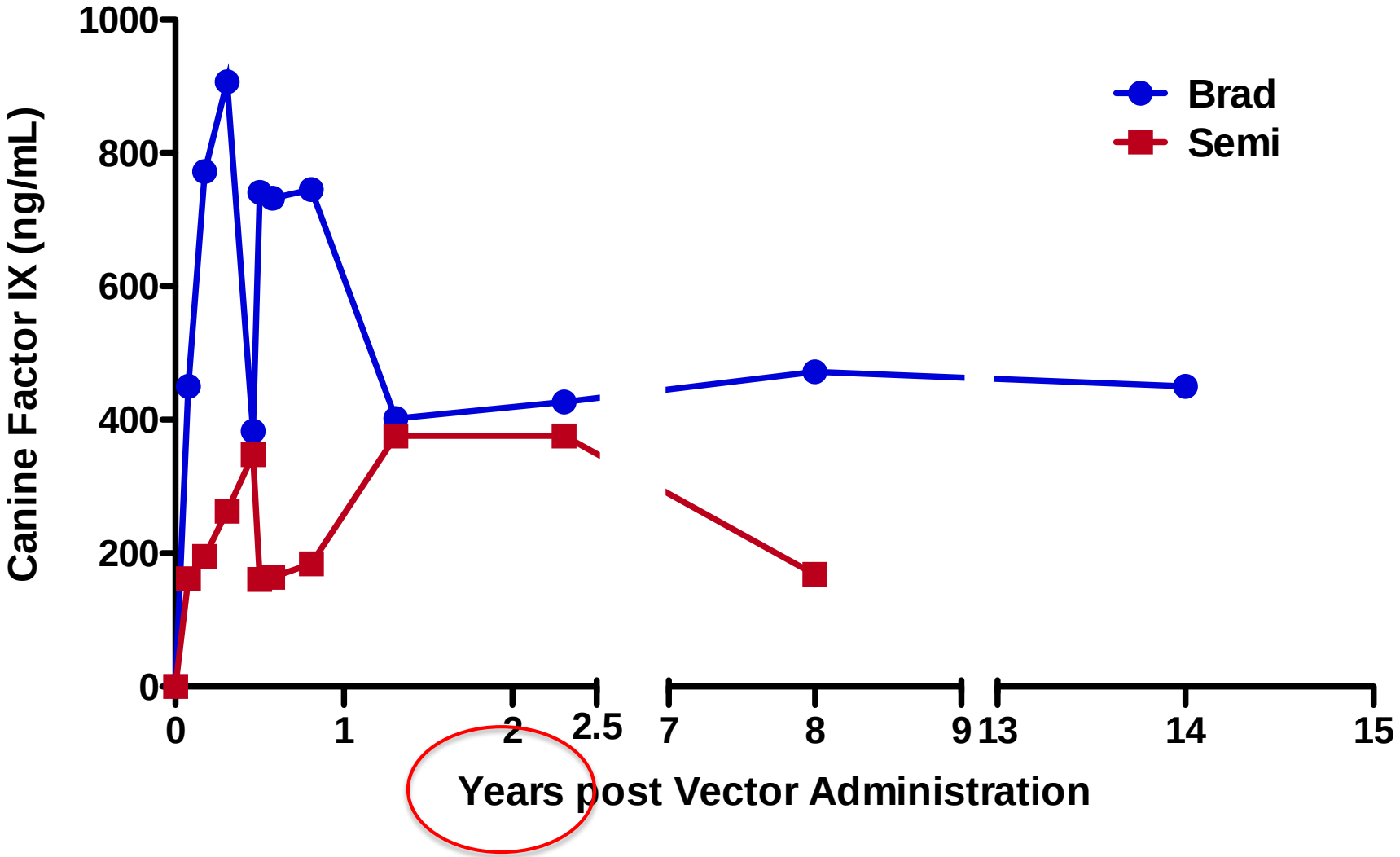


Safety

Numerous safety studies performed in rodents, dogs, non-human primates - no toxicity.

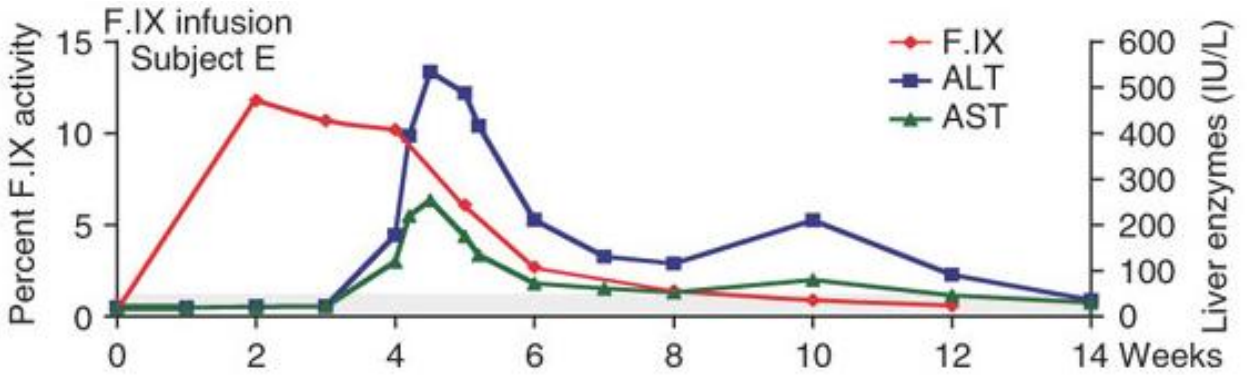
Blood 2002, Blood 2009

Long-term expression after single injection of AAV vector for liver gene therapy in HB Dogs

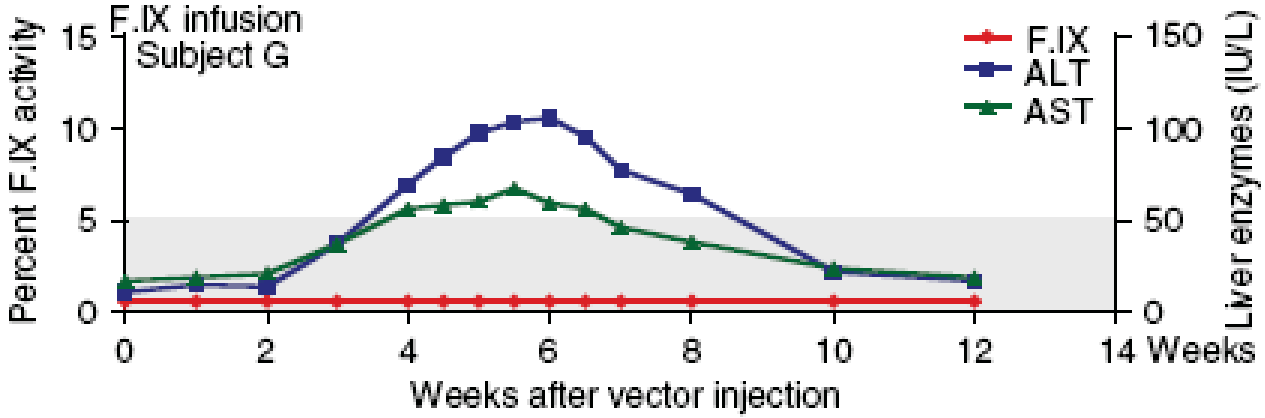


First trial: AAV2-liver-directed, transient expression
Increased liver enzyme, lost of FIX expressio
Vector-dose dependent

2 x10^{12} vg/kg



4 x10^{11} vg/kg

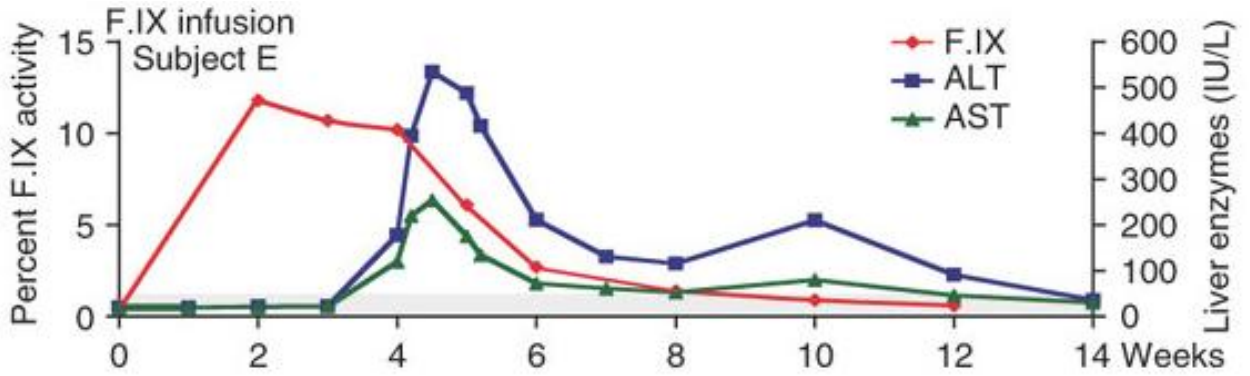


Vector genomes/kg body weight (vg/kg)

Manno et al Nature Medicine 12:342, 2006.

AAV capsid-proteins trigger T cell response
Cellular response is dose-dependent
Liver enzymes can be use as biomarkers

2 x10^{12} vg/kg



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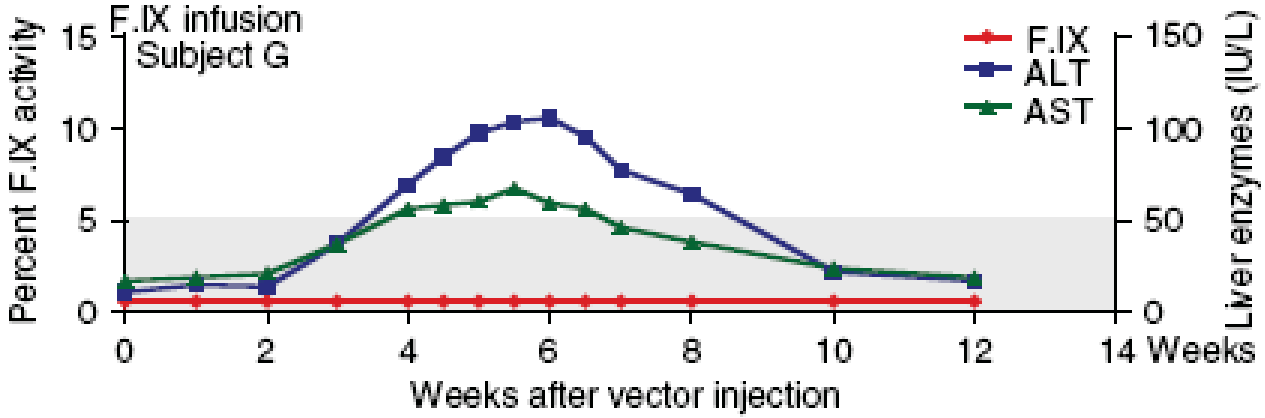
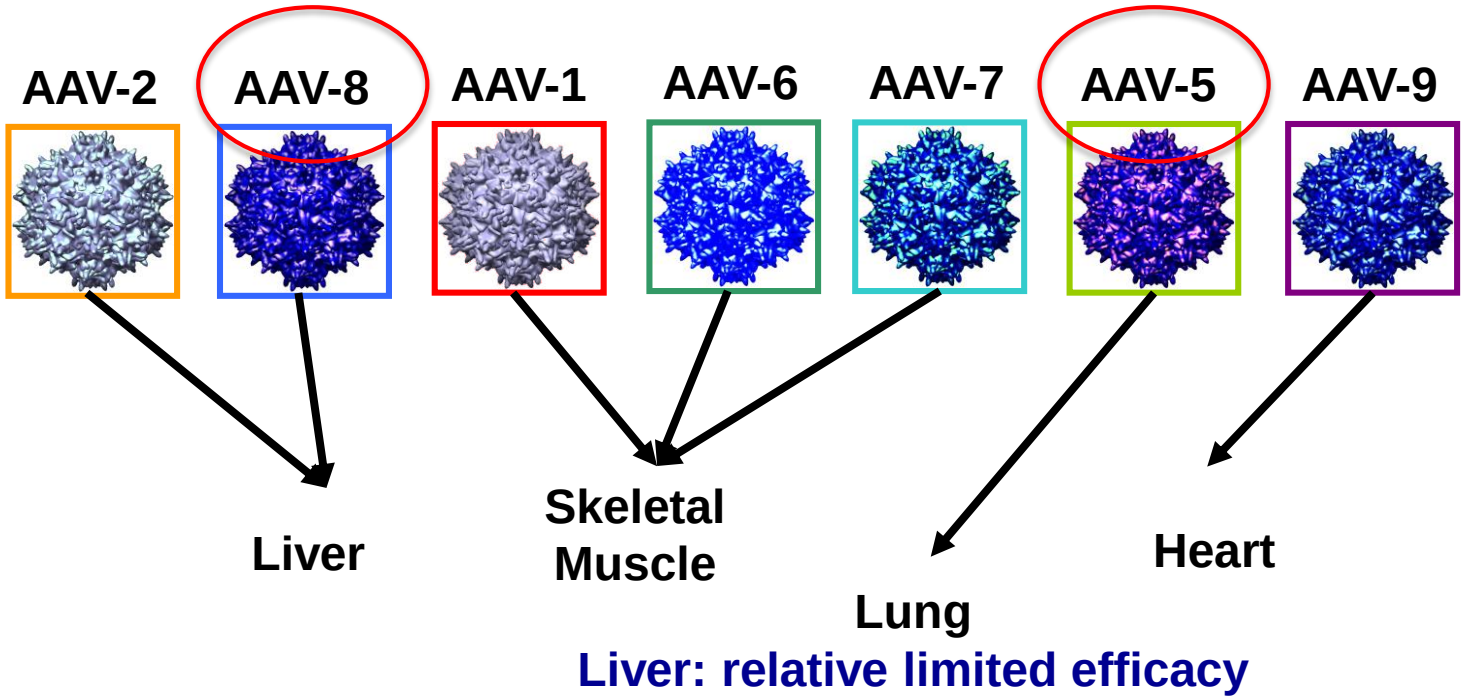


Manno et al Nature Medicine 12:342, 2006.

Lessons from early trials on intravascular delivery targeting the liver using AAV in hemophilia

- **The presence of neutralizing antibodies to the vector capsid, even at low levels, prevents gene delivery**
- **Immune responses mediated by the vector capsid that results in lost of transgene expression is restricted to humans and cannot be predicted in preclinical studies**
- **Monitor liver enzymes, frequently: use of steroids**
- **There is evidence that such complication is likely to be vector dose-dependent**

Efficacy and safety of AAV of alternate serotypes, capsid modifications



Only patients with undetectable or very low antibody titers to AAV capsid are enrolled

Completed and on-going gene therapy clinical trials

AAV vectors targeting liver-restricted expression

Sponsor	Subjects	Transgene	Serotype	Expression system
UCL/St. Jude CRH	10	FIX-WT	AAV-8 or similar	HEK 293 cells
Spark/CHOP	15	FIX-Padua		
Chatham/Baxalta	16	FIX-Padua		
uniQure	6	FIX-WT	AAV-5	Insect cells (baculo system)
Biomarin 270	9	FVIII-B domain deleted		

(distinct ratios of empty capsid, purification systems)

Intravenous injection of AAV serotype 8, expressing hFIX wild-type

The NEW ENGLAND JOURNAL of MEDICINE

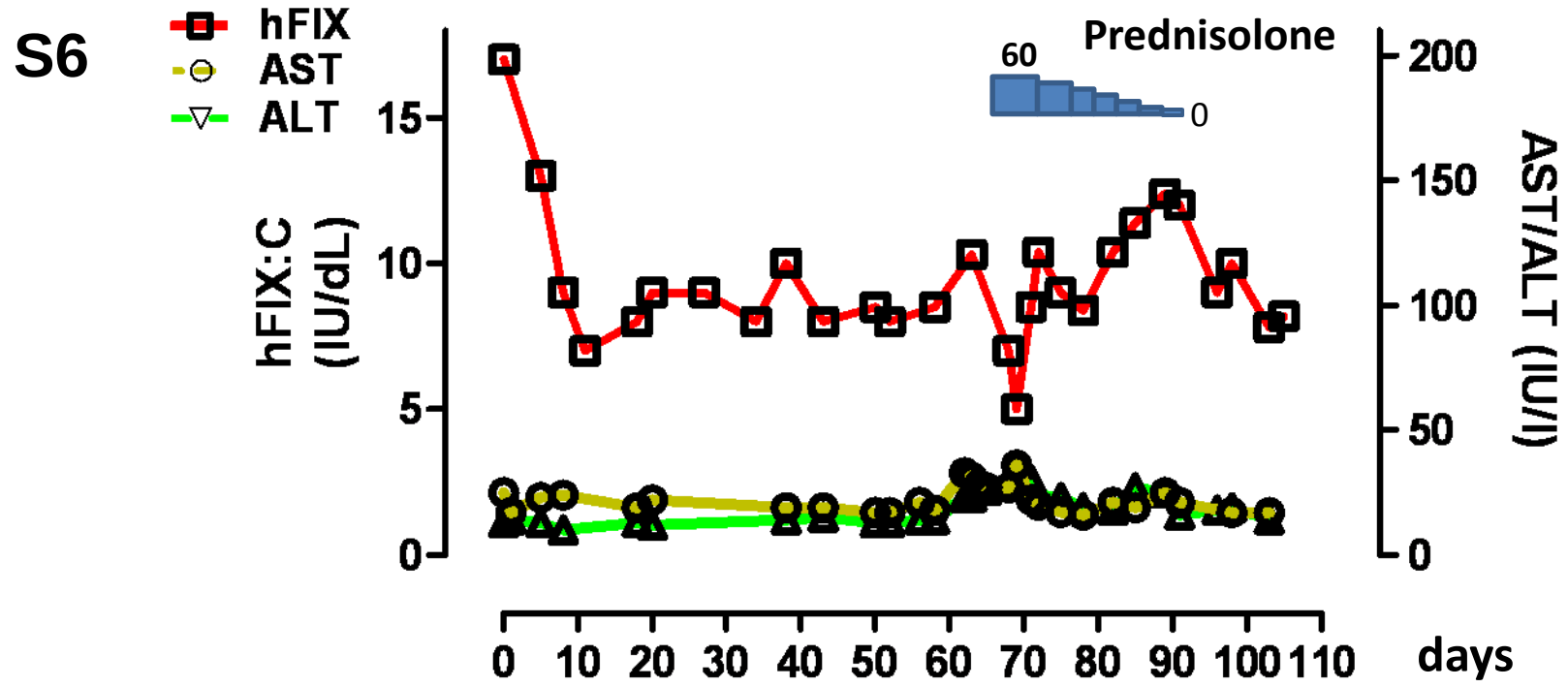
ESTABLISHED IN 1812 DECEMBER 22, 2011 VOL. 365 NO. 25

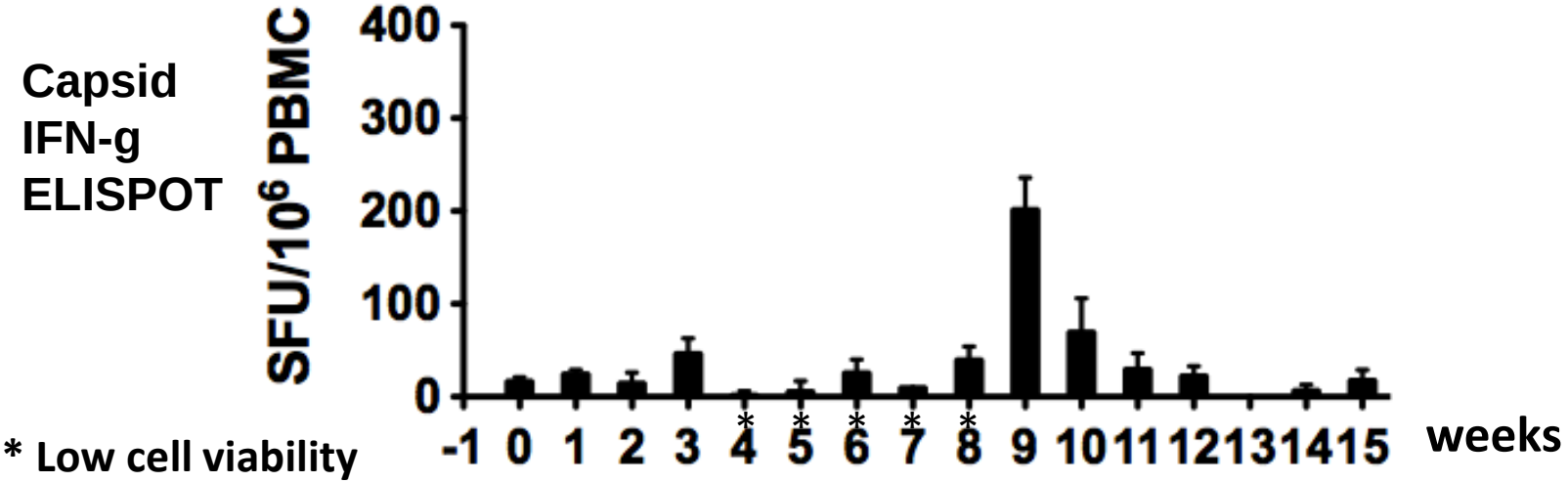
Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

Amit C. Nathwani, M.B., Ch.B., Ph.D., Edward G.D. Tuddenham, M.B., B.S., M.D., Savita Rangarajan, M.B., B.S., Cecilia Rosales, Ph.D., Jenny McIntosh, Ph.D., David C. Linch, M.B., B.Chir., Pratima Chowdary, M.B., B.S., Anne Riddell, B.Sc., Arnulfo Jaquilmac Pie, B.S.N., Chris Harrington, B.S.N., James O'Beirne, M.B., B.S., M.D., Keith Smith, M.Sc., John Pasi, M.D., Bertil Glader, M.D., Ph.D., Pradip Rustagi, M.D., Catherine Y.C. Ng, M.S., Mark A. Kay, M.D., Ph.D., Junfang Zhou, M.D., Yunyu Spence, Ph.D., Christopher L. Morton, B.S., James Allay, Ph.D., John Coleman, M.S., Susan Sleep, Ph.D., John M. Cunningham, M.D., Deokumar Srivastava, Ph.D., Etiena Basner-Tschakarjan, M.D., Federico Mingozzi, Ph.D., Katherine A. High, M.D., John T. Gray, Ph.D., Ulrike M. Reiss, M.D., Arthur W. Nienhuis, M.D., and Andrew M. Davidoff, M.D.

NEJM 2011, NEJM 2014

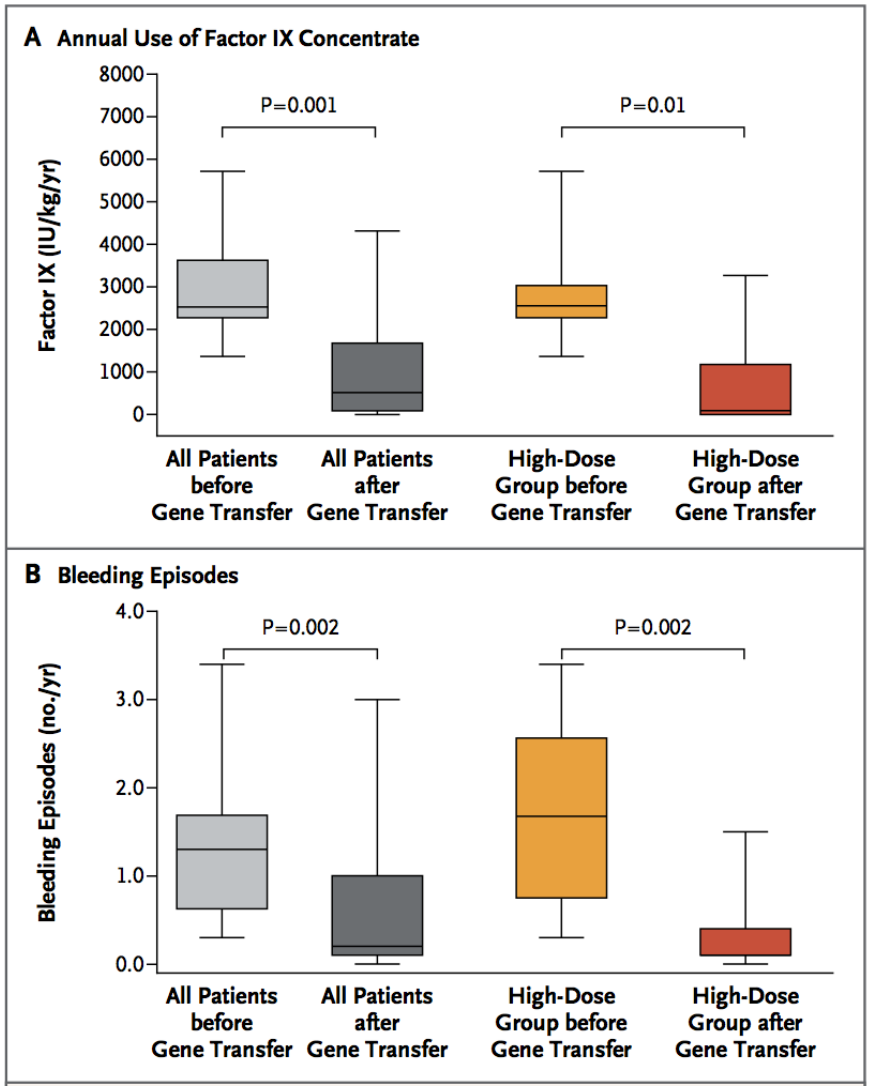
Rapid intervention in Subject 6 ($2x10^{12}$ vg/kg)
Mild liver enzyme elevation, Rescue FIX expression





NEJM 2011

Clinical Outcome: evidence of dose response in the efficacy: prophylaxis is feasible



UCL/St Jude's Trial: Update Nov 2016

- **High dose cohort (2 x 10^{12} vg/kg)**
  - **6 subjects**
  - **4 out 6 required steroids**

- **All cohorts (n=10)**
  - **FIX levels 2-8%**
  - **Follow up of 4 to 6.5 years**

- **Immune response to the AAV capsid is directly dependent on the vector dose *(no evidence at doses up to 6 x10^{11} vg/kg, n=4)***

Personal communication by A. Davidoff, SJCRH

Can we use lower the therapeutic vector dose?

Alternative serotypes

Transgene codon optimization

Hyperactive variants of clotting factor

FIX Padua (R338L): 8-10 fold higher specific activity

Table 1. Clinical Characteristics and Laboratory Data from the Family Members.*

Subject	Sex	Age (yr)	Activated Partial-Thromboplastin Time (sec)†	Factor IX Antigen (% of normal level)	Factor IX Activity (% of normal level)	Factor IX Activity-to-Antigen Ratio
II-1, proband	M	23	25.7	92	776	8.4
I-1	M	53	35.2	105	127	1.2
I-2	F	46	28.2	94	337	3.5
II-2	M	21	33.4	116	123	1.0
II-3	M	11	29.1	64	551	8.6

* II-1 refers to the proband, I-1 to his father, I-2 to his mother, II-2 to the older of his younger brothers, and II-3 to the youngest brother.
† The normal range for activated partial-thromboplastin time is 30 to 40 seconds.

Simioni P et al. N Engl J Med 2009;361:1671

FIX Padua (R338L): 8-10 fold higher specific activity

Robin Hood Strategy

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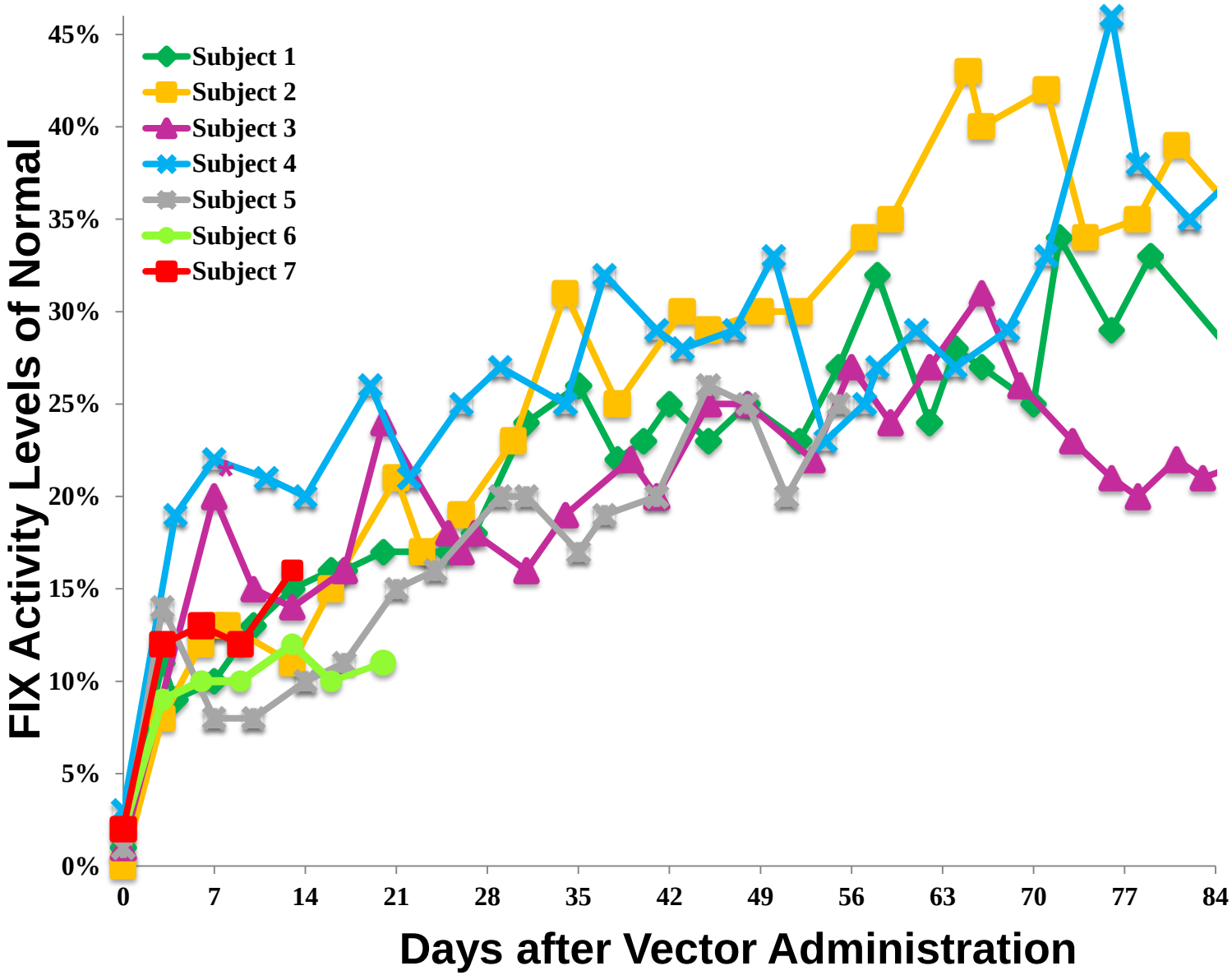
Preclinical studies in large animals: Finn et al Blood 2012, Crudele et al Blood 2015

AAV-FIX Padua trials

- **Low vector doses could avoid AAV-capsid-mediated immune responses**
- **Goal therapeutic levels without formation of antibodies to FIX variant**
- **AAV8 or Spark100 (comparable efficacy in preclinical studies)**

FIX:C in the first 12 weeks of the first 7 subjects: 5 x 10^{11}vg/kg



Spark Therap

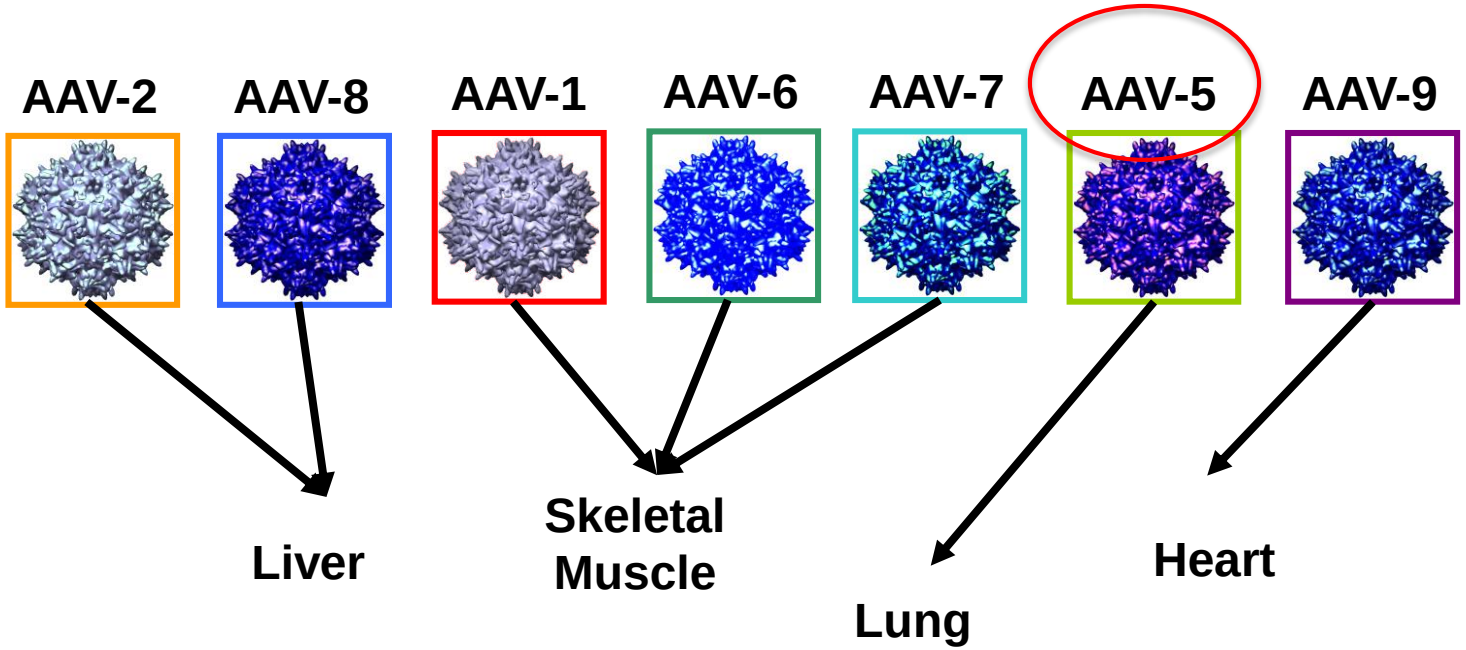
FIX Padua: Outcomes

1/7 subjects developed AAV-capsid mediated immune response, prednisone was sufficient to maintain therapeutic levels of FIX (ASH 2016)

Vector dose reduction is feasible but other factors may influence safety (HLA and expansion of pool of memory T cells specific for the vector capsid)

Together with Shire trial (total 15 subjects), no formation of inhibitor to FIX Padua

Efficacy and safety of AAV of alternate serotypes, capsid modifications and manufacture system?



Liver: relative limited efficacy

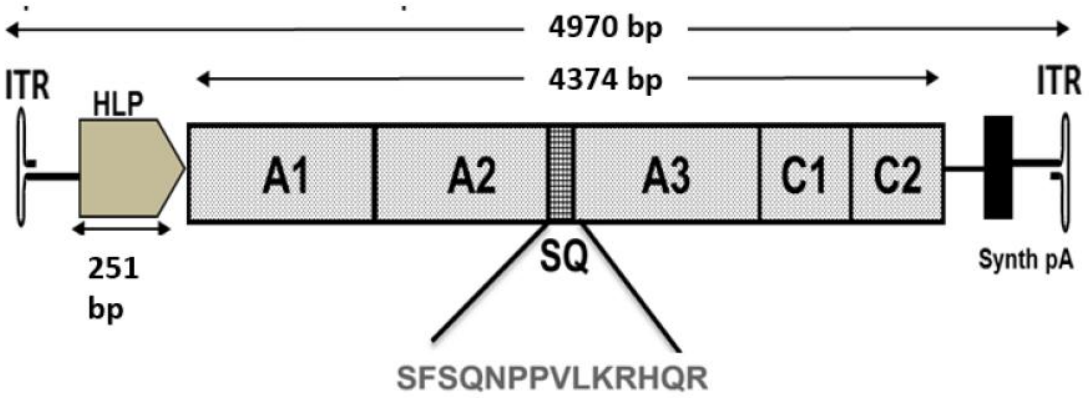
Gao et al PNAS, 2002

AAV serotype 5, FIX-WT, insect cells/baculo

uniQURE– WFH 2016 World Congress, July

Vector dose (vg/kg)	Subjects	FIX levels (% of normal)	Phenotype
5×10^{12}	1	No change	Annual Bleeding rates all: reduced by 68-80% 1/5 in prophylaxis
	1	2.9%	
	3	5.4-6.3%	
1 out 5 had increased transient elevation of ALT (after week 10 and received steroids) No sustained expansion of AAV5 capsid-specific T cell			

AAV-5 codon optimized FVIII B-domain deleted Insect cell lines/baculovirus expression system



BIOMARIN 270 – WFH July 2016

McInstosh Blood 2013

AAV-5 codon optimized FVIII B-domain deleted

BIOMARIN 270 – WFH July 2016

Vector dose (vg/kg)	Subjects	FVIII levels (% of normal)	High dose Cohort
6×10^{12}	1	No change	Annual Bleeding rates (from 20 to 5) After week 7 (no bleeds in 6/7)
2×10^{13}	1	> 2% (28 weeks)	
6×10^{13}	7	all > 10 %	
		Ongoing observation	FVIII 4/7 > 50% 2/7 > 150%

After the first subject in high dose cohort showed increased ALT at week 4, Prednisone was given prophylactically in all 6 patients (~10 weeks)
No data was shown on AAV5-specific T cell activation

AAV5-FVIII: Outcomes

No immune responses to FVIII

Subject 3 (the first in the high dose cohort): ALT

Increase to 1.5 fold baseline levels ~ 4 weeks, steroid therapy was initiated

Subjects 4-7 receive prophylactic steroid therapy starting week 4

No lost of expression of FVIII despite increase ALT

AAV5-FVIII: Problems

Unusual kinetics of expression (long time to reach plateau levels)

Supra-physiological levels were unexpected and the safety is yet to be determined

No clear dose-response (study design suboptimal)

Ongoing liver toxicity (subclinical) is a concern

Summary

35 subjects with HB (26) and HA (9) were enrolled in distinct clinical trials (AAV8, AAV5, Spark100)

Elevation of endogenous factor levels (>2%) are associated with improvement of the disease phenotype.

Some strategies are associated with predictable and consistent dose-response

AAV capsid-mediated T cell responses and lost of expression: vector-dose dependent and serotype-dependent

No sustained acute or chronic toxicity

No inhibitor formation to FIX (including 15 subjects with FIX Padua variant) or FVIII

Conclusion

AAV-mediated therapeutic levels of FVIII is feasible and the emerging safety data will be critical to define the best therapeutic strategies

Yes, we are doing better !

Alternative/Future Development

FVIII transgene with enhanced clotting activity: in other AAV systems (serotype/mammalian cell expression), mostly with modifications in the B domain

Peptide Linkers Substituted for the B-domain in FVIII variants

Variant Name	Category	Commercial Examples	PR(741)—Linker Sequence—(1649)EI	Ref
hFVIII-SQ	B-domain deleted	Xyntha, Eloctate CSL-627	—SFSQNPPVLKRHQR—	[12]
hFVIII-N8	B-domain truncated	NovoEight, N8 GP	—SFSQNSRHPSQNPPVLKRHQR—	[49]
pFVIII-OL		Obizur	—SFAQNSRPPSASAPKPPVLRHQR—	[30]
hFVIII-V3			—SFSQNATNVSNNSNTSNDSNVSPPVLKRHQR—	[50]
hFVIII-RH			—SFSQNPPVLKHHRQ—	[31]
hFVIII-ΔF			—SFSQNPPVLK—	
cFVIII-SQ			—SFSQNPPVSKHHQR—	[18]
cFVIII-ΔF			—SFSQNPPVSK—	

Siner and Samelson-Jones, JCI Insight October, 2016

Ref 50: McInstosh Blood, 2013

Alternative/Future Development

FVIII transgene with enhanced clotting activity: in other AAV systems (serotype/mammalian cell expression), mostly with modifications in the B domain

Side by side comparison of both manufacture system is needed

Understanding of ongoing liver damage (subclinical): is this vector serotype and/or manufacture related complications? Differences between FIX and FVIII (dose?)

Development of system to control expression in the event of supra-physiological levels (unlikely outcome in phase I/II)