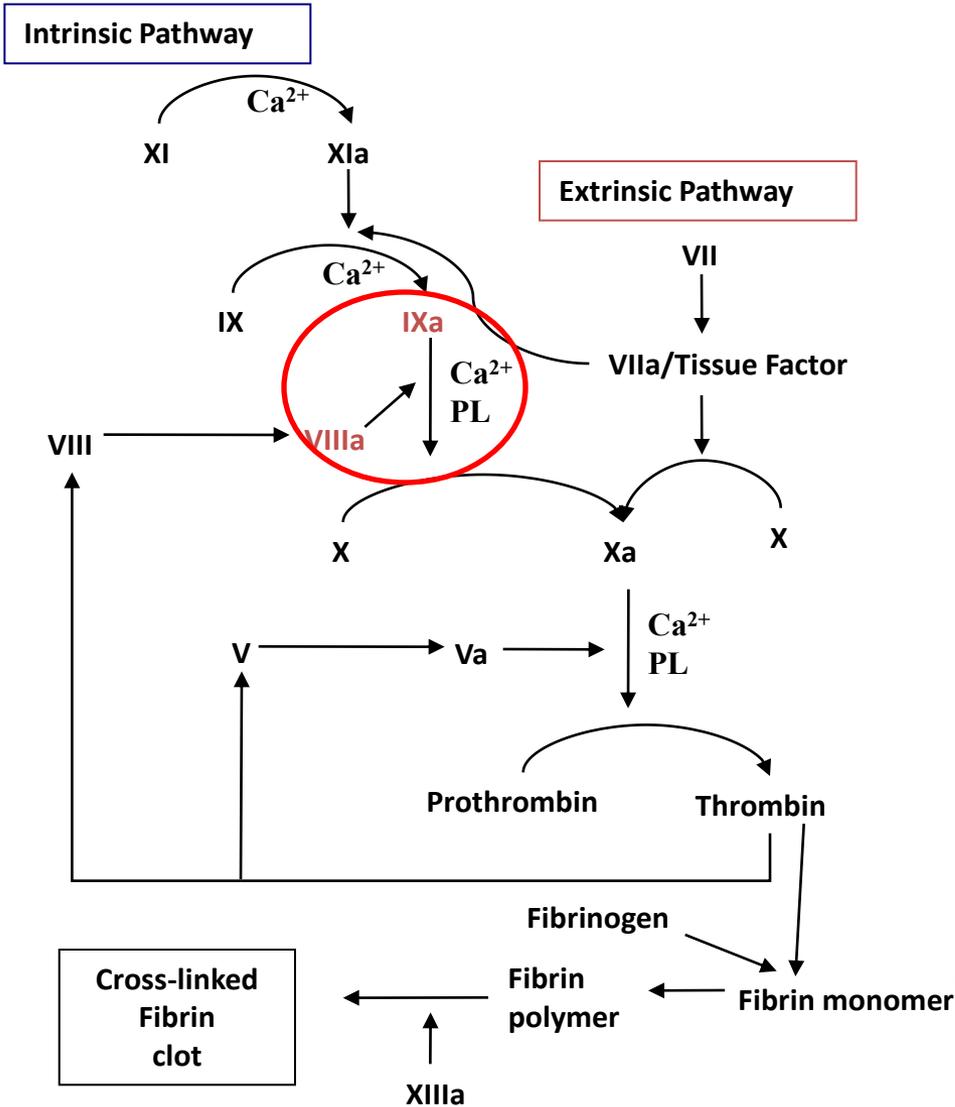


Coagulation and Hemophilia



Coagulation Cascade

- X-linked bleeding disorder caused by the absence of functional coagulation factor VIII (hem A) or factor IX (hem B)
- Characterized by frequent bleeds in joints and soft tissues, and less frequently into other critical closed spaces
- 1 in 5000 male births
- Severe <1%, moderate 1-5%, mild >5%

Severe knee bleed

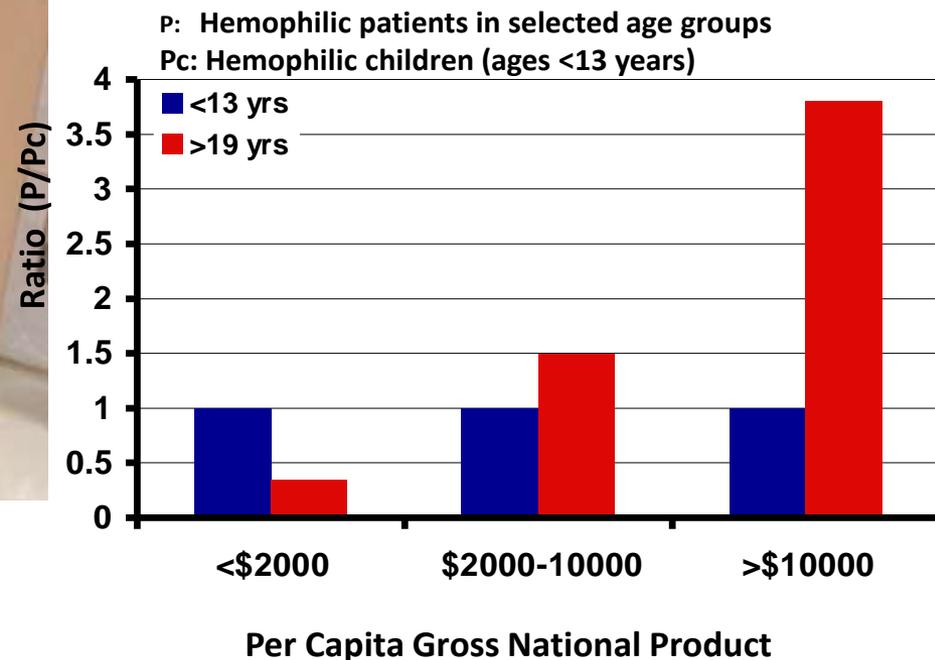


CURRENT TREATMENT OF HEMOPHILIA

IV infusion of recombinant or plasma-derived clotting factor concentrates



Estimated to be available
to ~20% of world's
hemophilia population



Key elements that make hemophilia an attractive model for gene therapy

- **Modest clotting factor levels required for therapeutic effect**
- **Latitude in choice of target tissue**
- **Precise regulation of expression not required**
- **Factor IX gene is small and fits easily into most viral vectors**
- **Small and large animal models exist**

First and second generation gene therapy strategies

	First generation trials		
	Vector	Target Tissue	Reference
Hemophilia A	Plasmid	Autologous fibroblasts	Roth et al., NEJM 2001
	Retroviral	Intravenous	Powell et al., Blood, 2003
Hemophilia A	Helper-dependent Adenoviral vector		-
Hemophilia B	AAV-2	Muscle	Kay et al., Nat Genet., 2000
Hemophilia B	AAV-2	Liver via hepatic artery infusion	Manno et al., Nat Med., 2006

Second generation trials or related pre-clinical strategies		
Vector	Target Tissue	Reference
Lentiviral	Ex vivo transduction of hematopoietic stem cells	1) Yarovi et al., Blood, 2003 2) Shi et al., J Thromb Haemost., 2007
Helper-dependent Adenoviral vector	Liver	Brunetti-Pierri et al., Hum Gene Ther., 2005
AAV	Skeletal Muscle by intravascular approach	Arruda et al., Blood 2010
AAV8	Liver via IV infusion	-

Gray shaded: clinical trial; blue shade: pre-clinical strategies

First generation trials all first in class, all demonstrated safety.

Phase I/II trial of AAV2-mediated liver-directed gene transfer for hemophilia B

Open-label dose-escalation design

Three dose cohorts:

8×10^{10} vg/kg

4×10^{11} vg/kg

2×10^{12} vg/kg

Hepatic artery infusion of vector

Measure: CBCs, LFTs, F.IX levels,
inhibitory antibodies



Subject Demographics

	A	B	C	D	G	E	F
Age (yrs)	63	48	21	20	27	31	28
Ethnicity	Cauc.						
HIV Ab	Neg	Pos	Neg	Pos	Neg	Neg	Neg
HCV Ab	Pos	Pos	Pos	Pos	Neg	Pos	Pos
HCV RNA	Neg	Pos	Pos	Pos	Neg	Neg	Neg
Liver Biopsy	N/A	F1	F0	F1	N/A	N/A	N/A

Anti-AAV

1:2

1:17

Risk of inadvertent germline transmission

Trial halted after gene shows up in semen

Nell Boyce, Washington

A patient in a gene-therapy trial has had the virus used to transfer the gene show up in his semen. The finding highlights the danger that gene therapy may inadvertently modify the genetic make-up of a patient's children.

The discovery was made in a trial at the Children's Hospital of Philadelphia, conducted in collaboration with researchers at Stanford University Medical Center in California. Officials at the Food and Drug Administration (FDA) halted the trial.

But the Recombinant DNA Advisory Committee (RAC), which advises the FDA on the safety and ethics of such experiments, announced on 6 December that the trial could continue, subject to special monitoring. The FDA is expected to follow this advice.

Gene therapy often uses viruses to transport therapeutic genes into cells. But these



Hopes and fears: could a therapeutic gene be inadvertently passed on to future generations?

months — the time it takes for sperm to mature. But Kay's team plans to enrol nine patients in the trial one after the other, so this

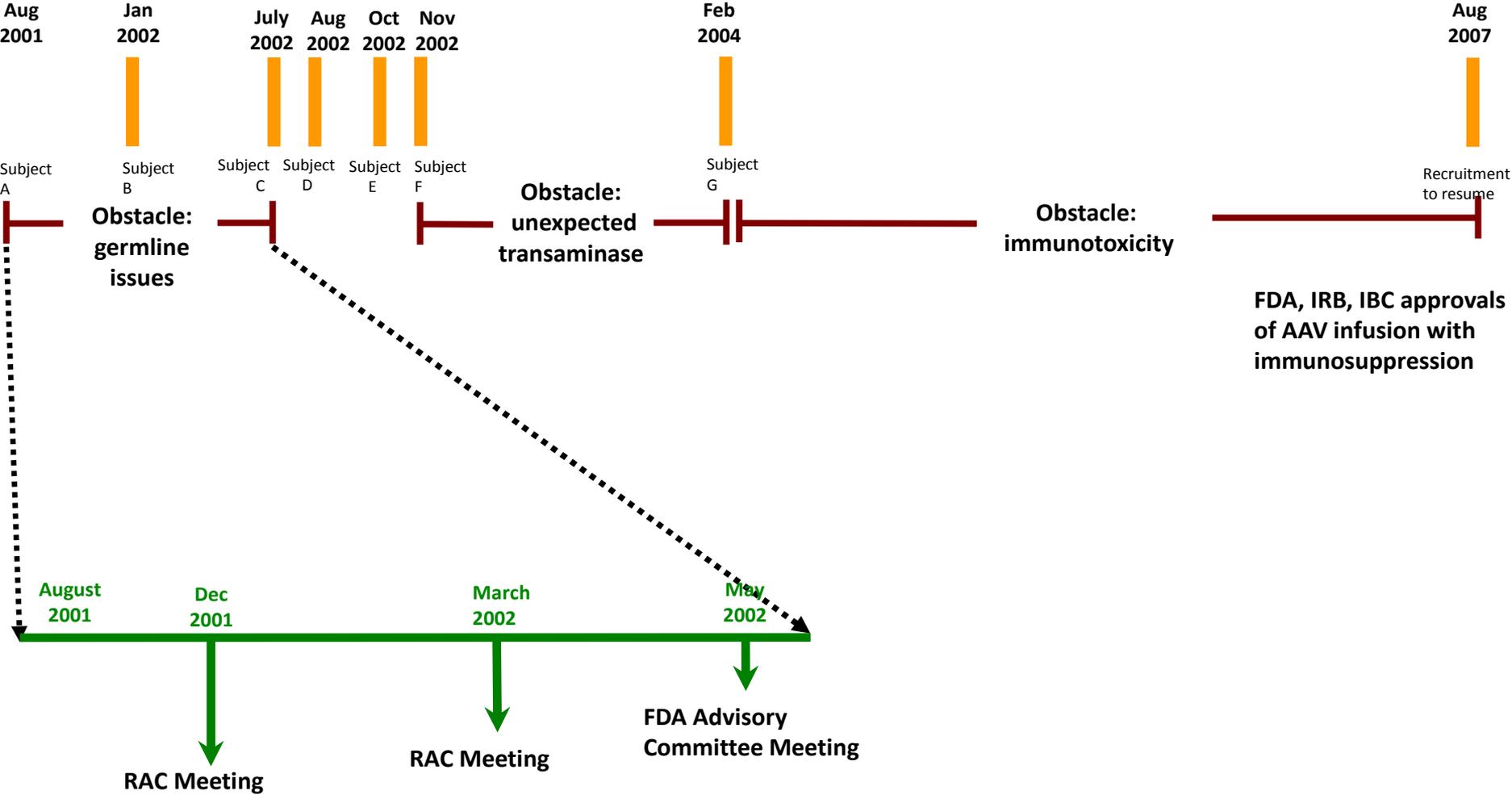
could extend it for up to five years, Kay asserts. He says the risk of children being affected is reduced because patients are advised to use condoms. "If you look at the realm of other risks that people take in drug studies, this one is relatively small," he says.

The RAC has agreed, recommending that the trial be allowed to continue, provided researchers conduct frequent tests on isolated cell types from patients' semen samples.

The ethical issues will deepen only if researchers find that an effective gene therapy also shows up in sperm. "We're dealing here with the grey area in which you may knowingly allow something but not deliberately do it," says Ruth Macklin, a bioethicist at the Albert Einstein College of Medicine in New York, and a member of the RAC. ■

Finding was not predicted by initial animal models or by human experience from the AAV-hF.IX muscle trial

Timeline of AAV-F.IX liver trial

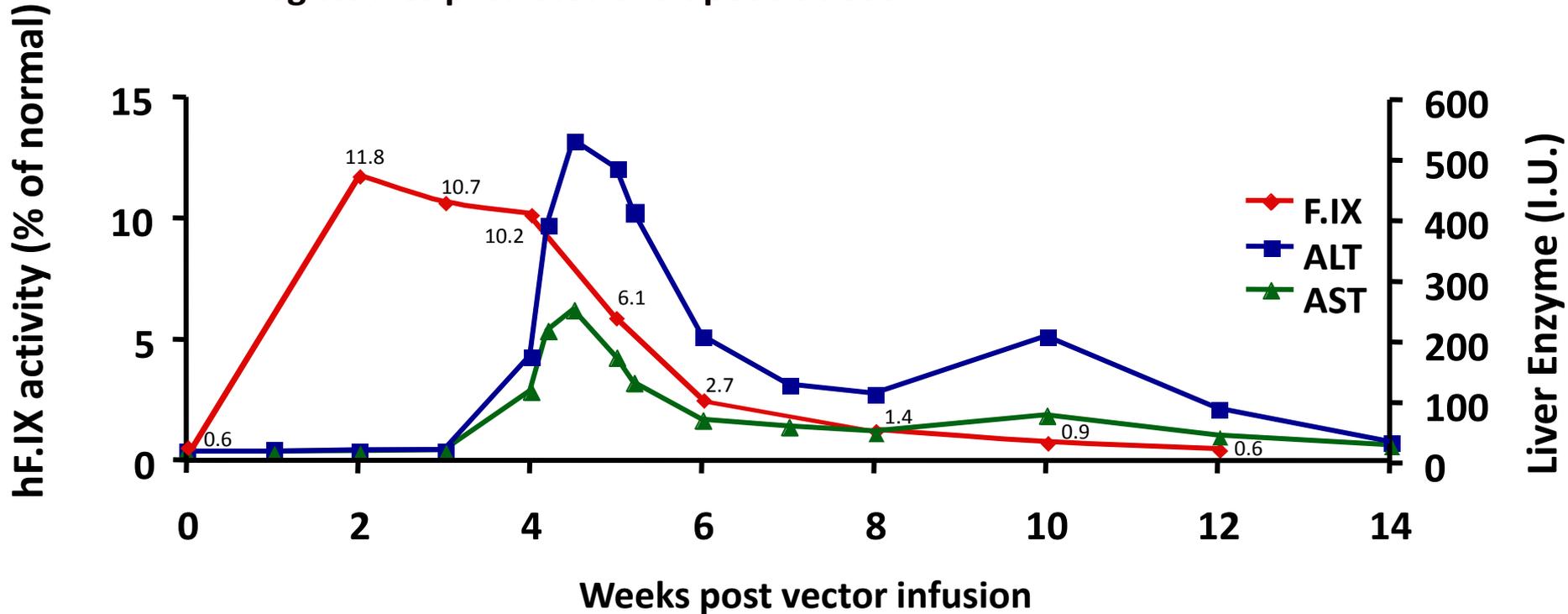


TRIAL PROTOCOL AMENDED TO MITIGATE RISK

- Monitor for presence of vector sequences in semen.
- Encourage contraception until vector sequences are no longer detectable.
- Encourage subjects to bank sperm
- We utilized model to assess risk with other AAV serotypes

Coagulation Studies: Subject E

Dog studies predicted therapeutic dose

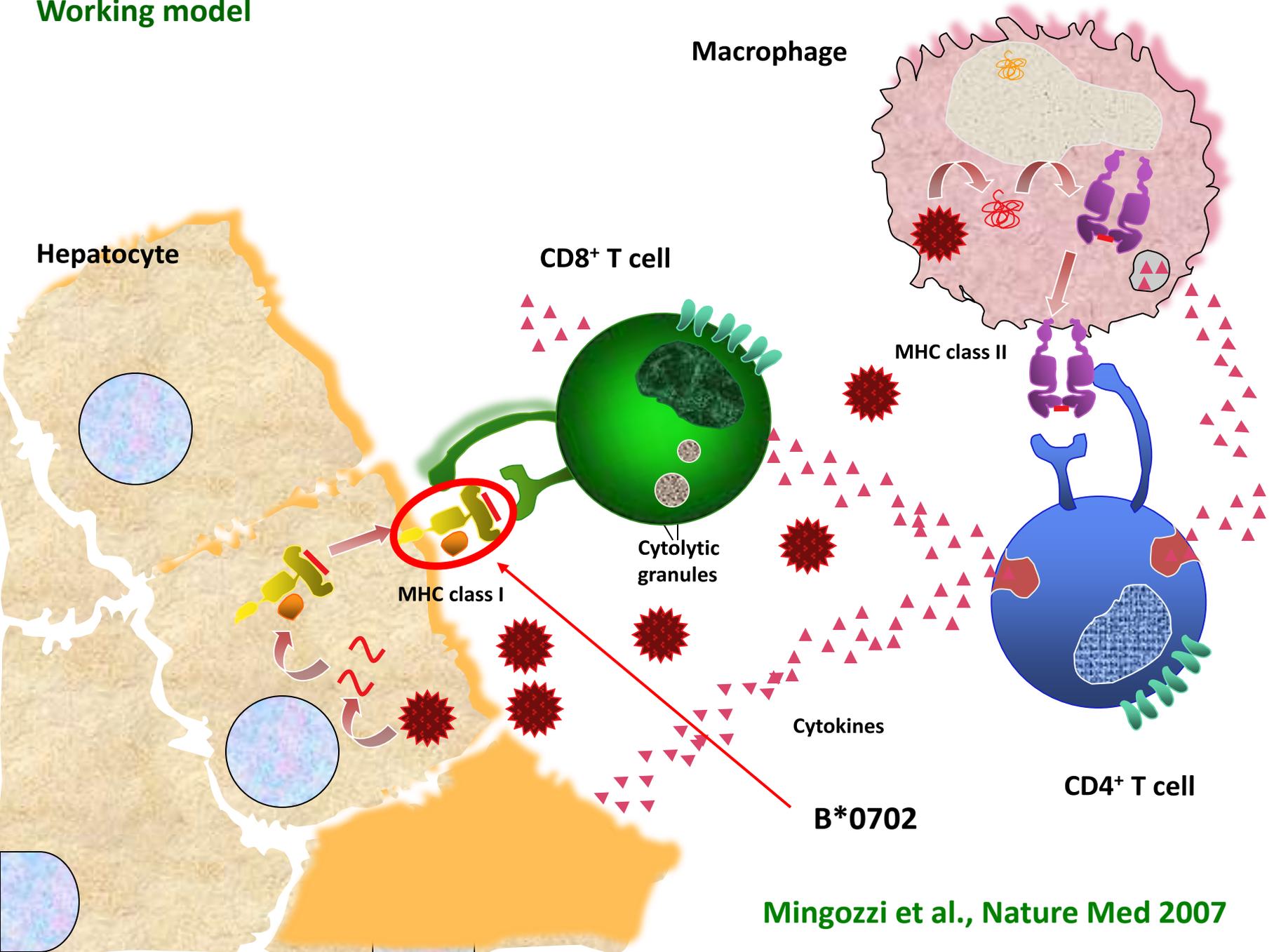


- All other chemistries and CBCs remained normal
- No F.IX inhibitor
- No evidence of HAV, HCV, HBV, EBV, CMV, Toxo

Observations from highest dose infusion in humans

- Identified therapeutic dose of vector
- Observed: ↓↓ in F.IX levels,
↑↑ liver enzymes
 - Began 4 weeks post vector infusion
 - Not seen in animal models

Working model

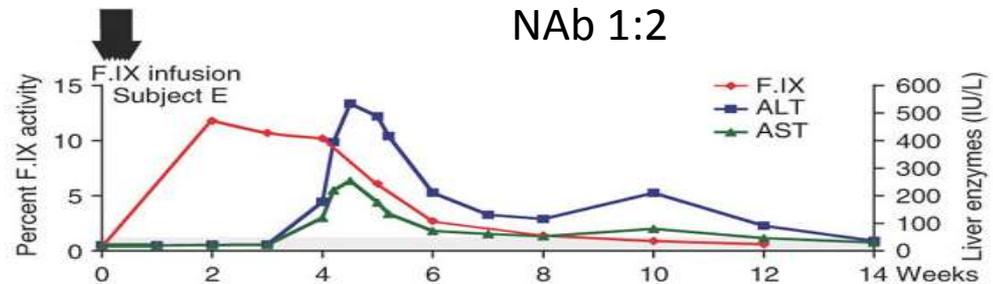


Cause of transaminitis & decrease in FIX levels

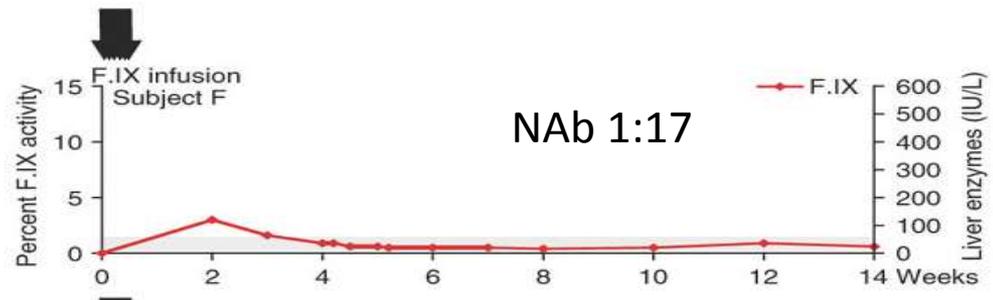
Underlying Cause	Prediction
Pre-formed capsid	Short-term IS will work
Rep/cap plasmid has been packaged	Short-term IS will <u>NOT</u> work
Alternative ORFs	Short-term IS will <u>NOT</u> work
Heparin binding site results in immunogenicity	AAV8 will <u>NOT</u> trigger immune response

Neutralizing antibodies to AAV block transduction

Subject E
High Dose
Therapeutic Level



Subject F
High Dose
NAb to AAV Blocked
Hepatocyte Entry



Advances in first trial

- **Vector transduces human liver and can direct therapeutic levels of expression**
- **But the host immune response limits duration of expression through CD8+ T cell response**
- **And neutralizing antibodies to AAV block transduction altogether at titers >1:5**
- **Risk of vertical transmission is low, mitigated by banking semen and using barrier birth control til semen negative**

UCL/St. Jude trial

- **Tests hypothesis that more efficient vector can direct gene expression at lower vector dose and thus avoid immune response**
- **Uses three features to generate a more efficient vector**
 - **AAV8 serotype**
 - **Self-complementary vector design**
 - **Codon-optimized F.IX construct to enhance translational efficiency**

AAV8-F.IX trial

University College London/Royal Free/St. Jude's

- **Peripheral vein infusion**
- **First subject infused in March 2010 at a dose of 2×10^{10} vg/kg**
- **9 subjects infused so far, no serious adverse events**

**More experience with sc vectors
has revealed a titering discrepancy
due to inefficiency of PCR in setting
of sc vectors**

When vectors re-titered using physical methods, they are similar to doses used in AAV2 trial

Dose cohort	Initial titers (vg/kg)	Re-titered vector (vg/kg)	AAV2-F.IX trial (vg/kg)
Low	2×10^{10}	2×10^{11}	8×10^{10}
Medium	6×10^{10}	6×10^{11}	4×10^{11}
High	2×10^{11}	2×10^{12}	2×10^{12}

Data on re-titering presented at RAC in Dec 2010

http://oba.od.nih.gov/rdna_rac/rac_past_meetings_2010.html#RAC2010

And published Fagone et al., Human Gene Therapy, 2011

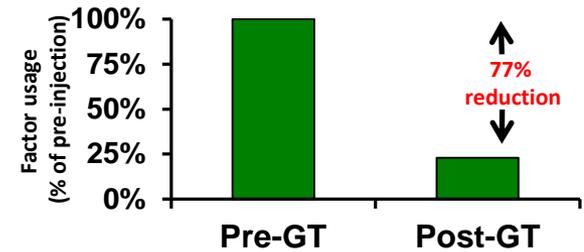
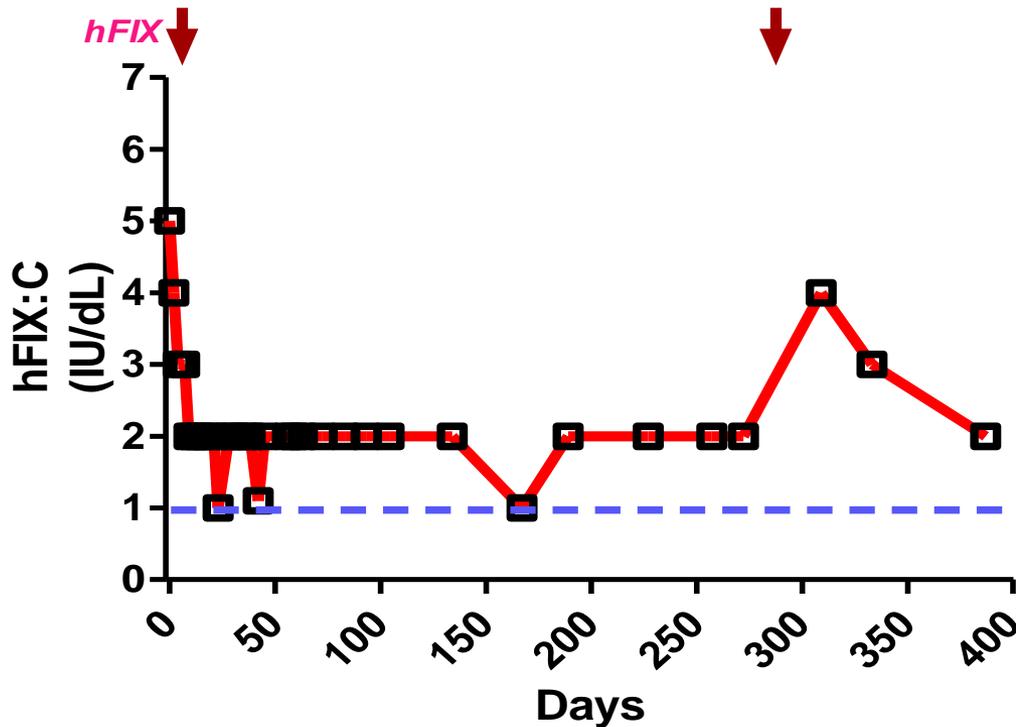
Factor IX levels in UCL/St. Jude trial

Dose (vg/kg)	Plateau Factor IX levels
2×10^{11}	~2%
6×10^{11}	2-3%
2×10^{12}	2-8%

NEJM 2011

Plasma FIX levels

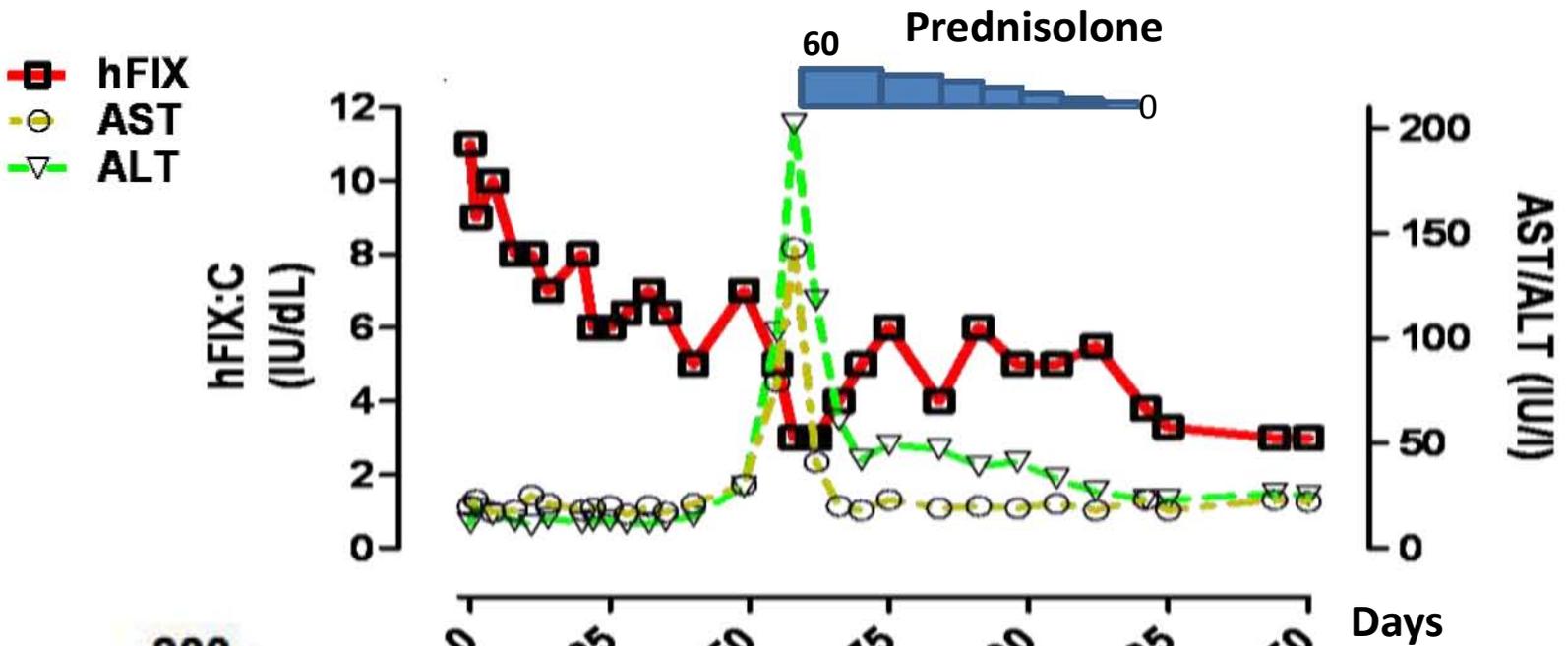
Low vector dose (2×10^{11} vg/kg): 1st subject (P1)



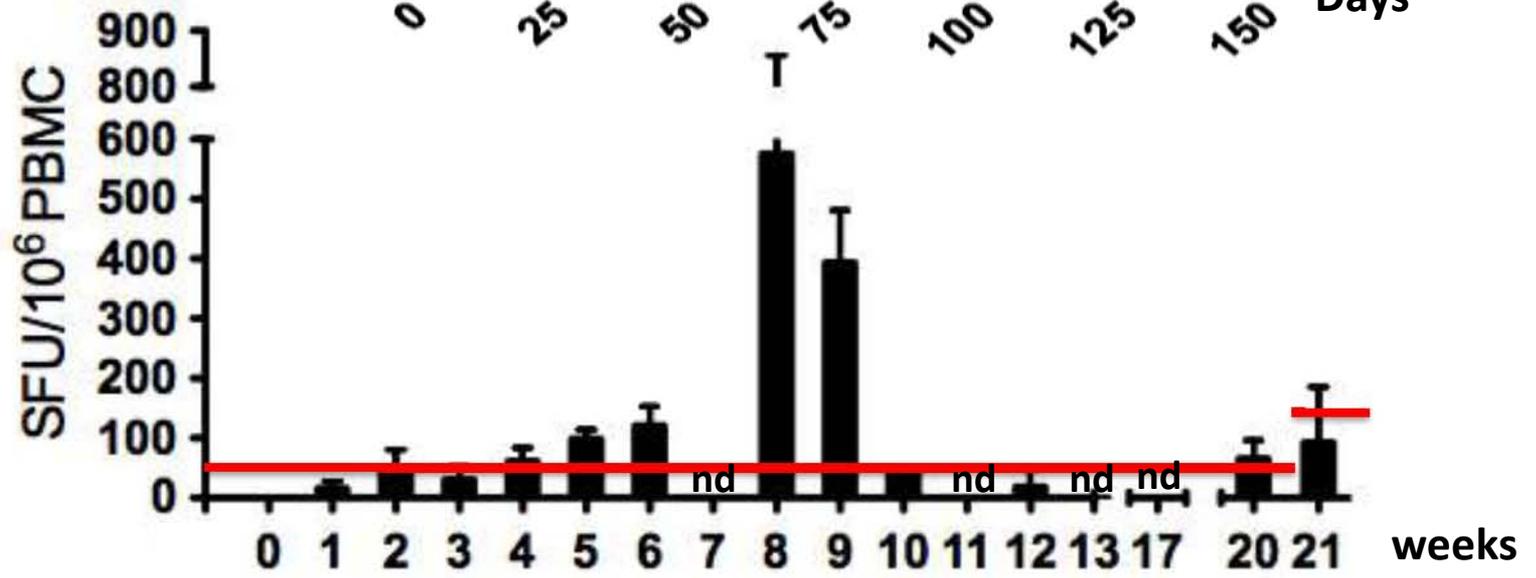
- Off prophylaxis >26 months → FIX level @ 1-2%
 - except prior to a minor surgical procedure
- No spontaneous bleeding episodes
 - received vector 26 months ago

Further dose escalation to 2×10^{12} vg/kg results in capsid T cell expansion and liver enzyme elevation in subject 5

S5

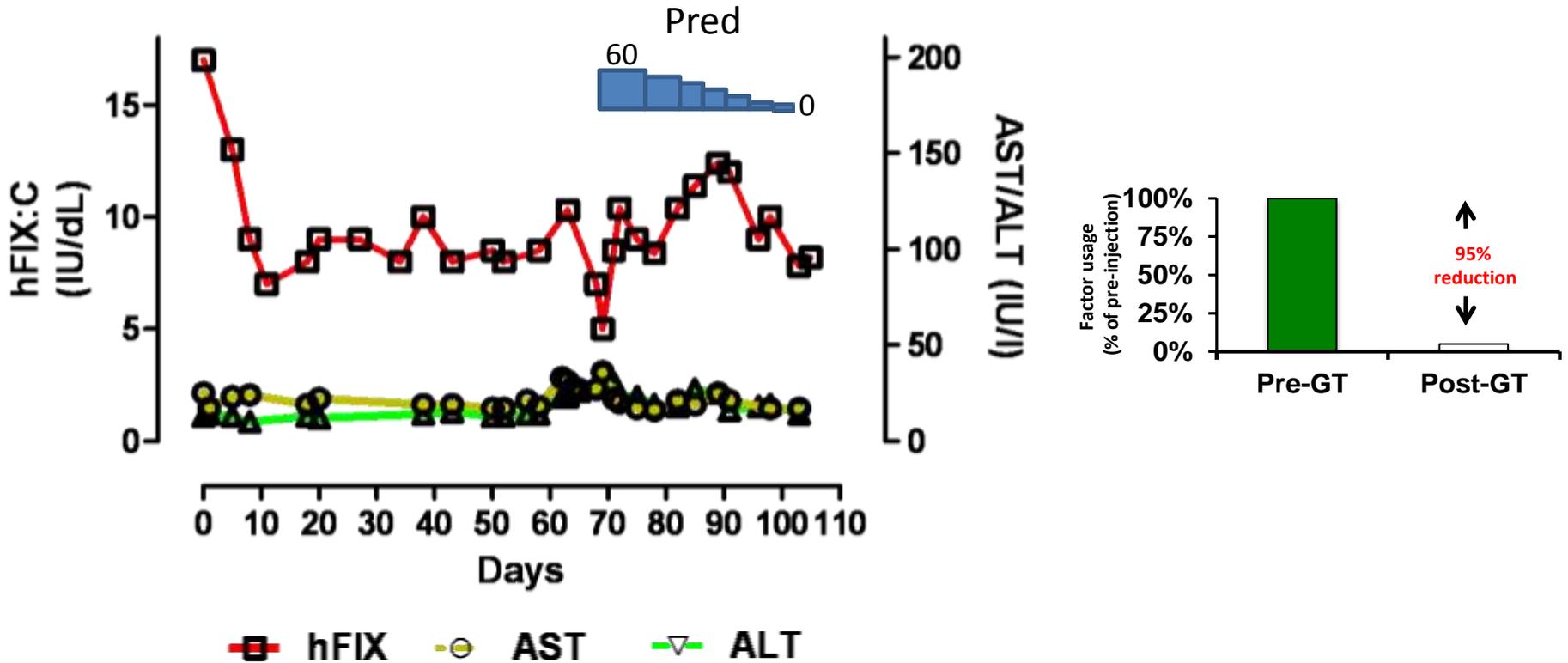


Capsid
 IFN- γ
 ELISPOT



Plasma FIX levels

High vector dose (2×10^{12} vg/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 off steroids
 - LFT's back to baseline, FIX level @ 8.5%
 - Received vector >1 year ago

High dose cohort

- All subjects achieved higher circulating F.IX levels, one in the range of 2%, the others in the range of 6%
- Transaminase elevation is asymptomatic, but serves as a marker of immune response
- Course of steroids appears to “rescue” at least some of the transduced hepatocytes from immune-mediated destruction
- Risk/benefit would favor a short course of steroids, if required, in exchange for a better circulating Factor IX level in most hemophilia patients

Advances in second trial

- Immune response to the vector can be managed by a course of high dose steroids
- Use of a capsid serotype (AAV8) with strong tropism for liver means vector can be infused intravenously

Next steps for UCL/St.Jude trial

- **Determine safety and efficacy of current vector at 2×10^{12} vg/kg, combined with immunomodulation**
- **Determine what percentage of adult subjects will require immunomodulation**
- **Determine whether reduction of empty capsid in product will improve safety and efficacy, and allow higher doses**

Next steps for field

- **Determine whether the same approach can be extended to F.VIII**
- **Determine how to dose individuals with antibodies to AAV**
- **Monitor for long-term side effects of AAV gene transfer into liver**
- **Devise treatment strategies for those with severe liver disease**

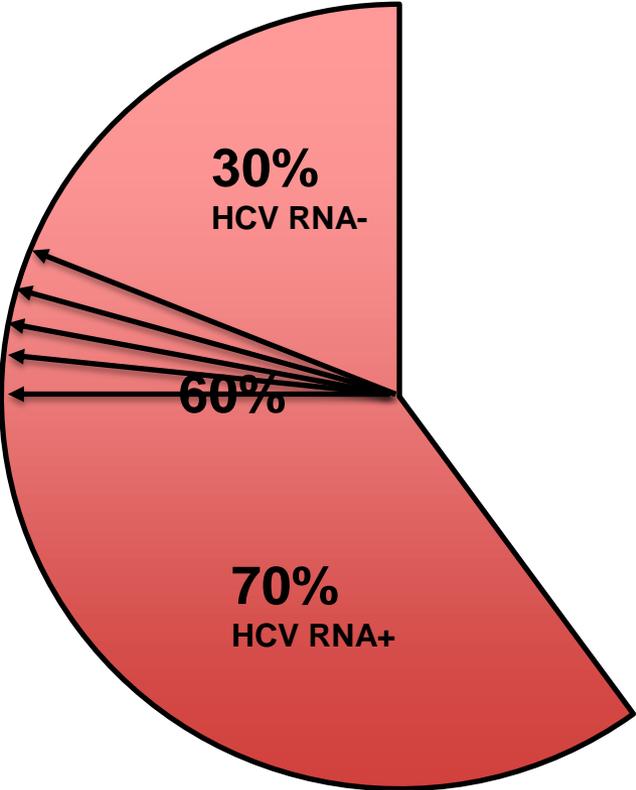
Where are we in 2012?

- **We have defined a group of patients who can expect long-term expression of F.IX after AAV-F.IX infusion**
 - **Adults**
 - **No or low-titer neutralizing antibodies to AAV**
 - **HCV RNA viral load negative, if steroids to be used**

All Adults with Hemophilia B

■ Pre-existing Anti-AAV N.Ab

■ No Anti-AAV N.Ab



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Typical Inclusion Criteria in AAV Gene Therapy Trials, Current and Proposed

Inclusion:

- **Severe Hem B: F.IX \leq 2%**
- **Age \geq 18 years**
- **HCV RNA viral load negative ¹**
- **HIV negative, or HIV positive and stable on HAART ²**
- **No previous history of F.IX inhibitor**
- **At least 20 exposure days to F.IX concentrate.**
- **Anti-AAV neutralizing antibody titer \leq 1:5**

¹ Earlier trial included subjects who were HCV RNA viral load positive but they are now excluded because of potential need for course of steroids.

² Some trials may include HIV-positive individuals who are stable with adequate CD4 counts on HAART