

# ***Retroviral Vector for FVIII Gene Transfer: Preclinical & Phase I***

---

Doug Jolly

Advantagene Inc.

ASGT Gene Therapy Stakeholders Meeting

April 7-8 2005

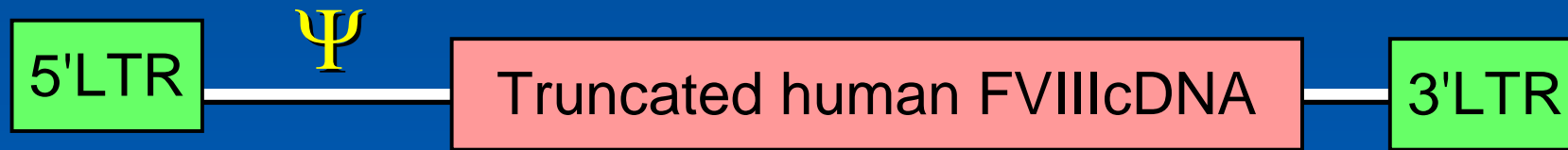
# Clinical Experience in GT Retroviral Vectors Ex Vivo

Start Year	Disease target	Cells transduced	Route	# of Sbjcts.	Vector Titer
93	HIV	Autologous Fibroblasts	IM	4	$10^6$
93	Cancer	Autologous Tumor	SC	24	$10^6$ - $10^7$
96	HIV	Autologous Hemopoietic Stem Cells	IV	10	$10^8$
98	Cancer	Allogeneic T cells	IV	24	$10^8$

# Clinical Experience with retroviral vectors, directly administered

Start Year	Disease target	Route of delivery	# of Sbjcts.	Product Titer	Max Total Dose
94	HIV	IM	230	$10^7$	$3.6 \times 10^8$
95	Cancer	Intratumor	30	$10^7$	$6 \times 10^8$
97	Cancer	Intratumor	16	$10^7$	$2 \times 10^8$
99	Hemophilia A	IV	13	$10^9$	$5 \times 10^{10}$

# Retroviral Vector Expressing Human Factor FVIII [hFVIII(v)]



- Amphotropic MLV vector made in human (HT1080) packaging cell line to confer complement resistance
- Genes for B-domain deleted hFVIII inserted
- Administered by peripheral vein injection
- High titer vector produced

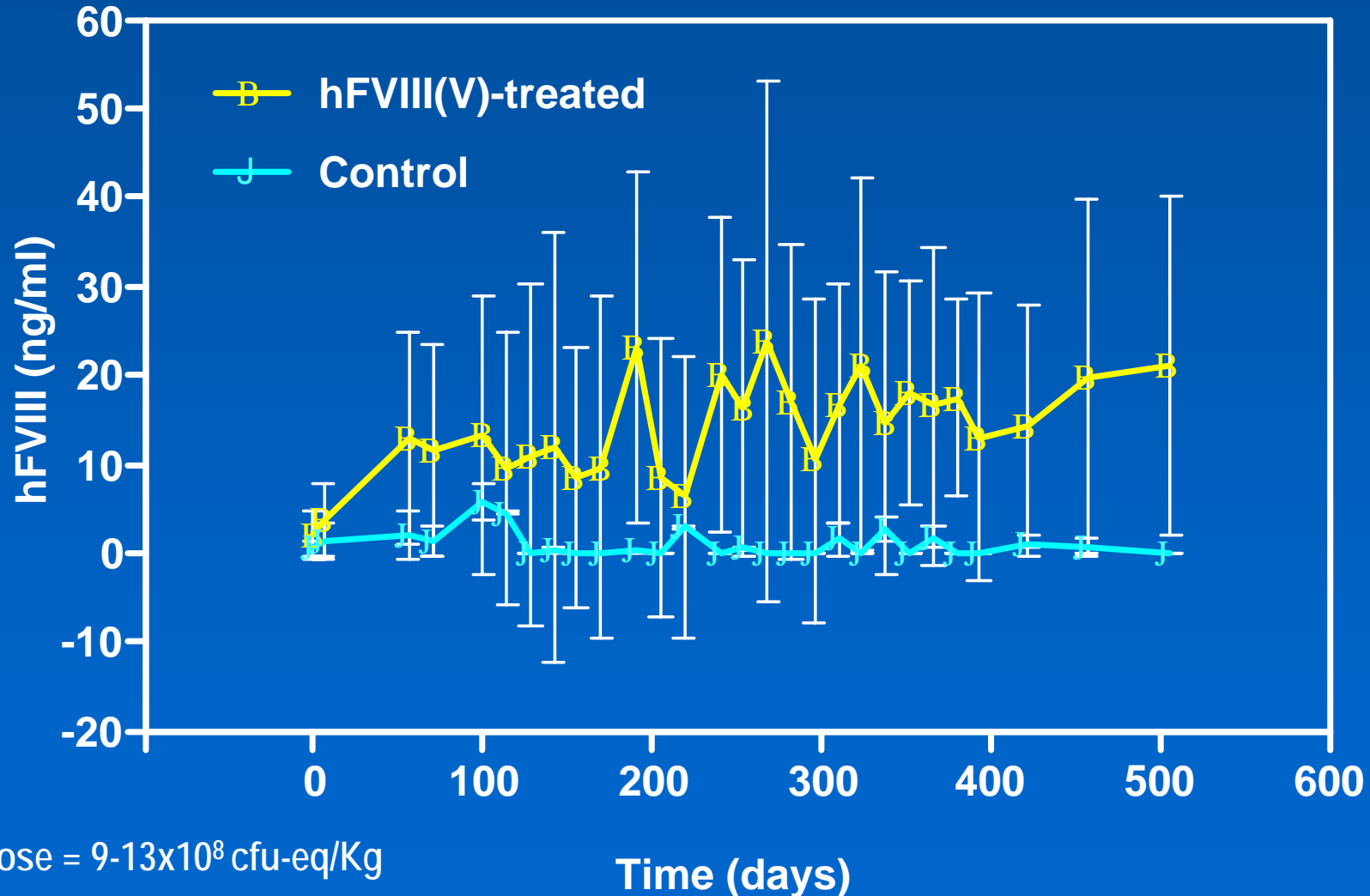
# Animal and Human Studies with FVIII RVV

Species	Parameters	Number Treated	Max Dose (Tu/kgx10e8)
Normal Mice	Toxicity	48	6 - 96
Normal Rabbits	FVIII in blood, Ab's, Toxicity, Biolocalization (inc. semen) Dose response etc.	Several 100	0.07 - 60
Normal Dogs	FVIII in blood, Ab's, Toxicity	2	6-9
Hemophilic dogs	Efficacy, Ab's Toxicity	16	4-18
Humans with Hemophilia A	Toxicity, Biological activity	13	0.28 - 8.8

# Animal and Human Studies with FVIII RVV

Species	Result summary
Normal Mice	No toxicity at highest dose ( $90 \times 10^8$ TU/kg)
Normal Rabbits	Reliable therapeutic levels of FVIII starting at $0.7 \times 10^8$ Tu/kg. Delayed appearance (20-30 days), long term expression (2 years). Vector DNA in liver and spleen Testes-low level PCR +, Semen-. AntiFVIII Ab's
Normal Dogs	One long term expression (1 year), One transient, but boostable
Hemophilic dogs	Long term FVIII protein in about 30%, lowered clotting times in almost all to 2years, anti-hFVIII - may mask hFVIII
Humans with Hemophilia A	No excess toxicity, most subjects made 1-3% at some point, no strong evidence of reduced factor use

# Levels of Human FVIII Persist After Treatment of Rabbits with hFVIII(V)



# Dose Response Study of hFVIII(V) in Rabbits

**Purpose:** To determine the relationship between hFVIII(V) dose and circulating levels of hFVIII in rabbits

**Study Design:**

Group	Number of Males	Dose Level (cfu-eq/animal)	Mean Relative Dose X 10 <sup>8</sup> (cfu-eq/kg)
1 (Control)	6	0	0
2 (Low)	6	1 x 10 <sup>7</sup> (1 x 10 <sup>9</sup> )	0.07 (3.5)
3 (Low-mid)	6	3 x 10 <sup>7</sup>	0.22
4 (Mid)	6	1 x 10 <sup>8</sup> (1 x 10 <sup>9</sup> )	0.7(3.5)
5 (Mid-high)	6	3 x 10 <sup>8</sup>	2.1
6 (High)	6	1 x 10 <sup>9</sup>	7

Note: Values in parentheses indicate additional hFVIII(V) administered on Days 111, 112, and 113 and at total cumulative dose (cfu-eq/kg)

# Dose Response Study of hFVIII(V) in Rabbits

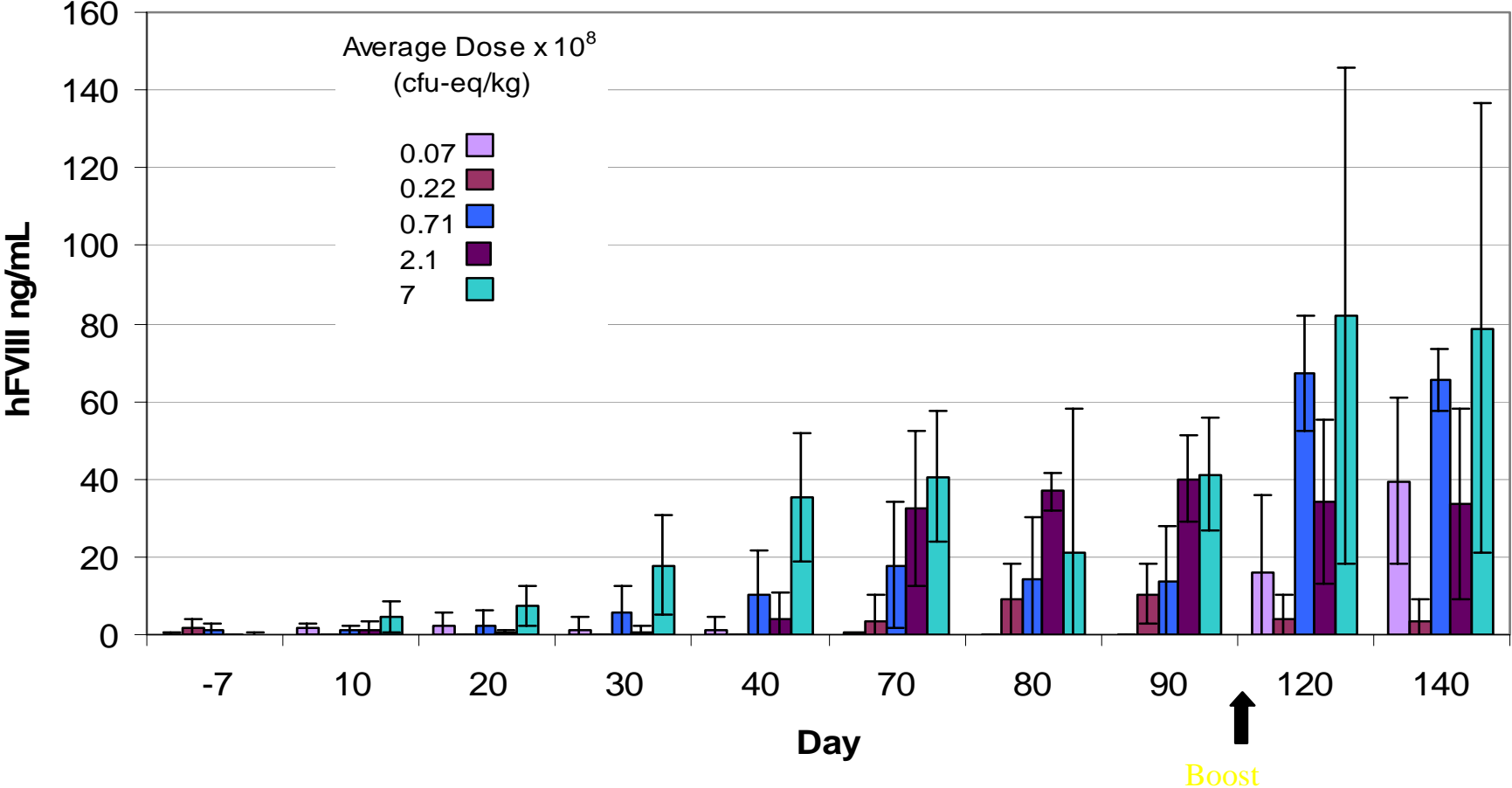
---

## Study Specifics:

- Animal Age: 8-10 weeks old
- Study Length: 6 months
- Treatment Schedule: 3 injections on each of days 1, 2 and 3
- Endpoints: hFVIII levels by ELISA, anti-vector antibodies, biolocalization of vector by PCR
- Safety Parameters: clinical observations, clinical pathology, body weights, macroscopic observations at necropsy

# DOSE RESPONSE TO hFVIII(V) IN RABBITS

Human Factor VIII Levels in Rabbits Given Different Doses of hFVIII(V)



## **In general, increasing levels of human factor VIII were seen with increasing hFVIII(V) dose**

- Despite inter-animal variability, there was a positive correlation between dose and the circulating level of human factor VIII
- The lowest dose associated with mean human FVIII levels of 14 ng/ml was  $0.71 \times 10^8$  cfu-eq/kg
- Expression of human factor VIII persisted through day 140
- A second hFVIII(V) treatment boosted the level of human factor VIII
- At a mean dose of  $2.1 \times 10^8$  cfu-eq/kg, all animals produced  $\geq 18$  ng/ml by day 70

# Hemophilic Dogs after hFVIII(V) Treatment

Whole blood clotting time in hemophilic dog #B28



Dose =  $14 \times 10^8$  cfu-eq/Kg

# Phase I Eligibility

- Severe hemophilia A (FVIII < 1%)
- Adult (> 18 years)
- No FVIII inhibitor - present or past
- On demand treatment
- Barrier contraception required
- If HIV positive, CD4 > 300 cells/mm<sup>3</sup> and not on reverse transcriptase medication
- If hepatitis C positive, no liver failure; no interferon or ribavirin within 3 months

# Phase I: Enrollment

- 13 subjects treated
- 5 dose levels (Tu/kg) :  $2.8 \times 10^7$ ,  $9.2 \times 10^7$ ,  $2.2 \times 10^8$ ,  $4.4 \times 10^8$ ,  $8.8 \times 10^8$ . 3 at each of first 4 doses, 1 at dose 5
- Median age:  $37.5 \pm 14.7$  years (range 18-55)
- Viral serology: 13/13 HCV+ , 5/13 HIV+
- Study duration 53 weeks (11 completed study, others left at 12, 24 weeks)

# Phase I trial results summary

- No serious adverse events
- FVIII levels between  $> 1\%$  (max 19%) seen in 23% of observations
- Dose levels well in excess of effective animal doses
- No inhibitor (anti-FVIII) observed, in fact clearance time for infused FVIII at 12 weeks was longer compared to baseline clearance time
- One transient semen PCR positive in 1/10 test samples at one time point in one subject was observed
- Powell et al Blood (2003) **102**:2038-2045.

# FVIII levels in sera of subjects at Dose levels 1 and 5

<b>Subject ID/Dose</b>	<b>Number of FVIII values <math>\geq</math> 1% /Number of observations</b>	<b>Study Days</b>	<b>FVIII Activity (%)*</b>
<b>Dose 1</b>			
<b><math>2.8 \times 10^7</math> Tu/kg</b>			
<b>02001</b>	<b>2/23</b>	<b>113, 315</b>	<b>1.0, 1.8</b>
<b>05001</b>	<b>1/8</b>	<b>93</b>	<b>6.6</b>
<b>05002</b>	<b>10/22</b>	<b>10 - 297</b>	<b>1.0 - 3.0</b>
<b>Dose 5</b>			
<b><math>8.8 \times 10^8</math> TU/kg</b>			
<b>05007</b>	<b>2/37</b>	<b>11, 56</b>	<b>1.4, 2.1</b>

# Questions and Experience from this trial

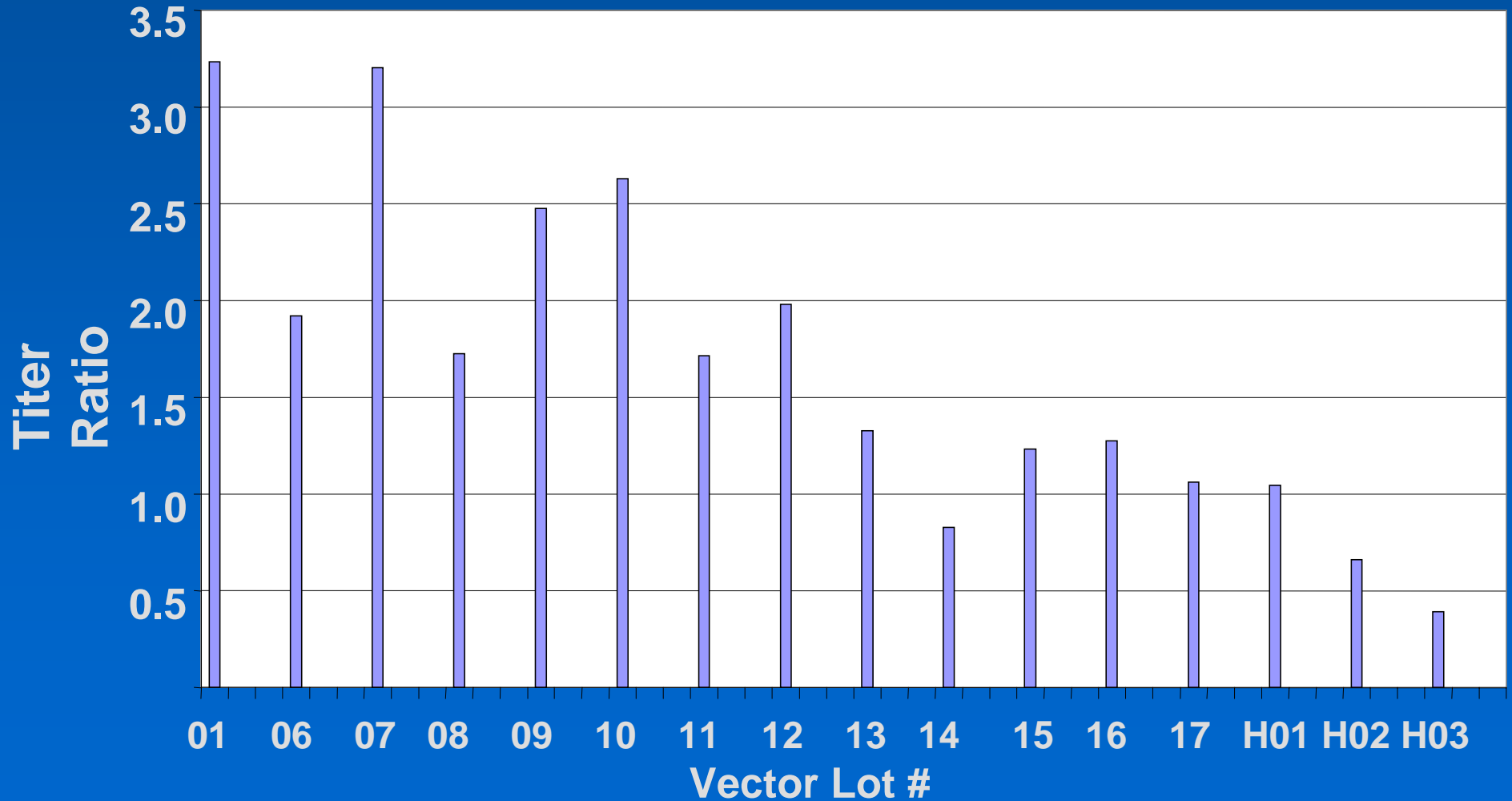
- Why did the vector work well in animals but scarcely in human subjects?
- PCL Performance and production
- Semen/testes studies
- Dosing Regimen 1vs 3 vs 9 injections
- Transduction events vs insertional mutagenesis
- Cost of trial
- Can you make money?

# Why did the vector work well in animals but scarcely in Human subjects?

- Animal models were not predictive
- Likely due to vector human interaction being different: complement was accounted for, possibly interaction with human restriction on MLV (maps to *gag*)- succeed with higher dose or modified vector?
- Lesson? Rumsfeld #4: unknown unknowns

# Different preparations of vector from the same PCL may have different properties

EXAMPLE: Ratio of PCR (DNA transduction) to FVIII Expression Titters

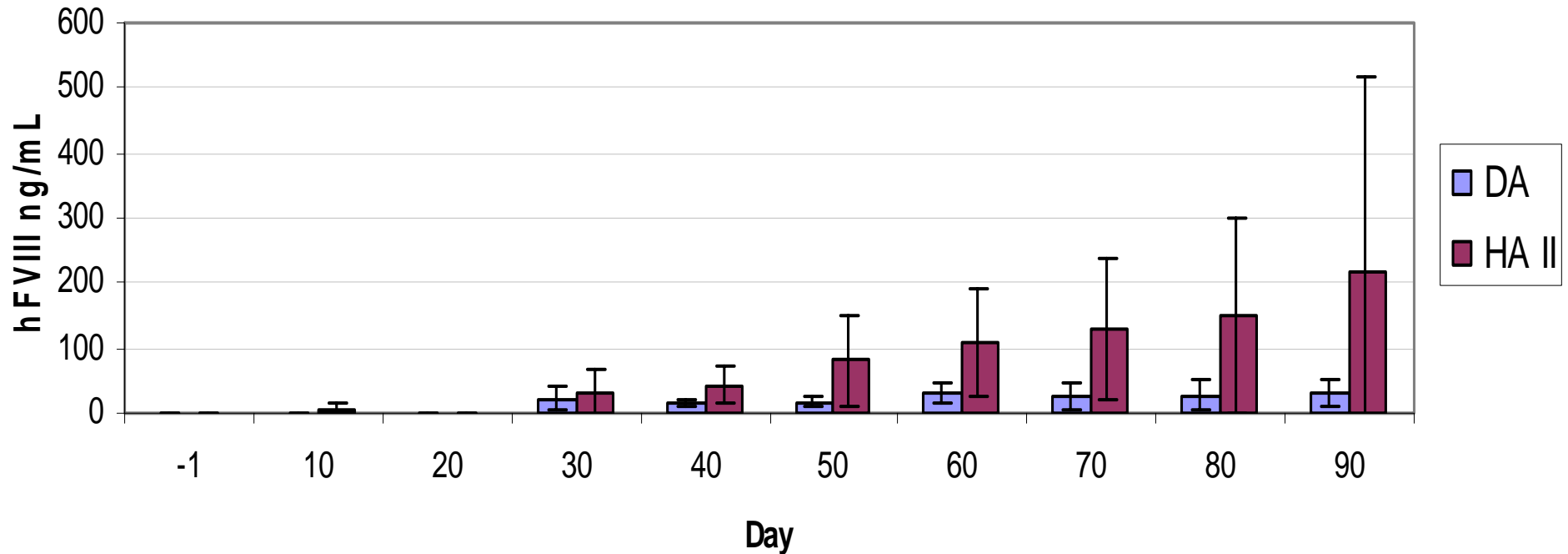


# Vector Production Issues

- Even well characterized vector may not be a consistent as you would like
- Never clear how variability in vector preparations may affect the outcome of preclinical or clinical experiments

# Same Titer (potency) from different PCL may not be the same in vivo

Mean Human Factor VIII Levels in Rabbits Treated with hFVIII(V)  
Produced by DA or HA II Cell Lines



# Semen

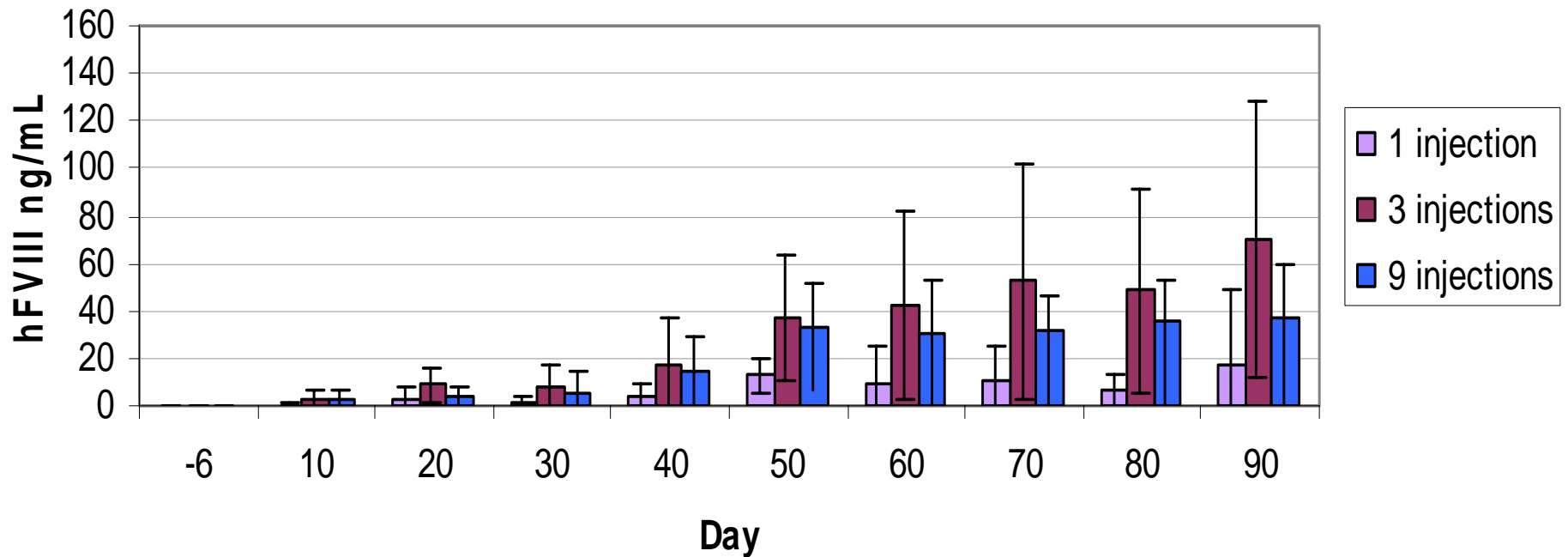
- Low level positivity in rabbit testes
- Extensive rabbit semen study was negative (Roehl et al. HGT 11:2529, 2000)
- We had 1PCR well/10 in one patient at one time point that was positive – Clinical hold that was difficult to get off as no more positives to test appeared.

# Clinical Trial Semen Data

SUBJECT ID	WEEK 0	2	6	9	11	17	29	53
02001	NT	Neg	NA, x 2	Neg	NA	Neg	Neg	NA
05001	NT	Neg	Neg	Neg	Neg	Neg	QNS	Off study (1)
05002	NT	Neg	QNS, x2	Neg	QNS	QNS	Neg	Neg
03001	NT	Neg	Neg	Neg	Neg	NA	NA	Neg
05003	NT	Neg	Neg	NA	Neg	Neg	Neg	Neg
03002	NT	Neg*	NA	NA	Neg	Neg	Neg	Neg
02002	NT	Neg	Neg**	Neg	Wk 13:Neg	Wk 20: Neg	Neg	Neg
05004	NT	Neg	Neg	Neg	Neg*	Neg	Neg	Neg
03003	NT	NA	NA	NA	NA	NA	Off study (2)	--
05005	NT	Neg	Neg	Neg	Neg	Neg	Neg	Neg
05006	NT	Neg	Neg	Neg	Neg	Neg	Neg	Neg
01001	NT	Neg	Neg	Pos <sup>TP</sup>	Wk 12, 13, 15: Neg	Neg	Neg	Neg
05007	NT	Neg	Neg	NA	Neg	Neg	Neg	Neg

# Multiple administrations make a difference

Human Factor VIII Levels in Rabbits Given  $1 \times 10^9$  cfu-eq/kg as 1, 3 or 9 Injections



# Integration and insertional mutagenesis

- In Fischer XSCID trial 1-2 x10e7 insertion events led to tumors in 3/10 children
- Efficiency of transduction is estimated (from rabbits) at around 1% of input in the FVIII protocol
- Subjects received 10e9 to 5x10e10 TU's, or 10e7 to 5x10e8 integration events. No evidence of insertional tumorigenesis in this cohort (started June 2000).
- Supports the idea that circumstances affect the insertional tumorigenic potential

\$\$\$\$\$\$

- Cost of program: Contract with third party supported early preclinical work \$9 million over 3years: later preclinical and clinical costs can be imagined.
- BUT it was easy to calculate that, if the product were to be “curative”, it would be a clinical and investment success.

# Overall Take-homes

- The better characterized your system is, the more likely you are to have a rational response to setbacks, and they will happen
- Preclinical is usually less expensive than clinical, but in the end you have to spend the clinical money also – “when” is the tough part
- Drug development and clinical trials take resources and a team.

# Contributors ...

## **Chiron**

### **Vector Development**

Douglas Jolly PhD

Carlos Ibanez PhD

Steve Chang Ph.D.

Judy Greengard Ph.D.

### **Vector Production**

K. Jon Kowal PhD, Nancy Sajjadi

Holger Roehl PhD

Paula Stemler PhD

### **Preclinical**

Martha Leibbrandt PhD

### **Clinical**

Veronica Cole LPN

Deborah Hurst MD

### **Development**

Don Gay, Dale Johnson PhD,

Biff Owen PhD

## **Clinical Investigators**

Jerry S. Powell MD

U. California Davis Medical Center,

Sacramento Gilbert White MD

U. of North Carolina Medical Center, Chapel

Hill Margaret Ragni MD, MPH

U. Pittsburgh Medical Center, Pittsburgh

Jeanne Lusher MD

Wayne State U. Medical Center, Detroit

Bruce Ewenstein MD, PhD

Harvard U. Medical Center, Boston

Arthur R. Thompson MD, PhD

U. of Washington Medical Center, Seattle

## **Hemophilic Dog Studies**

Timothy C. Nichols MD

Francis Owen Blood Research Lab,

U. of North Carolina Medical Center, Chapel

Hill