Hemophilia A gene therapy: AAV-mediated delivery of an enhanced F8 cassette to the liver produces supraphysiological levels of human FVIII *in vivo*

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WFH 2016 WORLD CONGRESS
Orlando, USA · July 24-28

Disclosures for: Brigit E. RILEY

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RESEARCH SUPPORT	Sangamo BioSciences
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SHAREHOLDER	
HONORARIA	
ADVISORY COMMITTEE	
CONSULTANT	

** Postgraduate Institute for Medicine*

INTRO SLIDE / AGENDA

- Background – Hemophilia A and AAV Gene Therapy
- Overview of AAV Factor 8 cDNA
- Approach to Optimizing AAV Factor 8 cDNA
- Evaluating the Optimized AAV Factor 8 cDNA
  - *in vitro*: HepG2 Cells
  - *in vivo*: Wild Type and Hemophilia A mouse models
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- Summary and Next Steps

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HEMOPHILIA A
AN IDEAL DISEASE FOR LIVER-DIRECTED GENE THERAPY

- Modest increases in Factor VIII levels (>1% of normal) have a positive impact on patient lives

- Adeno-associated virus (AAV) vectors have shown efficacy in preclinical and clinical studies; stable Factor IX (FIX) expression ~ five years in the clinic

- Lag in the clinic of AAV human Factor 8 (hF8) gene therapy – poor vector yields at clinical scale and dose required to achieve therapeutic FVIII levels

- We optimized an AAV hF8 cDNA vector cassette to improve both vector yields and liver-specific hFVIII expression

- Administration of the enhanced AAV hF8 cDNA vector *in vivo* resulted in 2-8X normal circulating hFVIII levels
  * **Higher circulating hFVIII levels will enable lower dose in the clinic**

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HEMOPHILIA A
LIVER-DIRECTED AAV F8 CDNA GENE THERAPY

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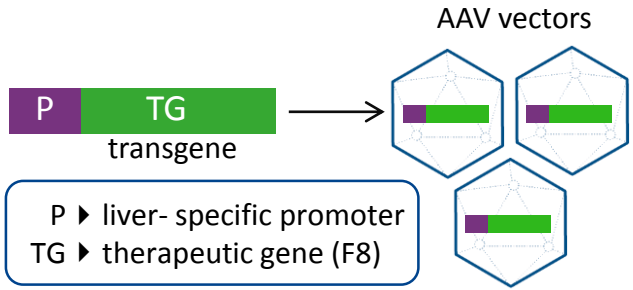
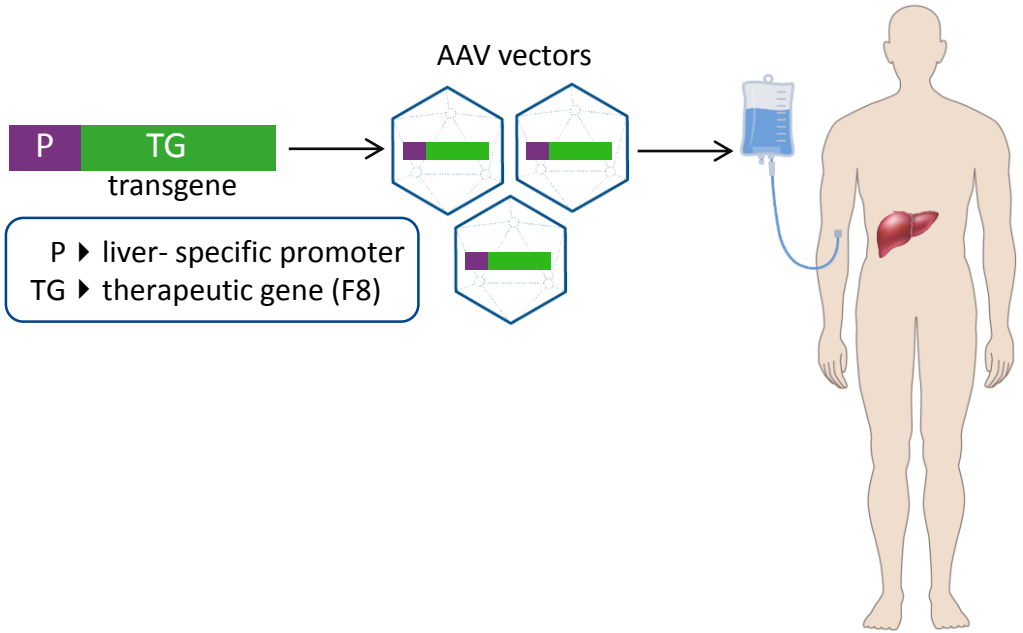
LIVER-DIRECTED AAV F8 CDNA GENE THERAPY

Transgene packaged into AAV vectors



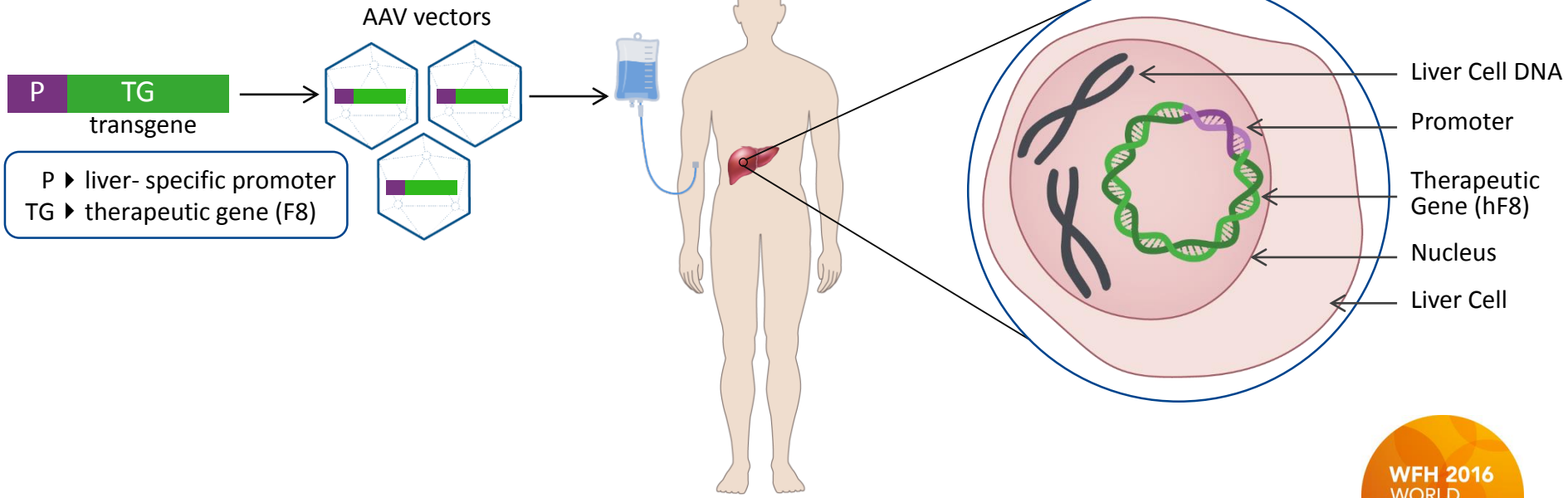
HEMOPHILIA A
LIVER-DIRECTED AAV F8 CDNA GENE THERAPY

Transgene packaged into AAV vectors → **AAV is delivered by a single infusion**



HEMOPHILIA A
LIVER-DIRECTED AAV F8 CDNA GENE THERAPY

Transgene packaged into AAV vectors → **AAV is delivered by a single infusion** → **AAV traffics to liver to deliver transgene into nucleus of liver cells**



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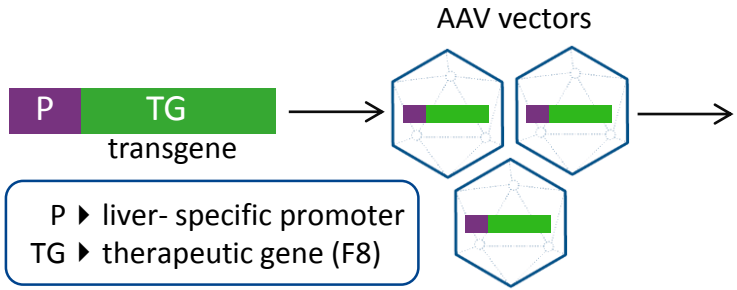

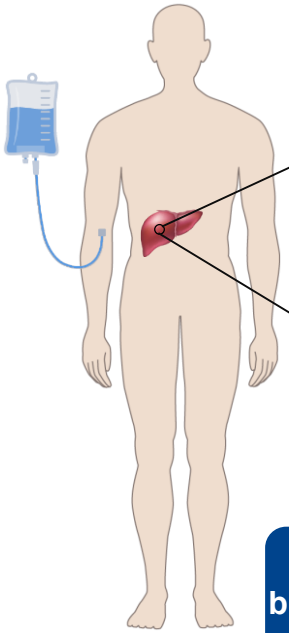

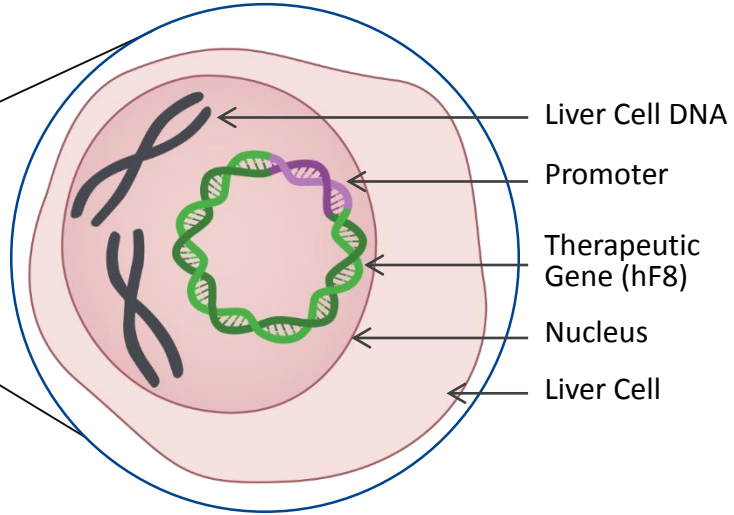

Transgene is expressed from the liver, but remains separate from the cell's DNA

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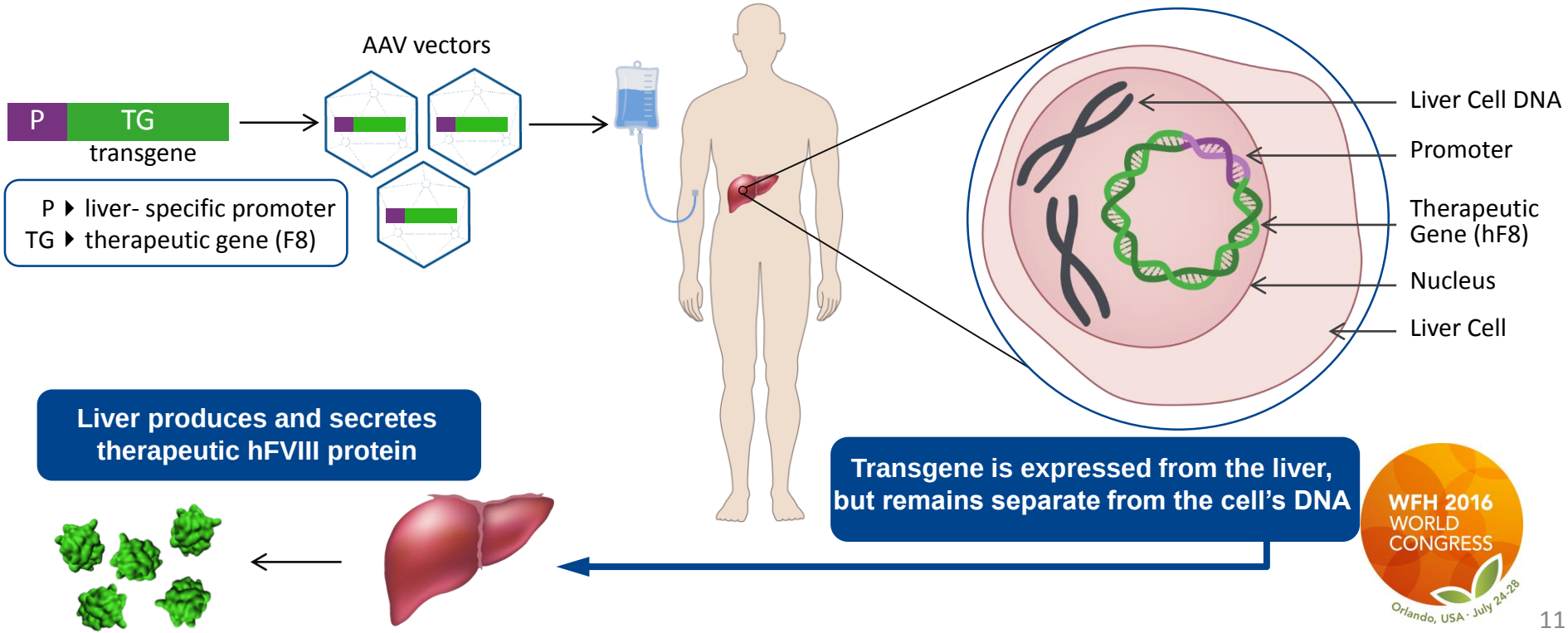
HEMOPHILIA A
LIVER-DIRECTED AAV F8 CDNA GENE THERAPY

Transgene packaged into AAV vectors → **AAV is delivered by a single infusion** → **AAV traffics to liver to deliver transgene into nucleus of liver cells**



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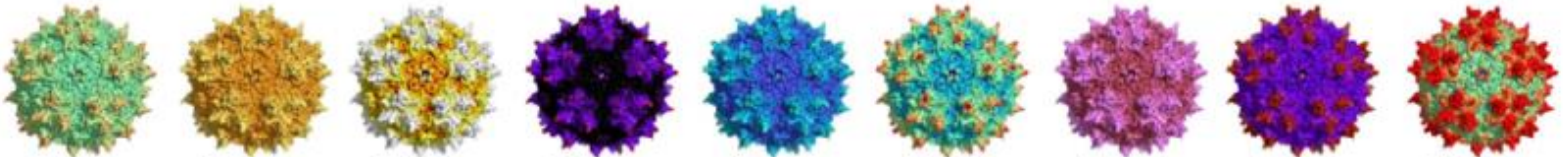
WILD TYPE AAV

- Single-stranded DNA virus which requires a helper virus for replication
- No pathology associated with AAV infection
- Tissue selectivity is determined by capsid composition

Tissue Selectivity of AAV Serotypes



AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9
Liver, Heart, Skeletal muscle	Liver, Heart, and muscle	Heart, Liver	Heart, Lung, Liver	Liver	Liver, Heart, Skeletal muscle	Liver, Skeletal muscle	Liver, Heart, Brain, Muscle	Liver, Heart, Brain, Lung, Skeletal muscle

Vance et al, DOI: 10.5772/61988

RECOMBINANT AAV

- Recombinant AAV (rAAV) has been used extensively for nearly 20 years as a gene therapy vector in preclinical and clinical studies

- Efficient transduction and long term, stable transgene expression in non-dividing tissues:
  - Liver, brain and muscle

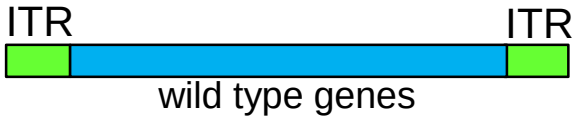
- Replication deficient

- High degree of stability which allows for rigorous methods of vector purification

- AAV vectors carrying capacity is small (~4.7 kb of DNA)

GENERATION OF RECOMBINANT AAV

Wild-Type AAV



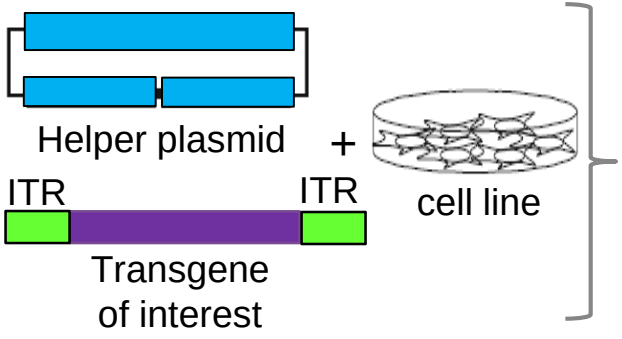
Replace wild type genes

Recombinant AAV

- Contains the transgene in place of wild type genes

Manufacturing rAAV

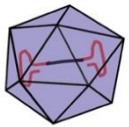
- Helper plasmid is supplied in trans, together with the transgene to a packaging cell line



Within Cell
- Capsid Assembly
- Virus Packaging

- Harvest crude rAAV extract
- Purify rAAV
  - Density gradient
  - Column purification

Highly purified rAAV



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WHAT IS OPTIMAL FOR AAV HF8 CDNA?

hF8 is not an ideal gene for AAV:

- Constrained by hF8 gene size
  - Optimal AAV transgene size is ~4.7 kb; full length hF8 is ~7 kb
- AAV dose required to achieve therapeutic hFVIII levels
  - Low efficiency of transcription/translation
- Low manufacturing yields of AAV hF8
  - Clinical scale manufacturing feasibility is limiting

Optimal AAV hF8 cDNA requires:

- Shorter coding sequence for hF8
  - Potential solution is the use of an optimized B-domain deleted sequence (BDD)
- An optimized robust liver-specific promoter module to drive hF8 expression
- Improved virus yields

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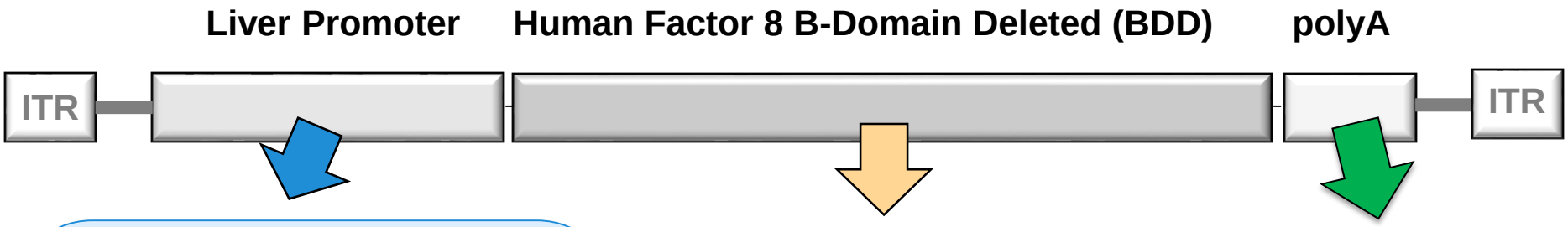
OPTIMIZATION OF AAV HF8 CDNA REQUIRED MULTI-FACTORIAL MODIFICATIONS



Promoter module modifications

- Assembled different permutations of liver-specific promoter elements
- Identified regions of the promoter module that could be improved upon
- For other elements a systematic mutational design ap

Transgene modifications

- Optimized the F8 cassette

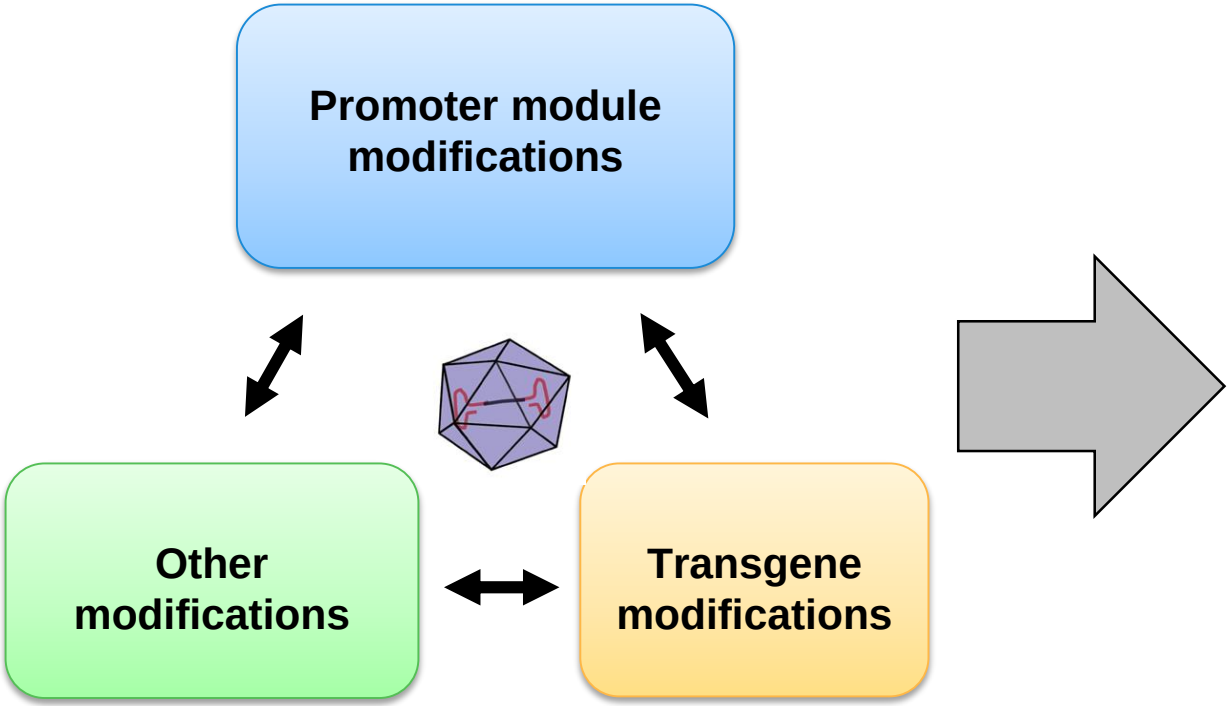
Other modifications

- Identified minimal synthetic polyA
- Removed un-necessary nucleic acids
- Reduced size

sequences
nsgene

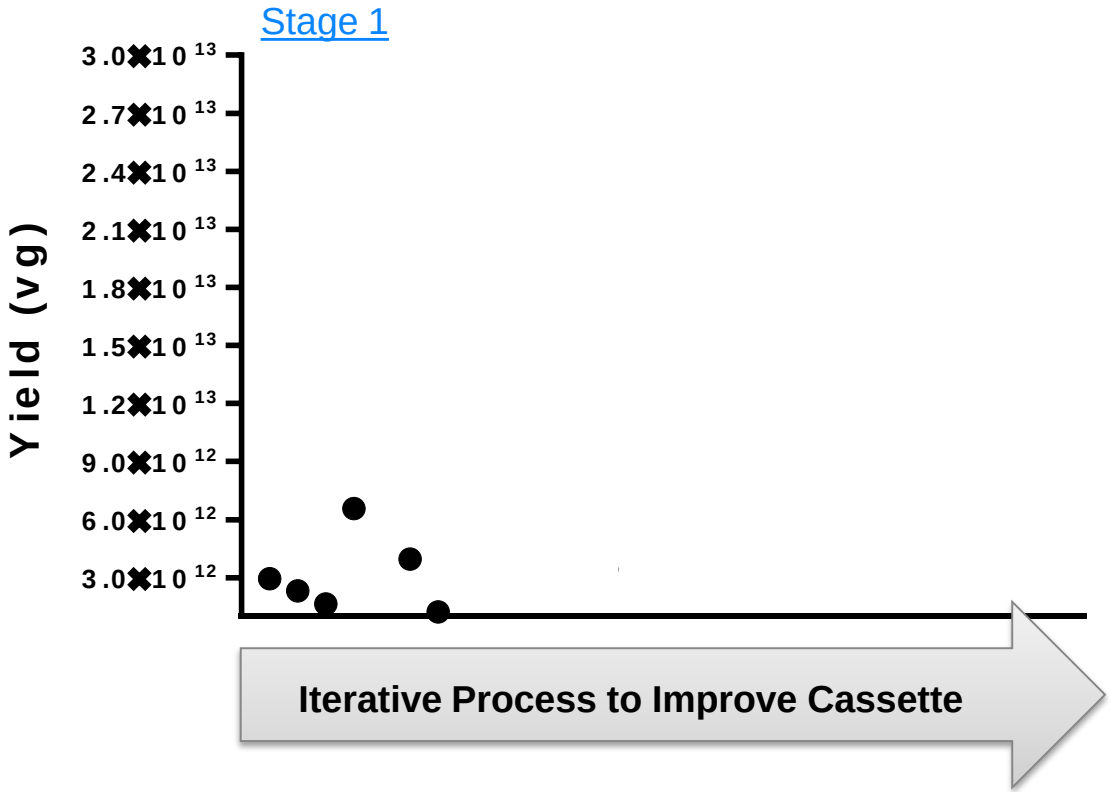
hFVIII protein has the same amino acid sequence as biologics currently in clinic

ITERATIVE PROCESS TO IDENTIFY AN IMPROVED AAV HF8 CDNA CASSETTE



- Virus Yields
- *In Vitro* secreted hFVIII levels (mRNA/protein) and activity
- *In Vivo* circulating hFVIII levels (mRNA/protein)

MODIFICATIONS IMPROVE VIRUS YIELD



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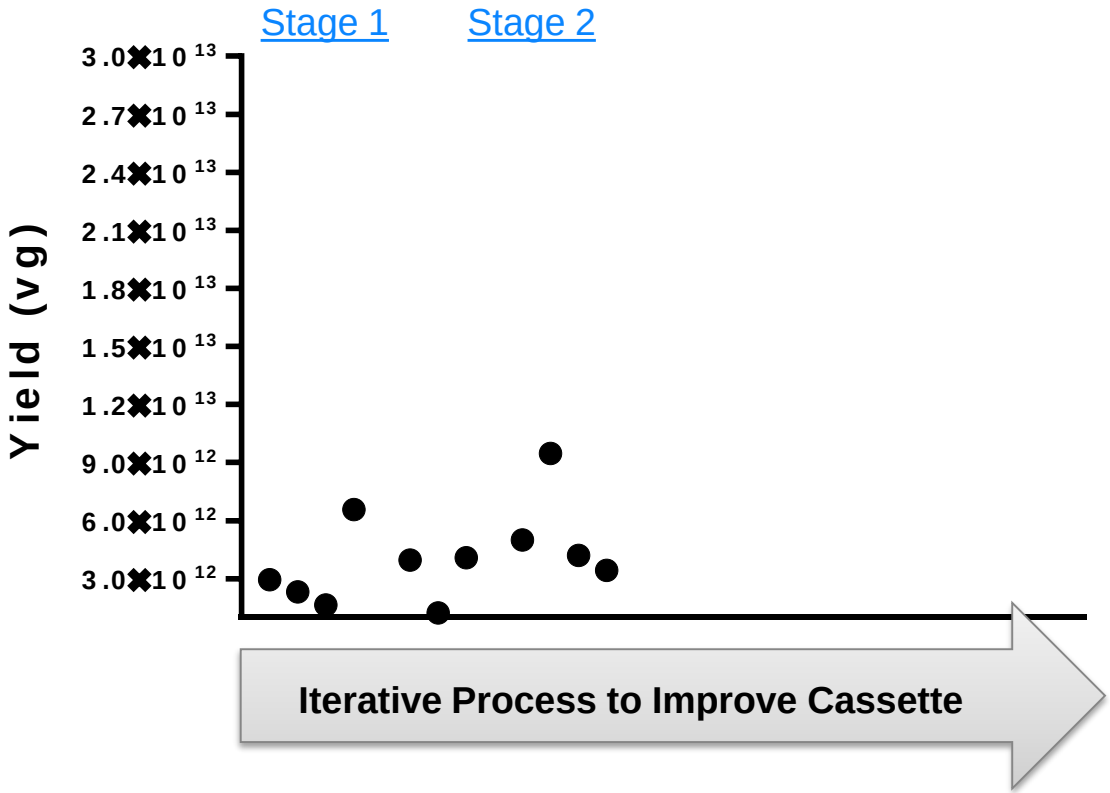

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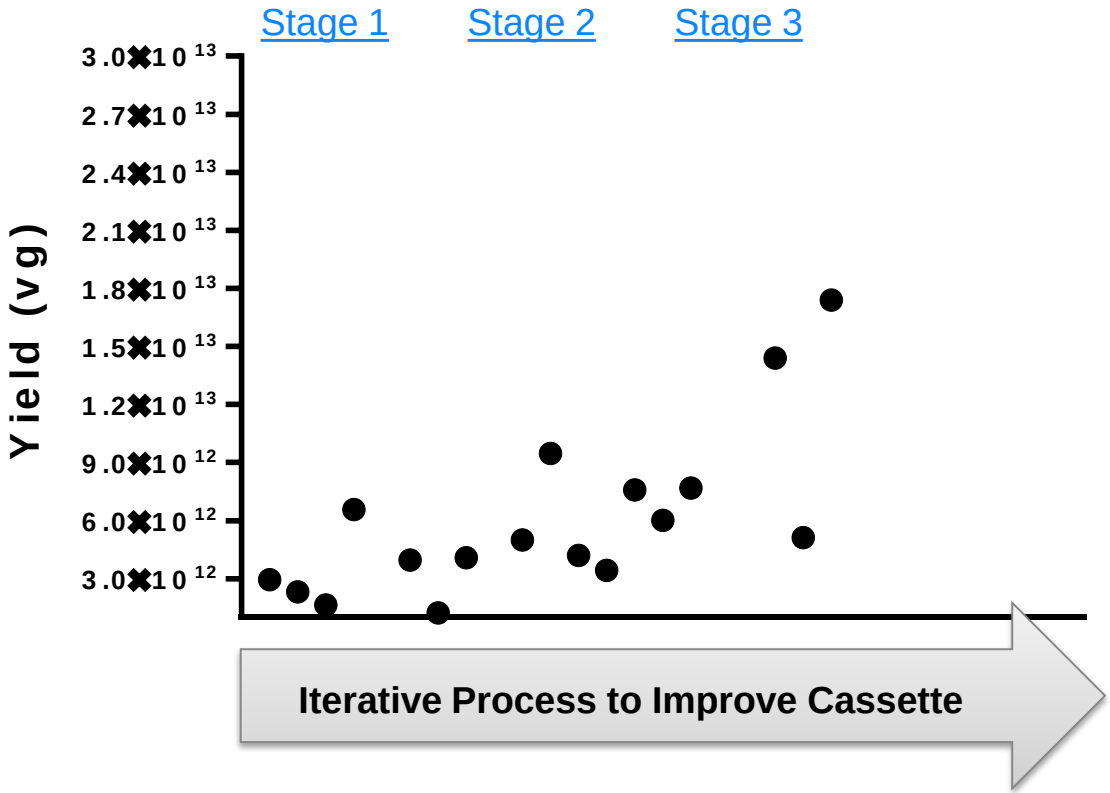

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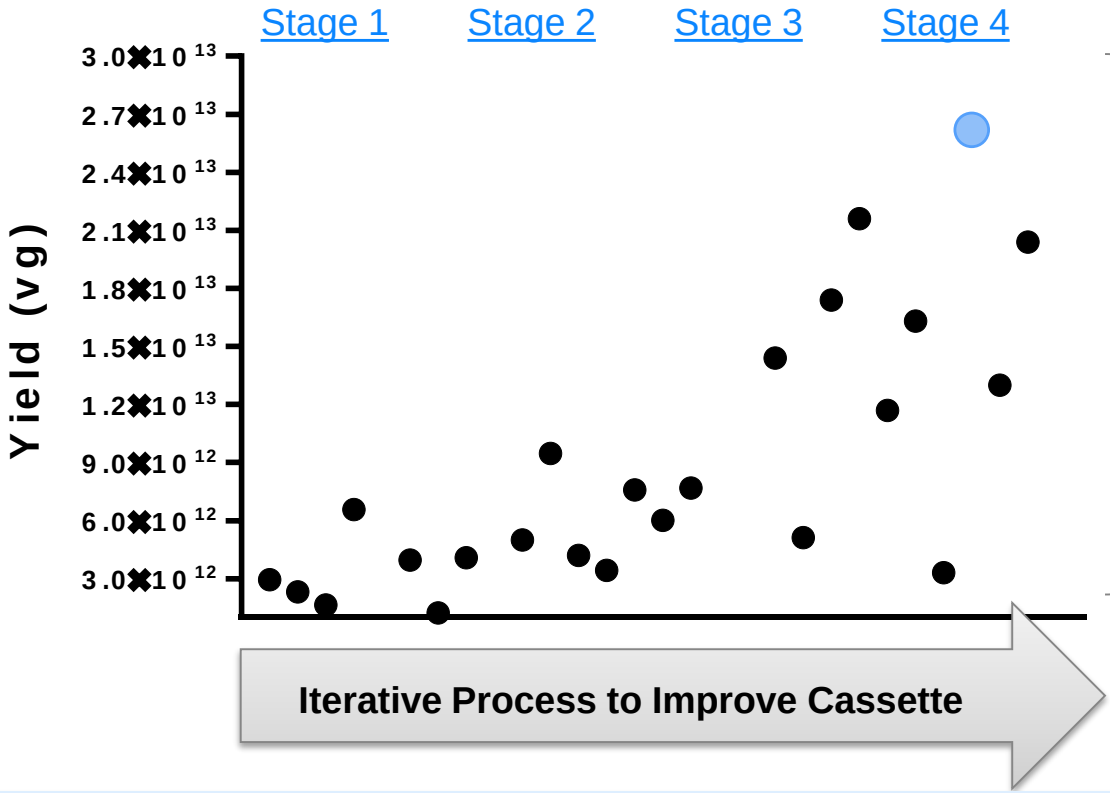

8-10 fold improvement at the cell factory scale

At clinical scale; greater than 5-fold improvement in vector yields

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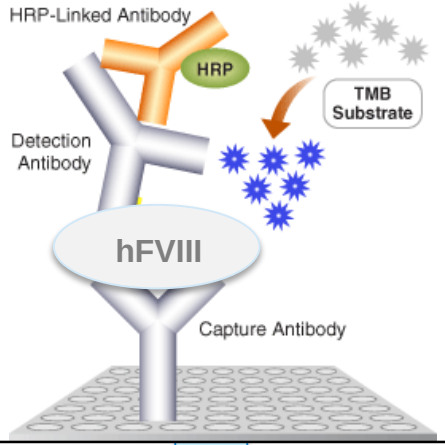
SEVERAL METHODS USED TO ASSESS HFVIII LEVELS AND ACTIVITY

Enzyme-Linked Immunosorbent Assay (ELISA)

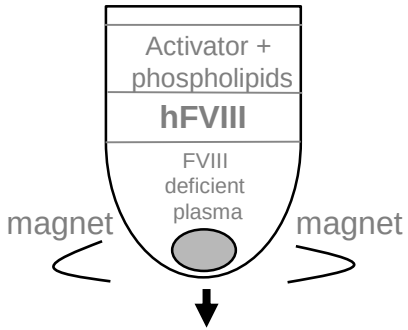


LEVELS

Specific for human FVIII

Clotting Assay

Add Ca^{2+}
Generation of Factor Xa and thrombin yielding clot formation



Clotting times

ACTIVITY

Not specific for human FVIII

Chromogenic Assay

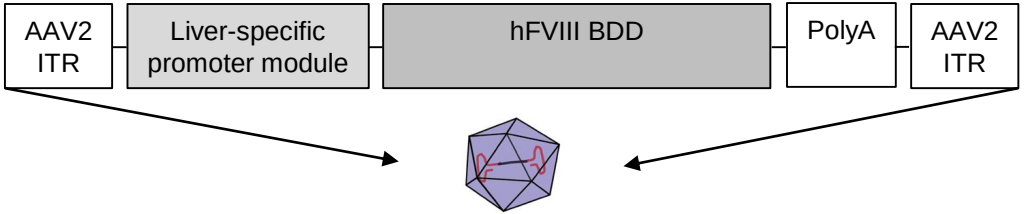
Factor X → (Factor IXa, Ca^{2+}, Phospholipid, hFVIII) → **Factor Xa**

Chromogenic substrate → (**Factor Xa**) → Chromophore 405 nm

ACTIVITY

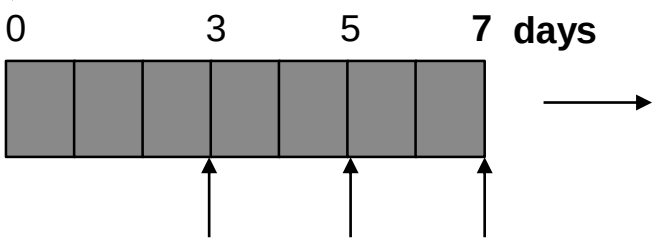
Not specific for human FVIII

IN VITRO: HEPG2 CELLS
EXPERIMENTAL DESIGN



Optimized rAAV hF8 cDNA cassette packaged into **AAV2/6**

Test article addition



Supernatant collection schedule

Endpoints

- ELISA for hFVIII levels
- Clotting assay for hFVIII activity
- Chromogenic assay for hFVIII activity

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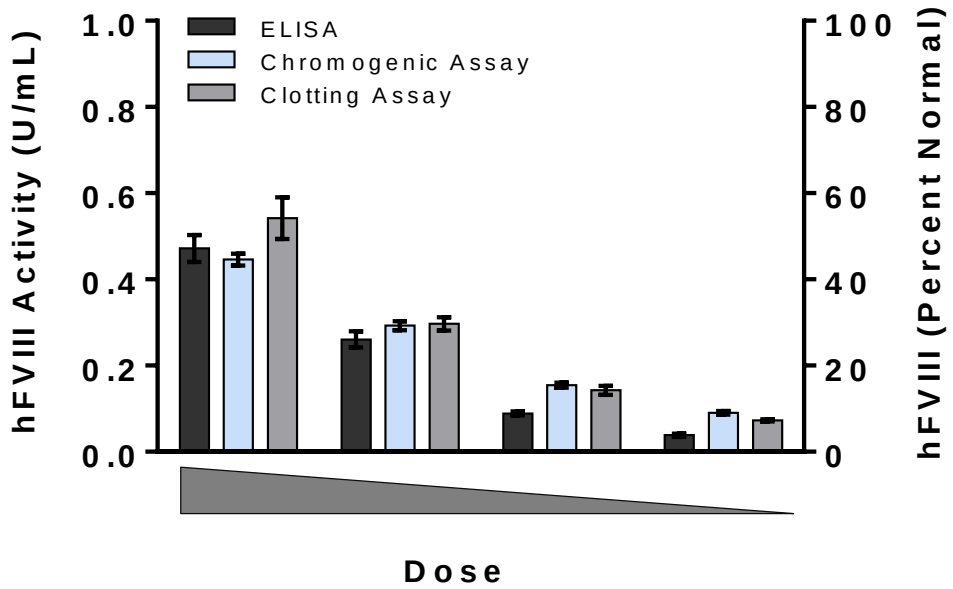
IN VITRO: HEPG2 CELLS
GOOD CORRELATION BETWEEN HFVIII ACTIVITY/LEVELS



Values will be reported as hFVIII (Percent Normal) for ELISA, Chromogenic or Clotting where 1 U/mL = 100% Normal

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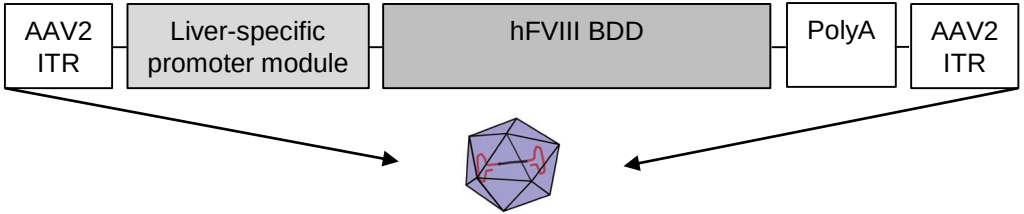
IN VIVO: WILD TYPE MOUSE DATA
EXPERIMENTAL DESIGN

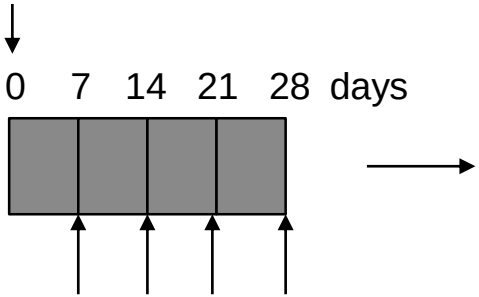


Optimized rAAV hF8 cDNA cassette packaged into **AAV2/6**

Dose

- **7.2E+12 vg/kg**

IV injection of test article



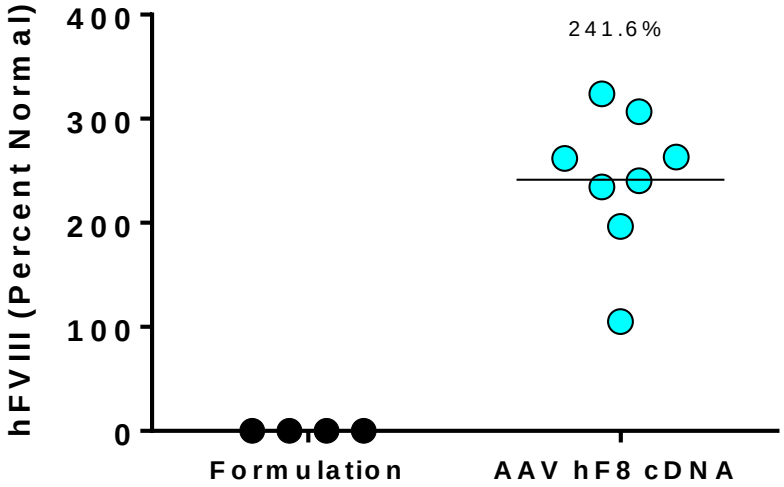
Endpoints

- ELISA for hFVIII levels
- hF8 mRNA tissue biodistribution

Immunosuppression regimen of cyclophosphamide (50 mg/kg)

IN VIVO: WILD TYPE MOUSE DATA
SUPRAPHYSIOLOGICAL LEVELS

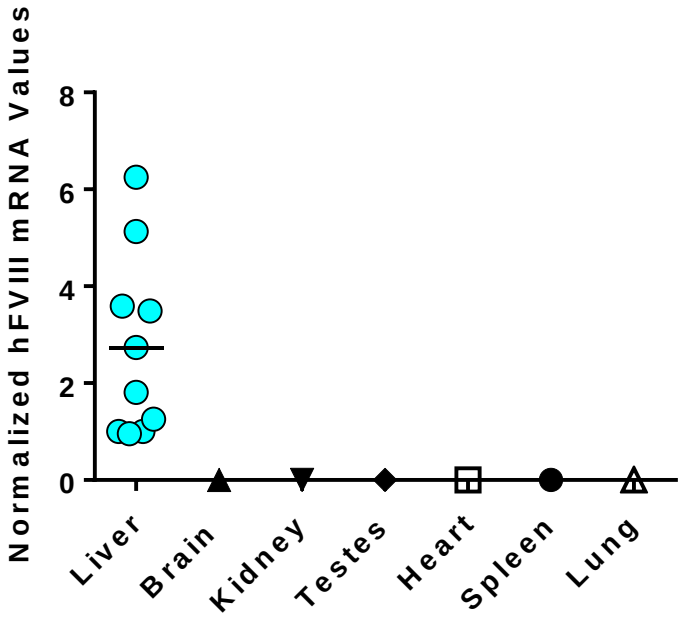
hFVIII Levels



Levels were determined by ELISA

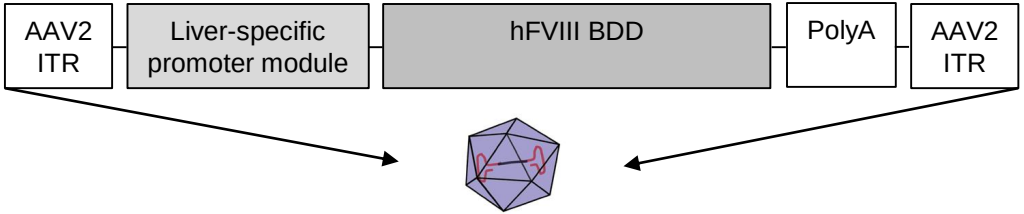
IN VIVO: WILD TYPE MOUSE DATA
HF8 EXPRESSION IS RESTRICTED TO LIVER

hF8 mRNA



Represents two independent mouse studies

IN VIVO: HEMOPHILIA A MOUSE DATA
EXPERIMENTAL DESIGN



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IV injection of test article

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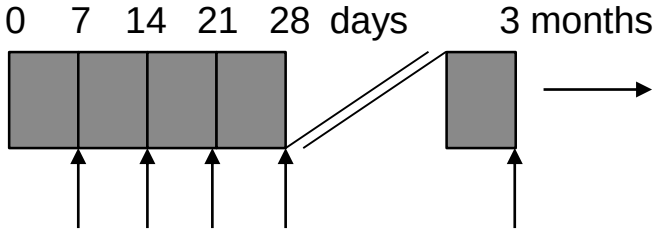

Plasma collection schedule

Endpoint

- Chromogenic assay for hFVIII activity
- Tail vein transection (TVT) for hemostasis

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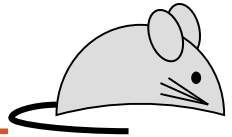
IN VIVO: HEMOPHILIA A MOUSE DATA
OVERVIEW OF HEMOPHILIA A R593C MICE



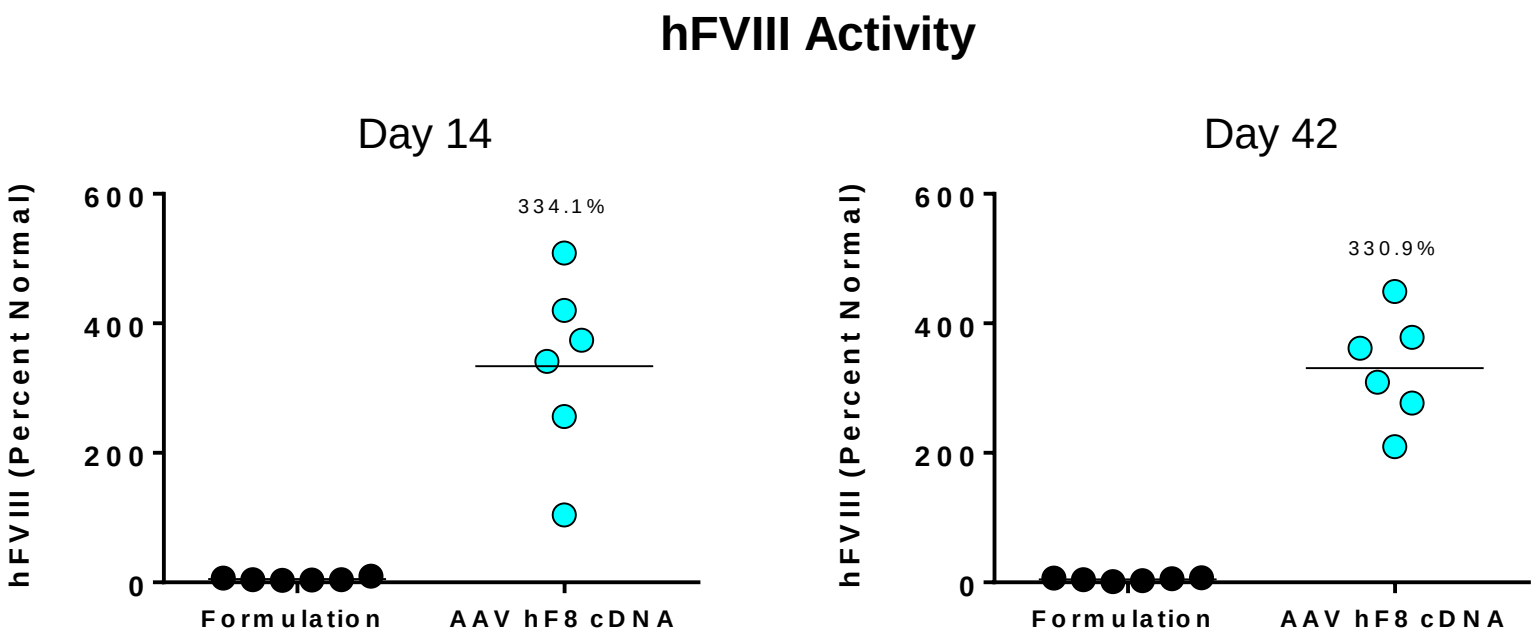
- Hemophilia A R593C mice are tolerized to hFVIII because they contain a hF8-R593C transgene under control of a murine albumin promoter

- hFVIII-R593C is frequently observed in Hemophilia A patients, and in mice produces no detectable hFVIII protein
  - Thought to be rapidly degraded in mice, with peptide fragments presented to the immune system

- Mice also contain a knockout of the mouse F8 gene and are deficient for endogenous mouse FVIII protein

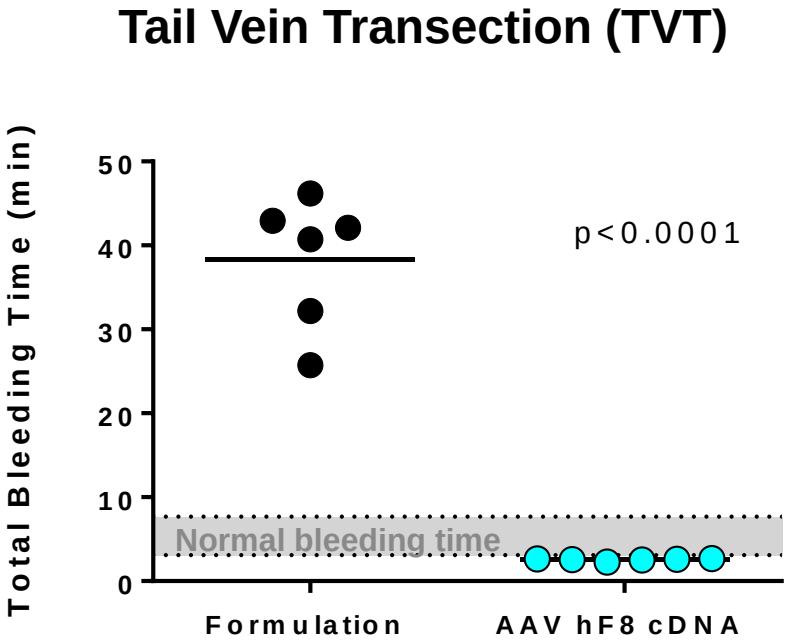
- Studies were conducted in collaboration with Dr. David Lillicrap at Queen's University

IN VIVO: HEMOPHILIA A MOUSE DATA
SUPRAPHYSIOLOGICAL LEVELS



Activity determined by Chromogenic Activity Assay

IN VIVO: HEMOPHILIA A MOUSE DATA
REDUCED BLEED TIME IN TREATED MICE



TVT method based on
Johansen et al., Haemophilia, 1-7, 2016.

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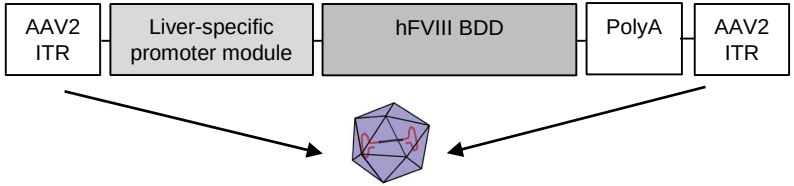
IN VIVO: NON-HUMAN PRIMATE DATA
EXPERIMENTAL DESIGN



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IV injection of test article

Doses
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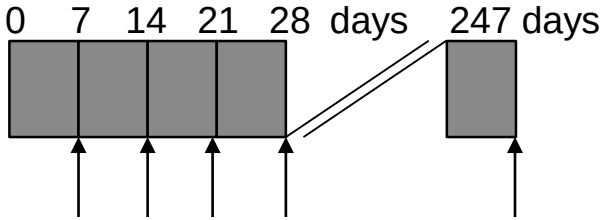
- **6.0E+12 vg/kg**

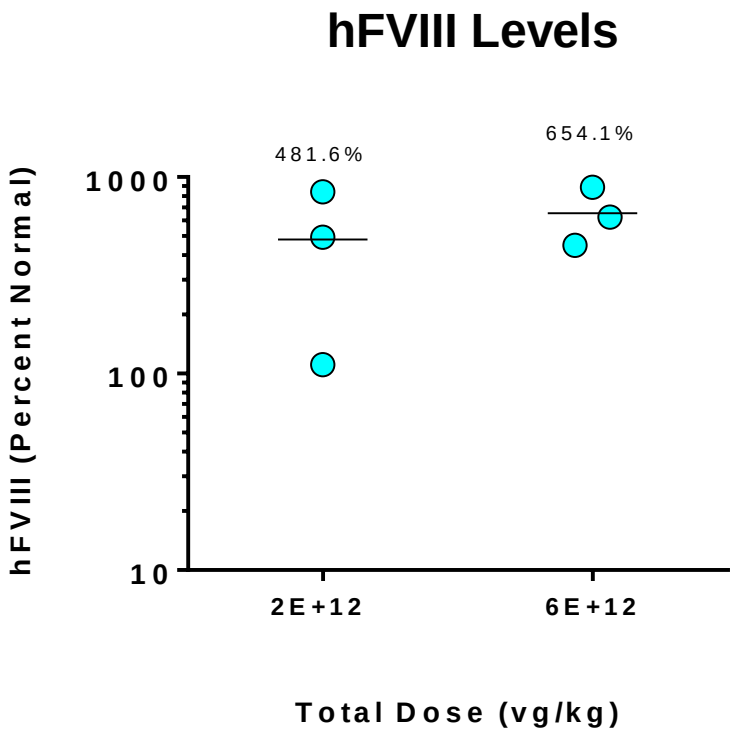


Plasma collection schedule

Endpoints
- ELISA for hFVIII levels
- Clotting for hFVIII activity
- Bethesda Units (BU) for inhibitory hFVIII antibodies
- Liver enzymes

Immunosuppression regimen of Rituxan and Solu-Medrol (10 mg/kg for both)

IN VIVO: NON-HUMAN PRIMATE DATA
SUPRAPHYSIOLOGICAL LEVELS

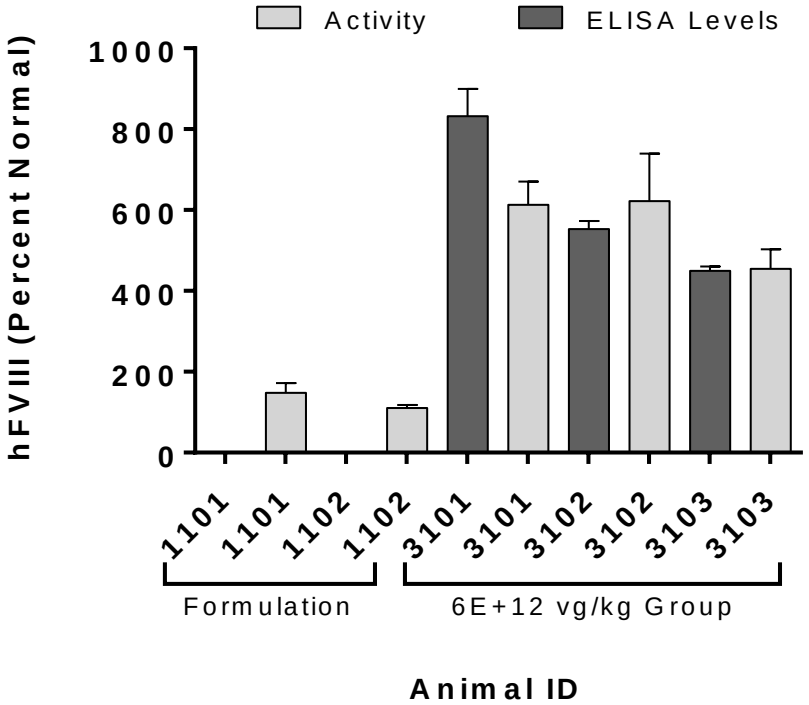


- Dose selection was based on published studies

Follow up dose-finding studies are aimed at determining the minimal effective dose for the clinic

Levels were determined by ELISA

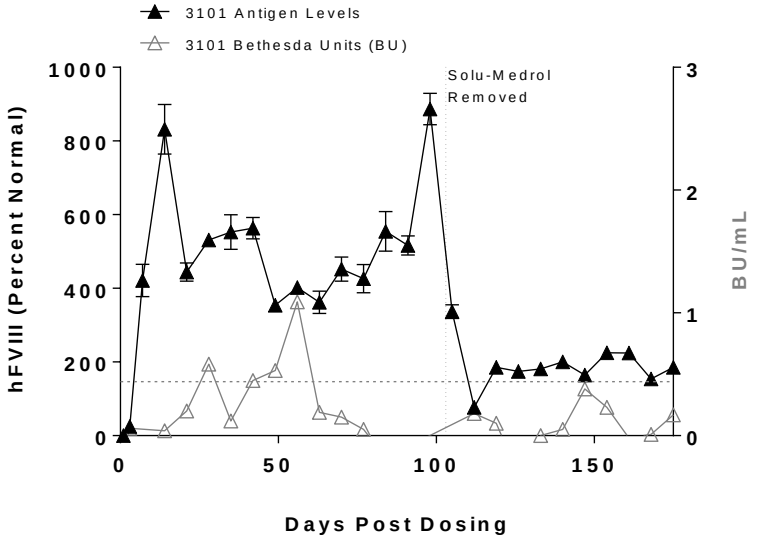
IN VIVO: NON-HUMAN PRIMATE DATA
GOOD CORRELATION BETWEEN ACTIVITY/LEVELS

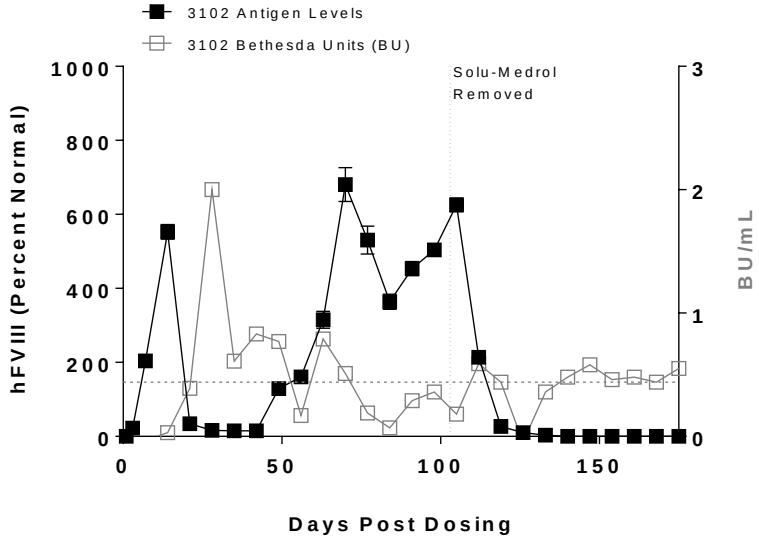


Shown in the Formulation Group:

- Detection of ~100 % Normal NHP FVIII activity
- No detection of NHP FVIII levels given the ELISA is specific for human FVIII

IN VIVO: NON-HUMAN PRIMATE DATA
DURABILITY





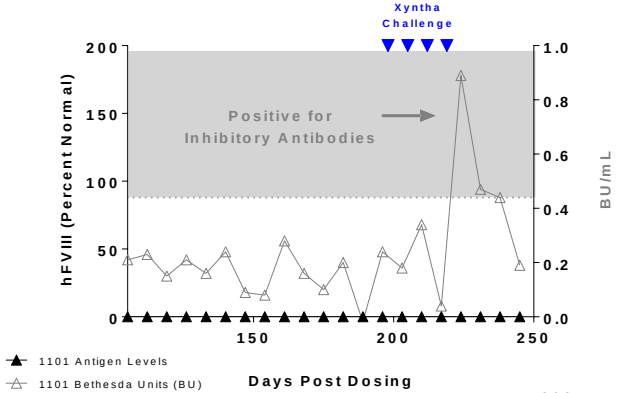
Stable hFVIII levels for over 8-weeks in the absence of all immunosuppression

Levels were determined by ELISA

IN VIVO: NHP IMMUNE TOLERANCE CHALLENGE DURABILITY

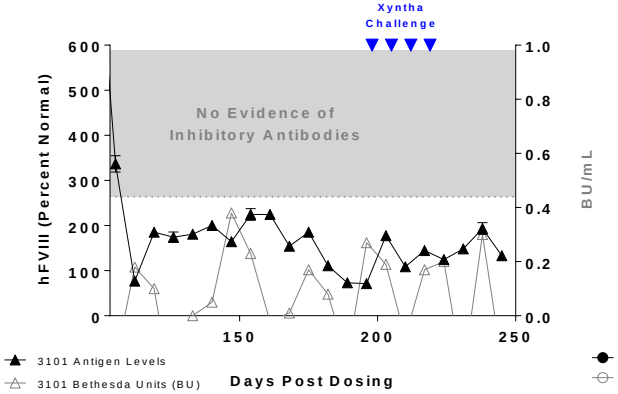
- A simplified view of tolerance is induction of B-cell/T-cell anergy and/or apoptosis in the presence of sustained levels of agent (hFVIII)
- Do the sustained hFVIII levels produced from the rAAV-hF8 *prevent* re-induction of neutralizing antibodies?
- rAAV-hF8 treated NHP were challenged with hFVIII biologic
- hFVIII biologic challenge consisted of 4 weekly infusions of 25 U/kg of Xyntha®

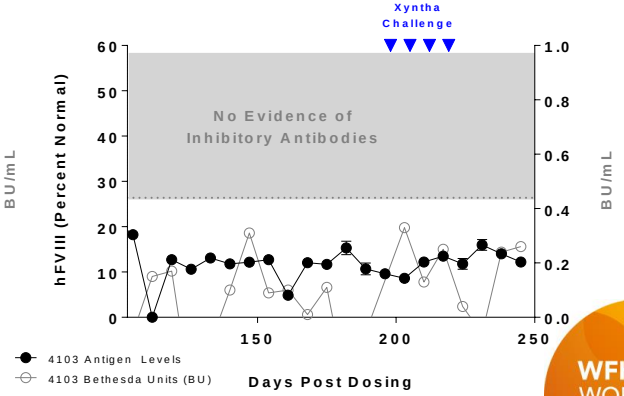
IN VIVO: NHP IMMUNE TOLERANCE CHALLENGE
NO APPEARANCE OF INHIBITORY ANTIBODIES



Control Group

AAV hF8 cDNA Dose Groups





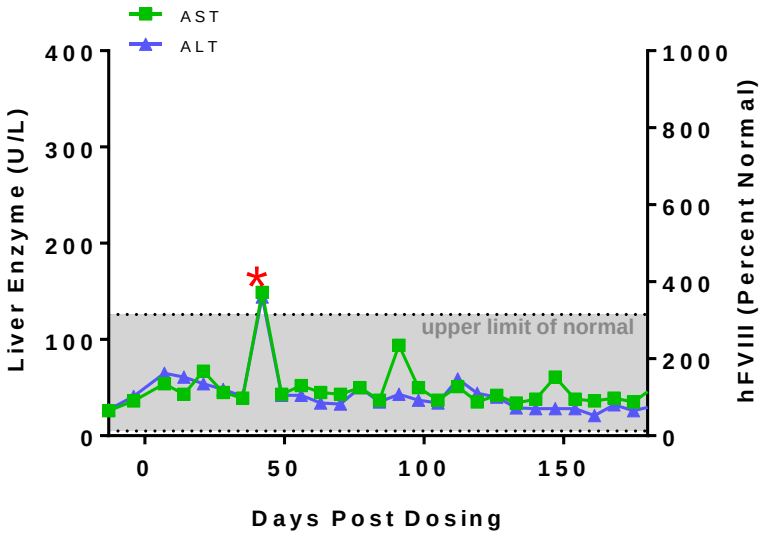
hFVIII levels ~150%

hFVIII levels ~10%

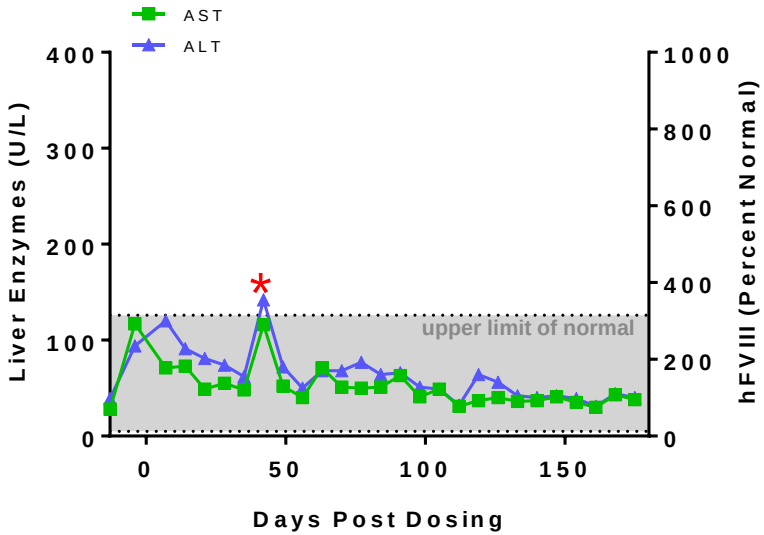
+ Regions shaded gray are above the BU cutpoint thus positive for inhibitory antibodies

IN VIVO: NON-HUMAN PRIMATE DATA
WELL TOLERATED

Control Group



High Dose Group



***Elevated levels observed post-liver biopsies (day 41)**

ALT = Alanine Aminotransferase, upper limit of normal,126 U/L
AST = Aspartate Aminotransferase, upper limit of normal,120 U/L

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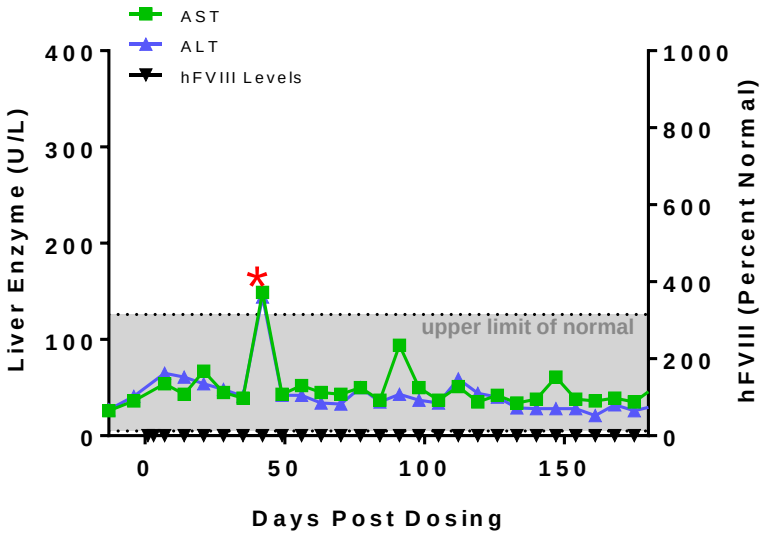
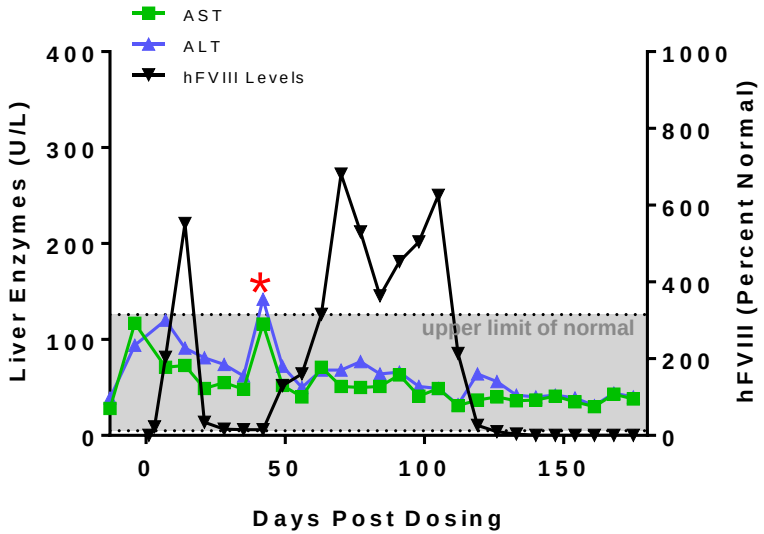
IN VIVO: NON-HUMAN PRIMATE DATA
WELL TOLERATED

Control Group



High Dose Group



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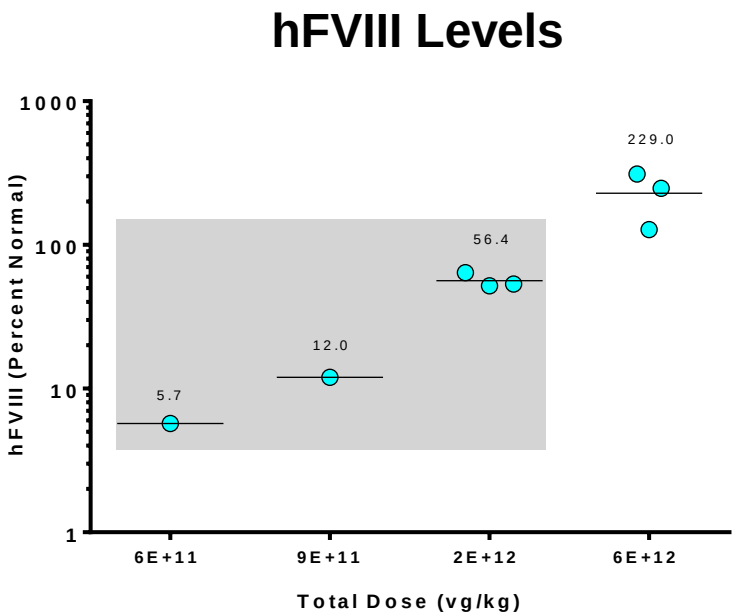
IN VIVO: NON-HUMAN PRIMATE DATA
PRELIMINARY RESULTS DOSE-FINDING STUDY



Preliminary results. Dose-finding study demonstrates high hFVIII production from GMP-clinical scale manufacturing process

Levels were determined by ELISA

SUMMARY AND FUTURE PLANS

- Administration of AAV hF8 cDNA, engineered to improve vector yields and liver-specific hFVIII expression, resulted in supraphysiological levels *in vivo*
  - Wild type mice
  - Hemophilia A R593C mice
  - NHPs

- Good correlation between assays used to measure circulating hFVIII protein
  - Levels by ELISA and activity by Chromogenic or Clotting assays

- Sustained hFVIII levels from the rAAV-hF8 prevented re-induction of neutralizing antibodies with biologic challenge suggestive of induced tolerance (even in the context of a xenogeneic setting)

- Ongoing studies are aimed at determining the minimal effective dose

- Goal of filling the IND, second half of 2016

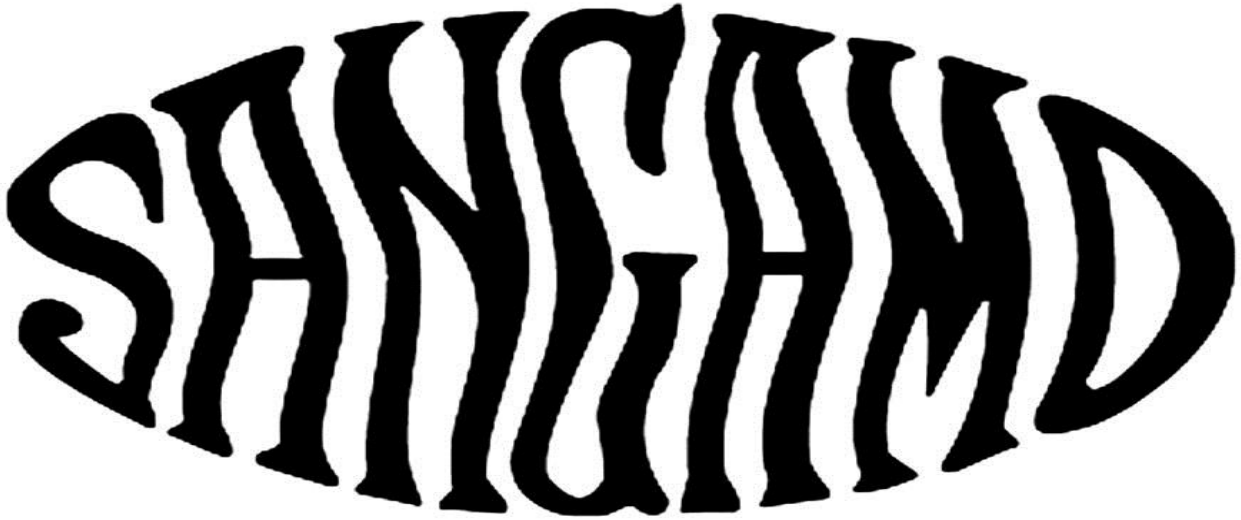
ACKNOWLEDGEMENTS

Judy Greengard
Lisa King
Eudean Garces
Stephen Ballaron
Daniel Richards
Melanie Butler
Carolyn Gasper
Kathy Meyer
Dale Ando

Richard Surosky
Alicia Goodwin
Andrea Kang
Tim Gabriele
Hung Tran
Jennifer Huang

Mike Holmes
Jeff Boonsripisal
Derek Liu
Rainier Amora
Lei Zhang

David Lillicrap
Christine Hough
Dominique Cartier
Kate Nesbitt
Courtney Dwyer
Kassandra Herbert


SANGAMO