CASEBIA THERAPEUTICS

Therapeutic Levels of FVIII Generated by CRISPR/Cas9-Mediated In Vivo Genome Editing in Hemophilia A Mice

Alan Brooks, Karen Vo, Dariusz Wodziak, Rangoli Aeran, Keith Abe, Cornell Mallari, Valerie Guerrero, Christopher Cheng[#], Andrew Scharenberg

Casebia Therapeutics, San Francisco, CA and Cambridge, MA [#]

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Abstract #428

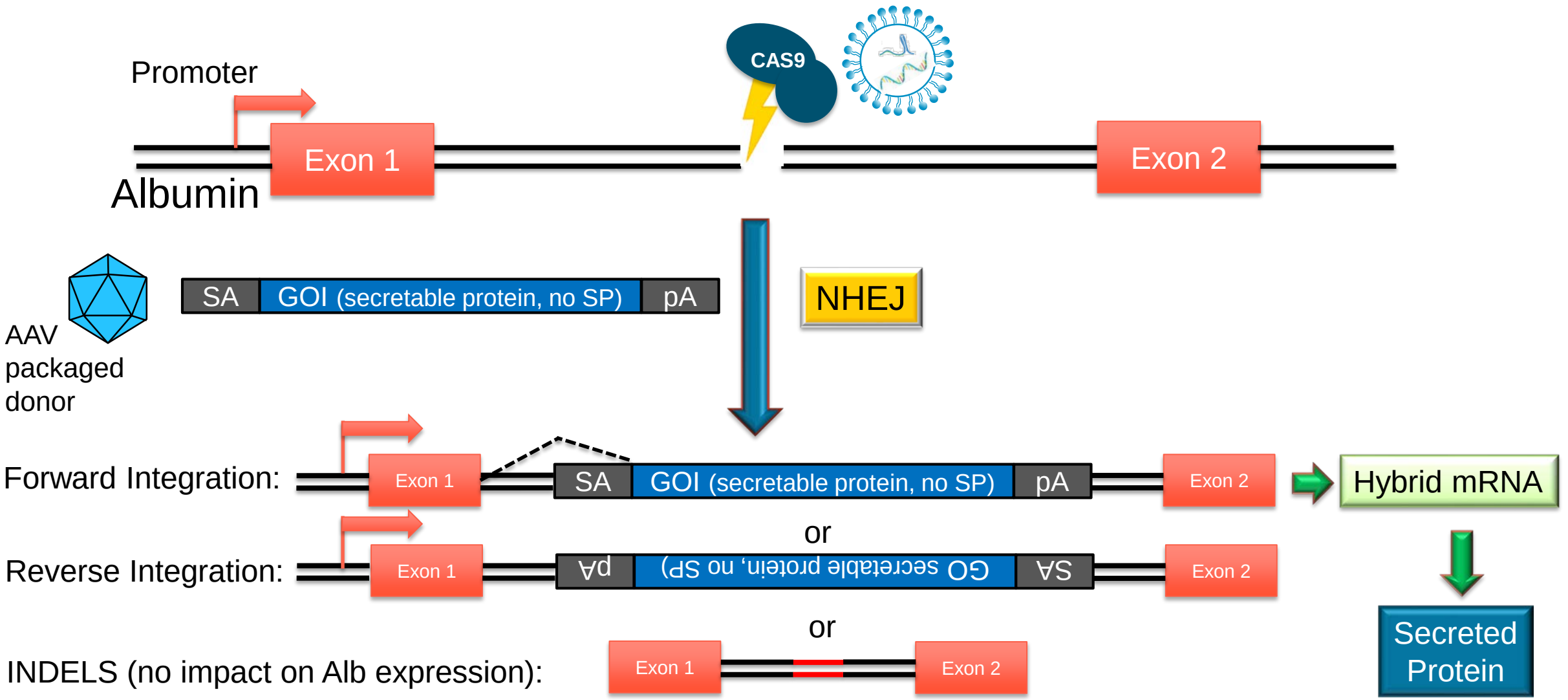
Disclosures

All authors are employees of Casebia Therapeutics LLC

Hemophilia A - Background

- Genetic disorder caused by non-functional FVIII gene resulting in deficiency of active FVIII protein
- Protein replacement is the SOC:
  - 1 to 3 x weekly i.v. infusions
  - Peaks and troughs of FVIII resulting in less than ideal protection
- Ideal therapy is a constant level of FVIII in the normal range (50-150%) to provide continuous hemostasis
- AAV based gene therapy shows promise in clinical trials but may have limitations:
  - Life-long persistence of episomal based vectors un-proven
  - Only 1 treatment possible (due to nAB to AAV) with variable and unpredictable FVIII levels
  - Concern about utility in pediatrics due to vector dilution
- **Approach presented:**
  - Expression from FVIII gene integrated at a defined site in the genome
  - Potential for titration to the normal range which may represent the ideal curative therapy
  - Absence of liver specific promoter/enhancer minimizes risks of insertional gene activation

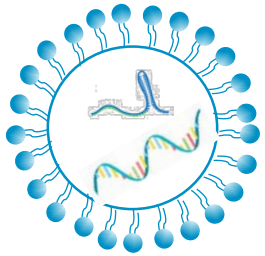
Targeted Gene Insertion Mediated by CRISPR-Cas9



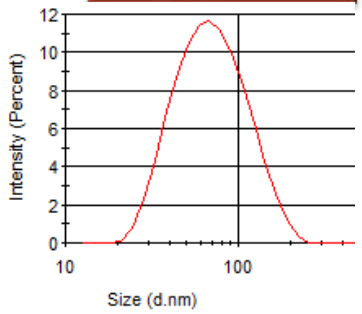
In Vivo LNP Delivery of spCas9 mRNA /gRNA Targeting Albumin

- Cationic lipid, neutral lipids, PEG-lipid
- Single gRNA (chemically modified)
- spCas9 mRNA (with NLS)

Self assembly (Precision NanoSystems flow cell)



Dynamic Light Scattering



LNP

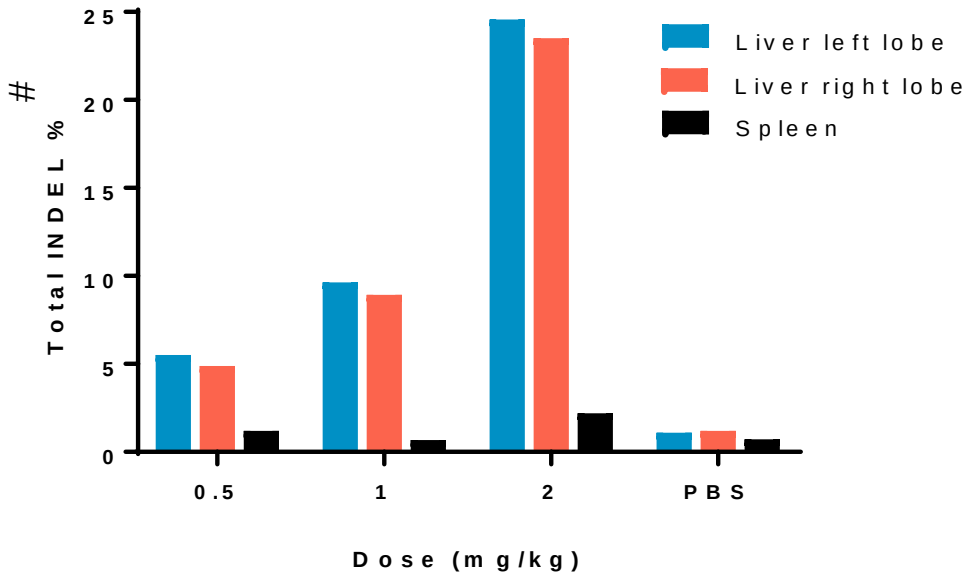
- 50 – 90 nm
- RNA concentration determined by Ribogreen
- Dose mice by total RNA concentration (sgRNA + mRNA)

Visit Poster Abstract #494 for more details

sgRNA 1 targets intron 1 of mouse albumin

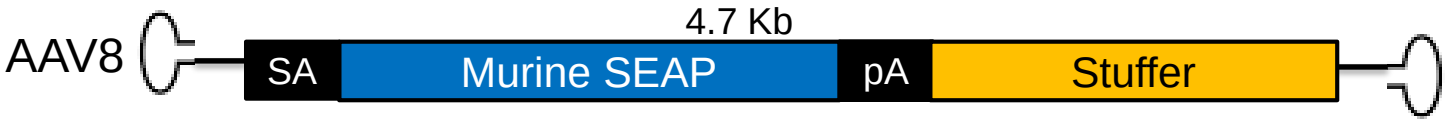
HemA Mice

I.V. LNP
On-target INDELS at day 7



Genomic DNA from liver or spleen analyzed by TIDE

Repeat LNP Dosing

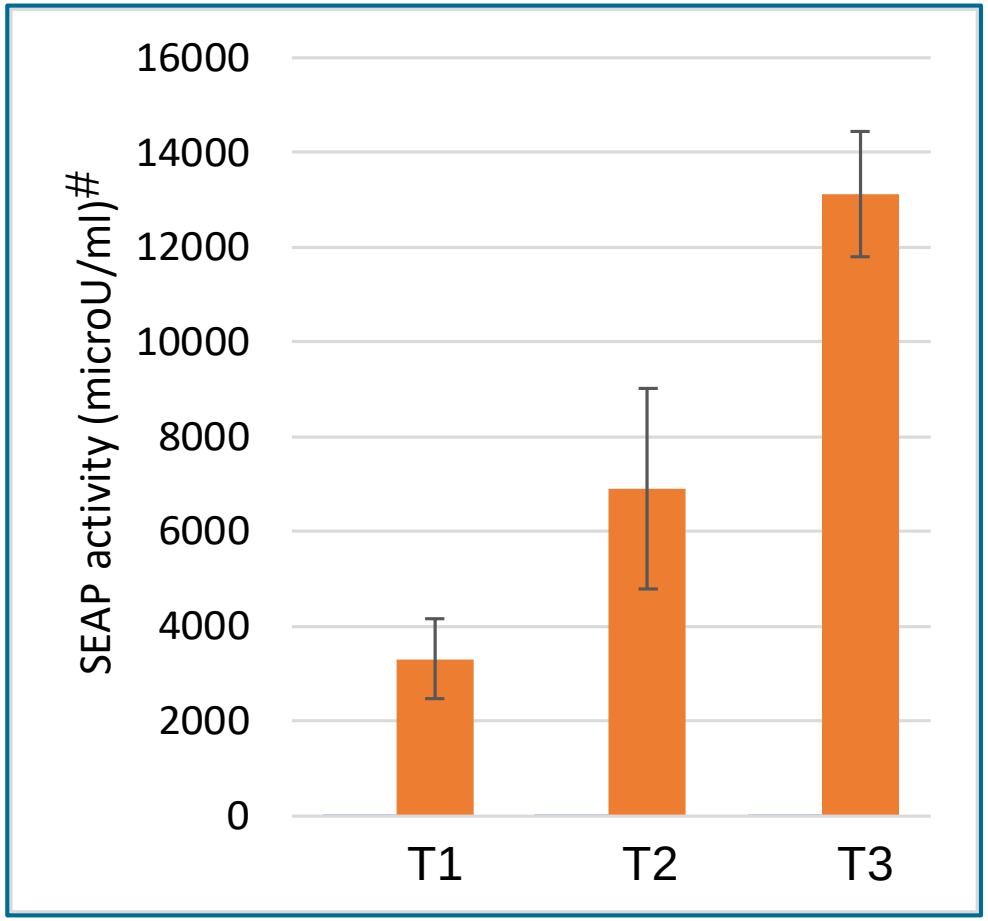


- 2e12 vg/kg injected in HemA mice

Repeated LNP dosing, gRNA 1 (2mg/kg)
- T1= 0 days
- T2= 28 days
- T3= 49 days

SEAP activity in plasma measured weekly after each LNP dose
- Mean SEAP from 3 to 4 weekly assays plotted

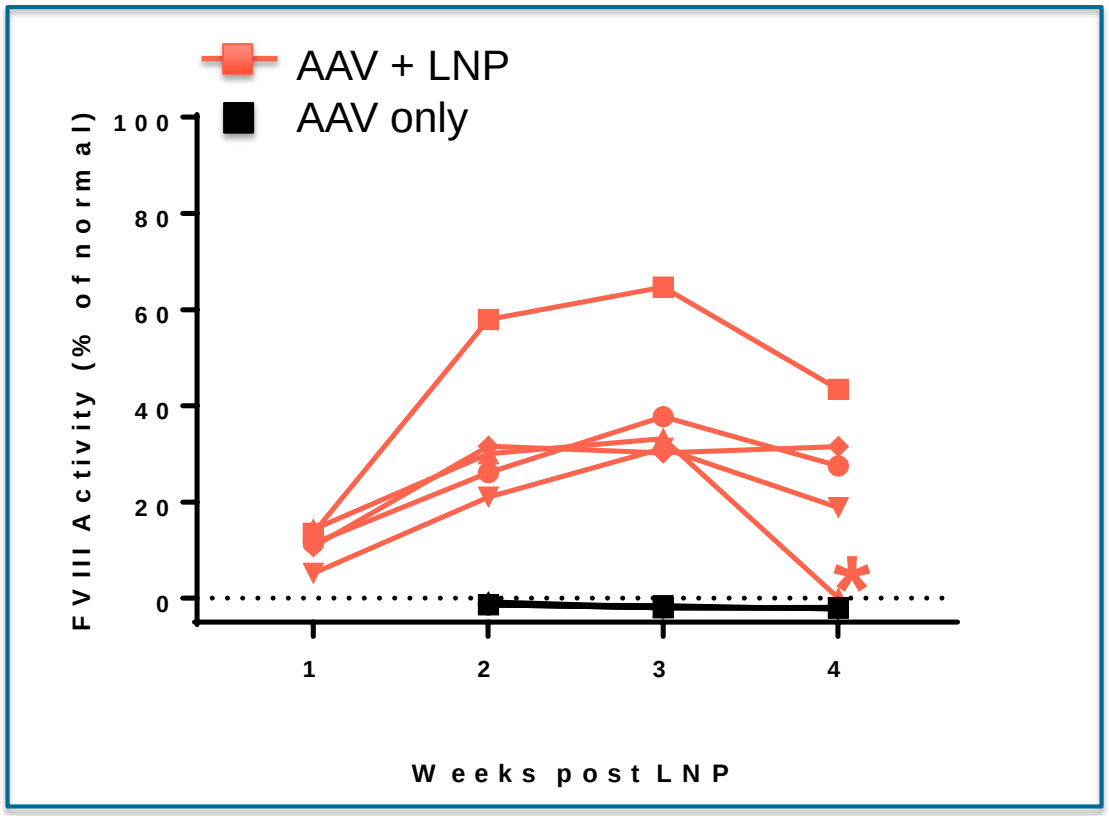
- Sequential increases in gene expression after each LNP dose
- This modality has the potential to tune the desired expression level after a single dose of AAV



Background SEAP activity in mice that received AAV only was 0

Therapeutic Levels of FVIII After Genome Editing in HemA mice

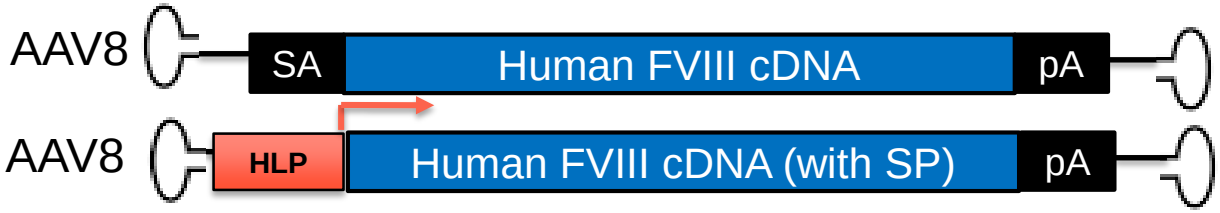
HemA Mice	AAV-FVIII + LNP (2 mg/kg) –sgRNA1 AAV-FVIII only



* Antibodies to human FVIII present

- Therapeutic FVIII activity achieved, approaching the normal range
- No FVIII when only the AAV-FVIII donor was injected demonstrating that cleavage of the genome required
- Antibodies against the foreign human FVIII protein develop in some mice

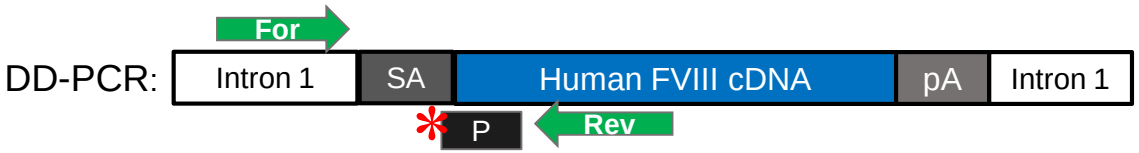
Targeted Integration Correlates to Cellular Distribution of Hybrid mRNA



HemA Mice	AAV8-FVIII (2e12 vg/kg) + LNP (2mg/kg) AAV8-**HLP**-FVIII (2e12 vg/kg)

Day 10: FVIII activity in blood

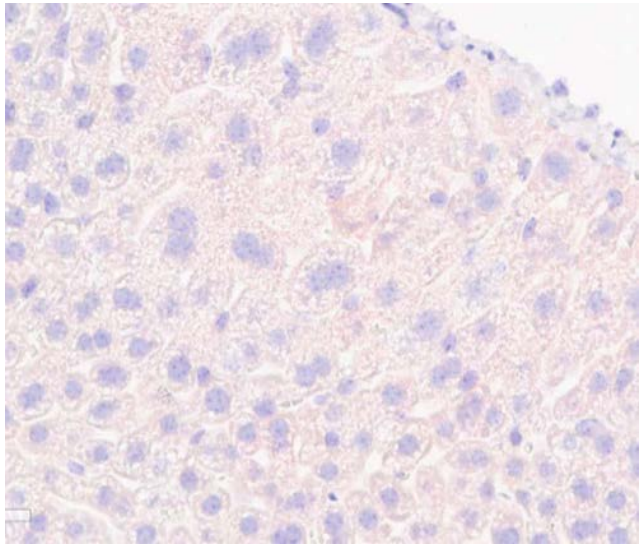
Day 25: BaseScope™ and Targeted Integration by DD-PCR

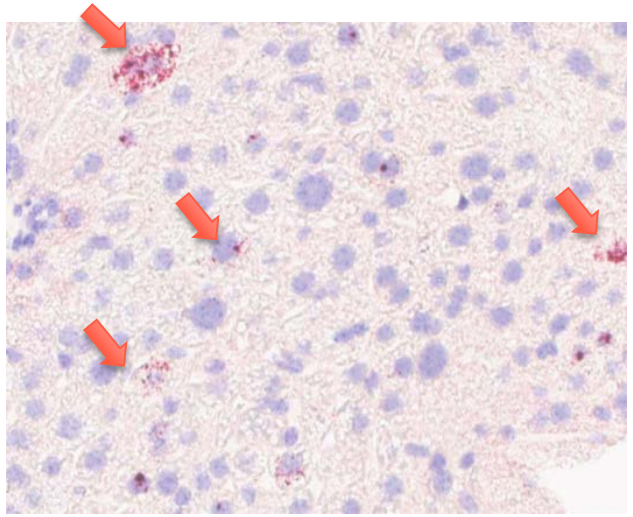


Group (n=5)	FVIII activity on day 10 (% of normal)	Targeted Integration (% per haploid genome)
AAV8-FVIII +LNP	26 +/- 9	2.5 +/- 0.6
AAV8-**HLP**-FVIII #	28 +/- 5 (peak on d30)	-
Naïve mice	0	0

HLP promoter driving same FVIII CDS (but with signal peptide)

BaseScope™ for hybrid mRNA in liver

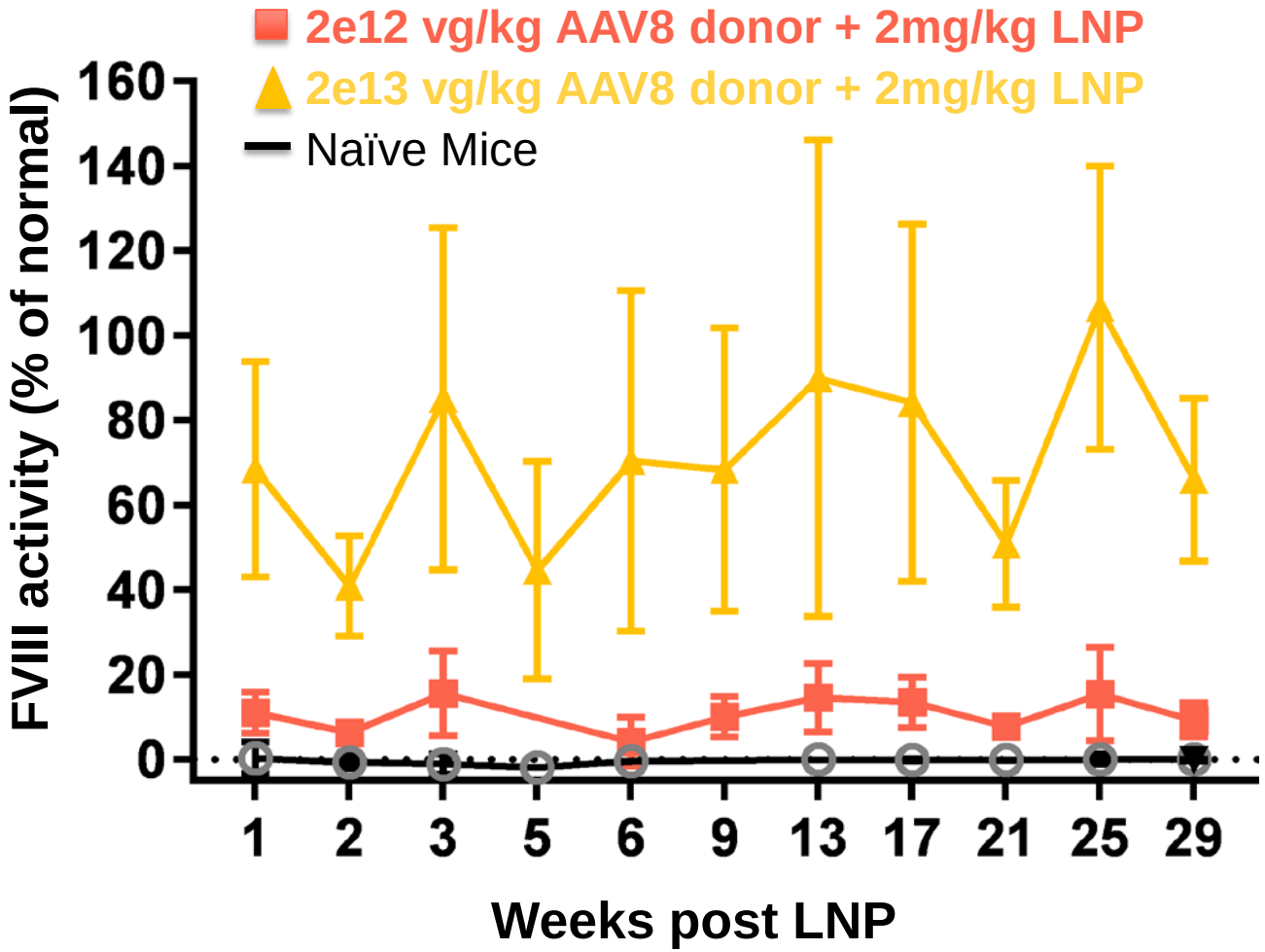




2% to 4% of cells positive

Long Term Expression of FVIII

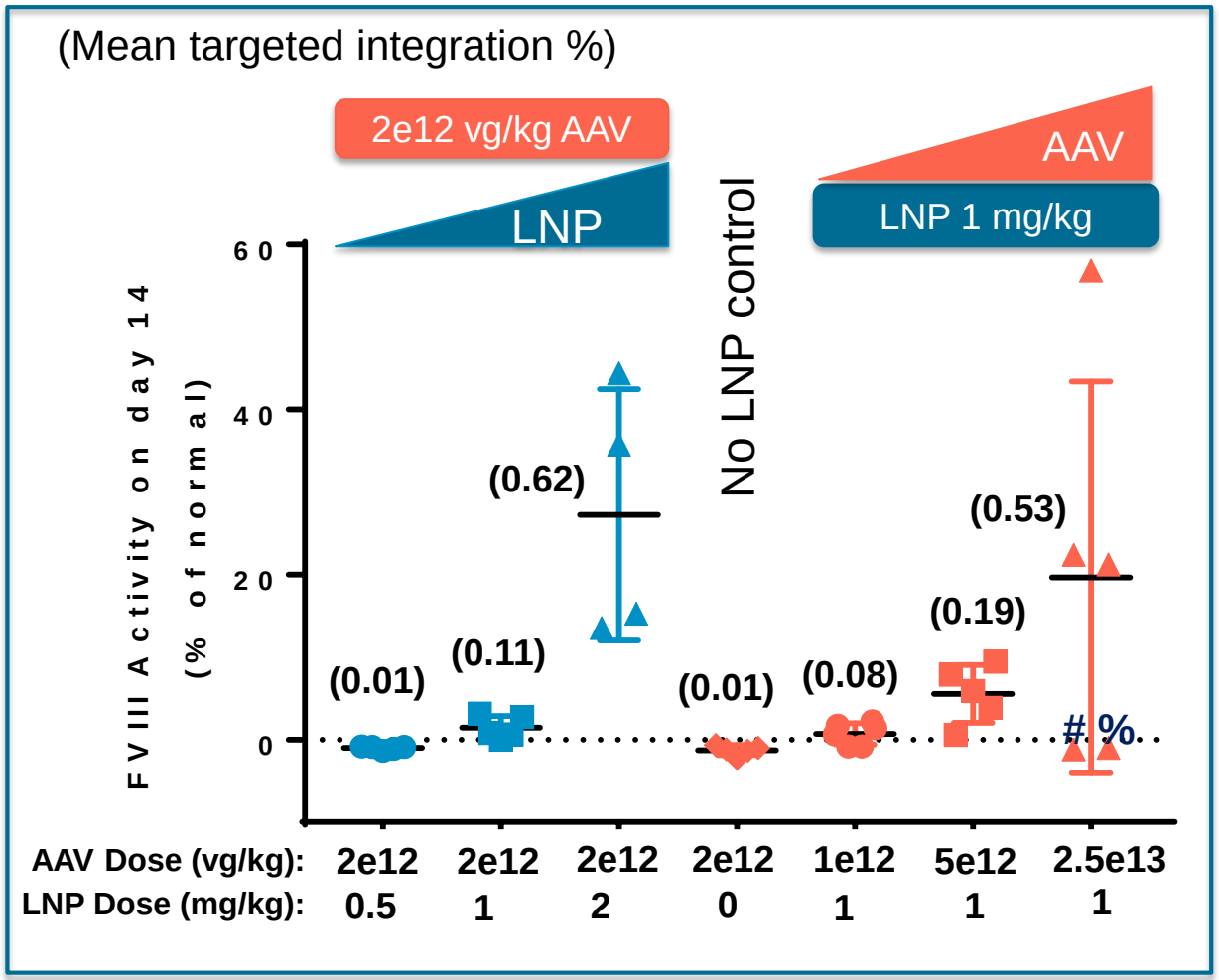
- Immune deficient NSG mice to prevent antibodies to human FVIII
- FVIII detected with human FVIII specific capture-activity assay



- Stable expression to 7 months and continuing to monitor
- FVIII levels dependent on dose of AAV donor

AAV and LNP Dose Response in HemA Mice

+/- LNP



- FVIII level can be modulated by LNP dose
- Higher doses of AAV enable FVIII expression at lower LNP doses
- FVIII levels correlate to targeted integration frequency

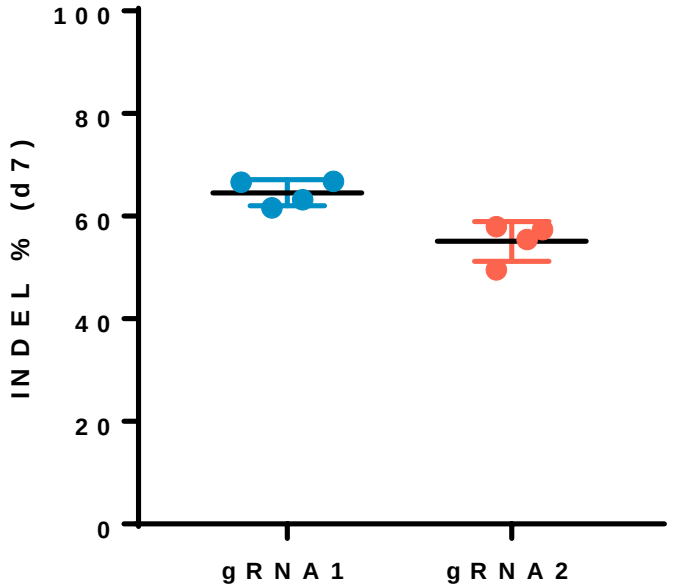
% Mouse had antibodies to human FVIII on d14
# Mouse had no INDELS indicative of failed LNP delivery

Selection of gRNA can Impact Expression

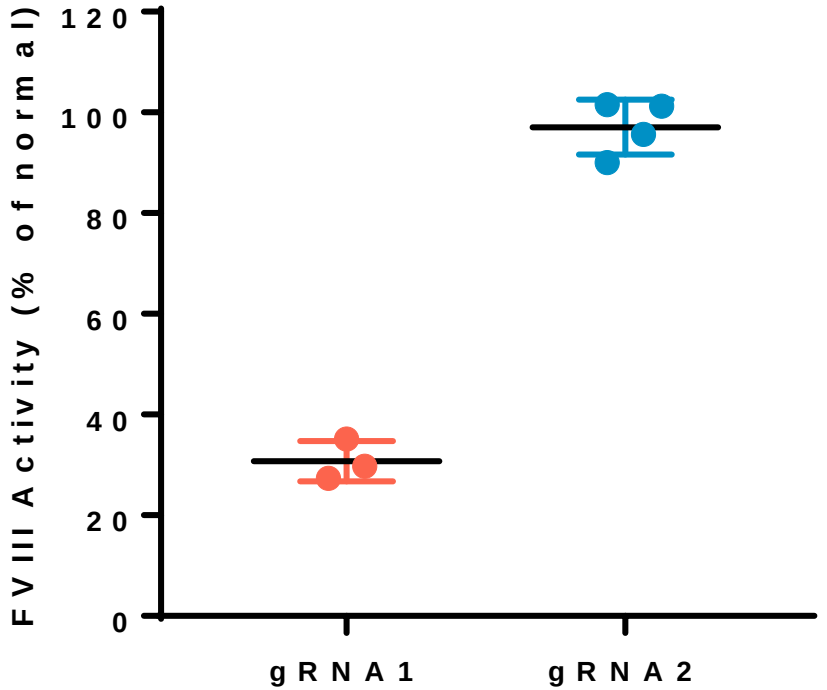
HemA Mice	LNP only (1.5 mg/kg) AAV-FVIII (2e12 vg/kg) + LNP (1.5 mg/kg)

Optimized LNP, two gRNA targeting Albumin intron 1
- gRNA1 (used for results in previous slides)
- gRNA2

Mice dosed with LNP only



FVIII Day 8 post LNP



- 5-fold improved expression using gRNA2 vs gRNA1
- Improvement with gRNA 2 was not due to higher INDELS
- 100% FVIII with low variability achieved with 2e12 vg/kg AAV8 donor
- Measurement of targeted integration is planned

Summary and Conclusions

- CRISPR-Cas9 mediated targeted integration of FVIII at the albumin locus generates therapeutic levels of FVIII in mice, up to 100% of normal
- Low levels of targeted integration are sufficient for curative levels of FVIII
- Expression was stable (7 months and continuing)
- Targeted integration correlated to FVIII levels and was dependent on dose of both AAV and LNP
- FVIII level could be modulated by LNP dose
- Transient delivery of the nuclease as a LNP packaged mRNA has the potential for repeat dosing to “dial-in” FVIII levels while minimizing off-target risk
- Further optimization and evaluation in higher species in progress

Acknowledgments

Co-Authors

Karen Vo

Dariusz Wodziak

Rangoli Aeran

Keith Abe

Cornell Mallari

Valerie Guerrero

Christopher Cheng

Andrew Scharenberg

Additional Contributors

Chandra Patel

Luis Gamboa

Greg Cost

Gene Uenishi

Christopher Cheng

Faye Wu

Samantha Chin

Scott Munzer

Karolina Kosakowska

Kui Wang

Patrick Au

Mihail Rokas

Jim Burns