Preclinical Evaluation of Combined AAV and Nanoparticle delivery of piggyBac® DNA Modification System for Durable Transgene Expression in the Growing Neonatal Murine Liver

Jingjing Jiang, MD, PhD
Associate Director, Gene Therapy

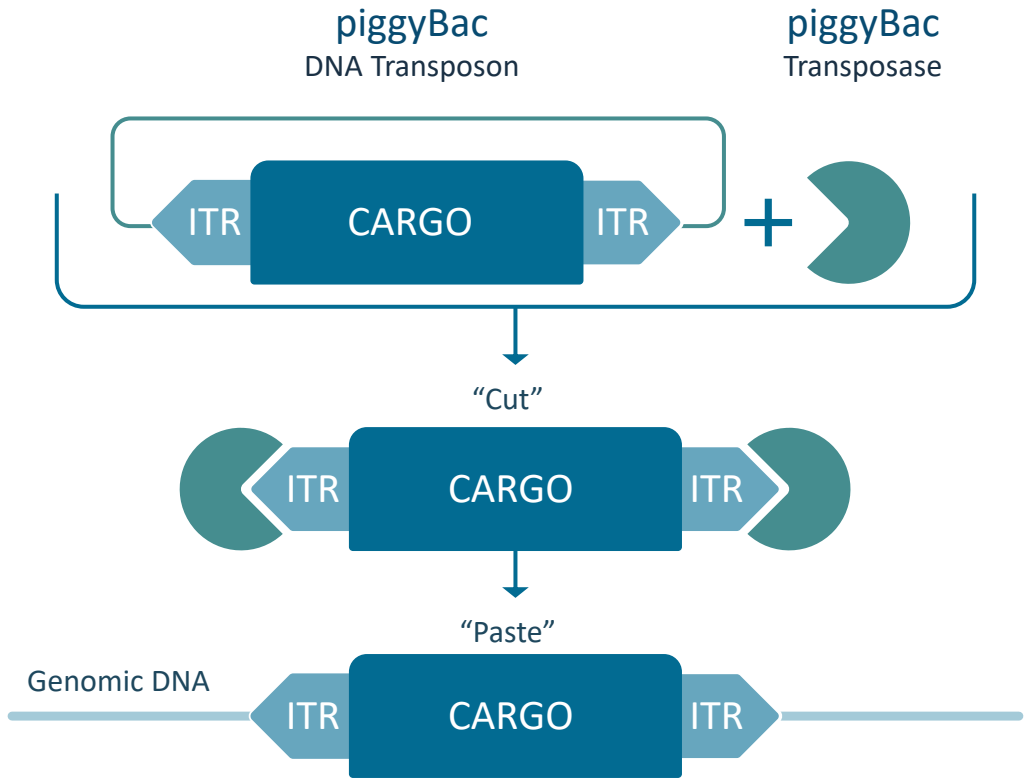
POSEIDA THERAPEUTICS

Disclosures:

Jingjing Jiang is a full-time Employee at Poseida Therapeutics, Inc.

piggyBac®: A Versatile DNA Delivery System for Developing Gene Therapy Products

- Permanent DNA integration and stable expression
- Very large cargo capacity (~200 kB)
- Works in a wide variety of cell types
- Favorable insertion profile
- Works with multiple delivery modalities including AAV and/or nanoparticle technologies
- Multiple safety benefits

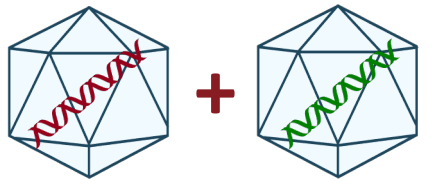


POSEIDA THERAPEUTICS

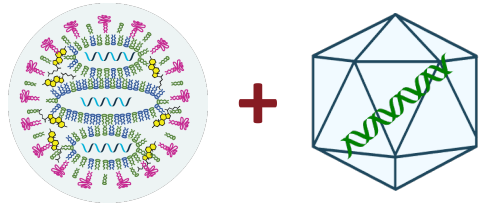
Poseida: Combinations of AAV and Nanoparticle Nucleic Acid Delivery Range from Fully Viral to Fully Non-Viral

The goal of our in vivo gene therapy program is to enable single treatment cures of genetic diseases by combining the piggyBac® DNA Modification System with Poseida's proprietary gene delivery platforms

Viral → **Non-Viral**

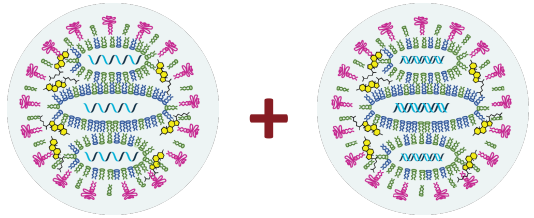


AAV (SPB-DNA)
AAV (PB-DNA)



Nanoparticle (SPB – RNA)
AAV (PB-DNA)

P-OTC-101



Nanoparticle (SPB – RNA)
Nanoparticle (PB – DNA)

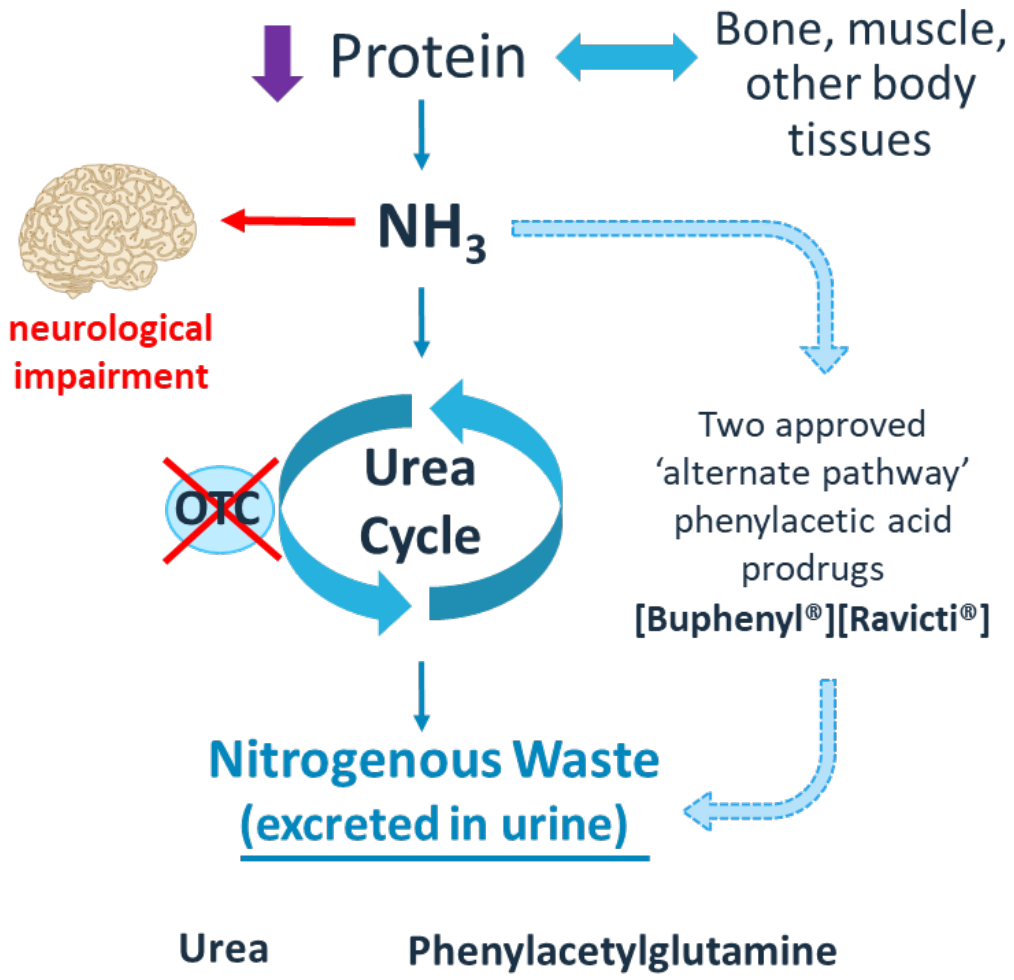
P-FVIII-101

POSEIDA THERAPEUTICS

Ornithine Transcarbamylase (OTC) Deficiency

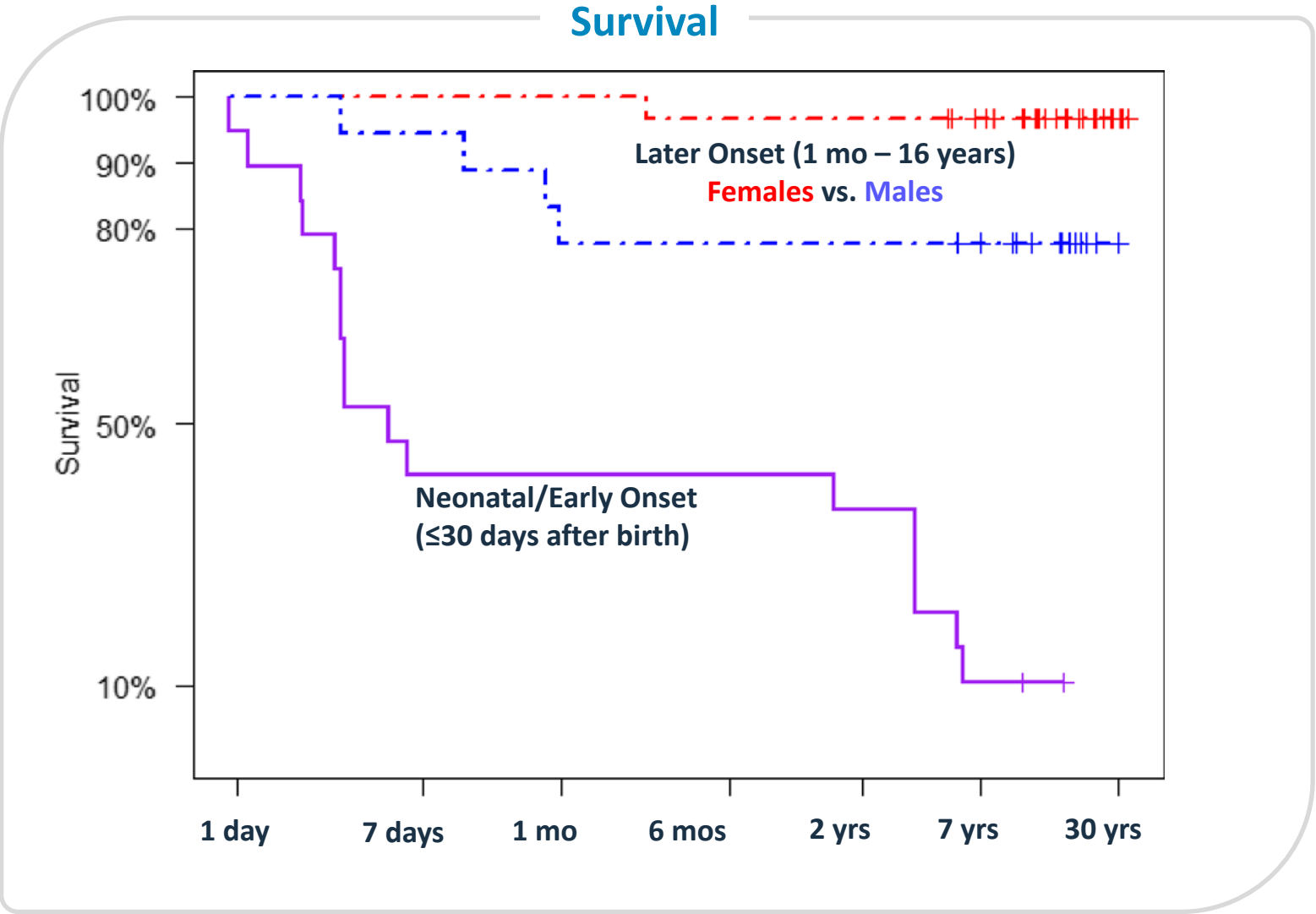
OTC Deficiency

- X-linked ultra-orphan metabolic disorder
- Most common urea cycle disorder subtype and most common cause of 'early onset' illness
- Causes hyperammonemia crises which may result in neurological impairment or death
- Dietary protein restriction & alternative pathway drugs inadequate for early onset illness
  - Infants/children at risk for crises despite maximal medical Rx
  - Liver transplantation is the emerging standard of care



POSEIDA THERAPEUTICS

Early Onset/Severe OTC Deficiency:
Major Unmet Need and Opportunity for Benefit

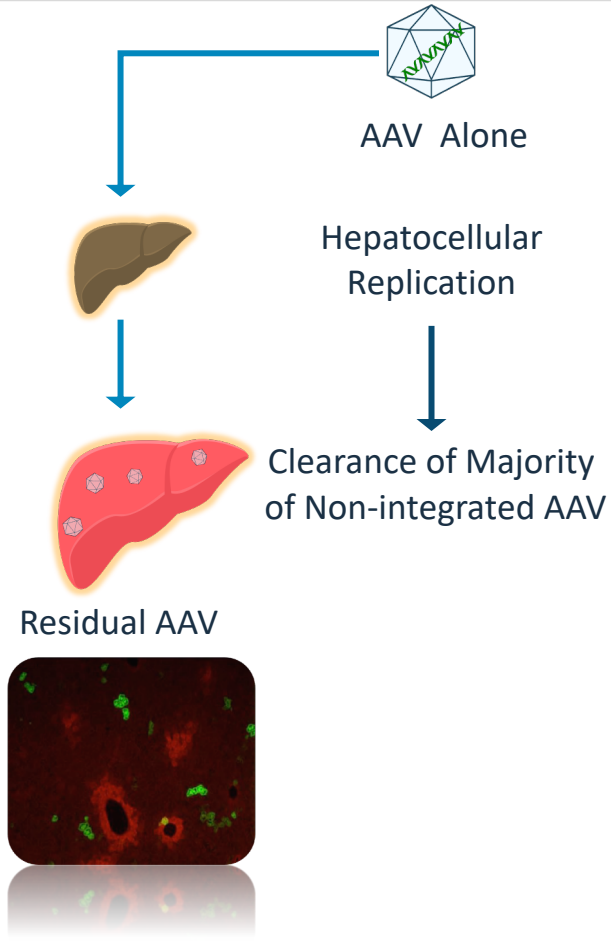


Brassier et al., Orphanet Journal of Rare Diseases , 2015 (French series spanning 1971-2011)

POSEIDA THERAPEUTICS

Shortcomings of Current Approaches

- AAV alone
  - Episomal, diluted with cell division
  - Not well-suited for durable, high-level expression in rapidly growing tissues
- Liver transplantation
  - Expensive
  - Inaccessible to many
  - Infants/children at risk for lethal crises while they grow sufficiently to render it feasible, or while on waiting list
  - Lifetime immunosuppression-related cost & morbidity

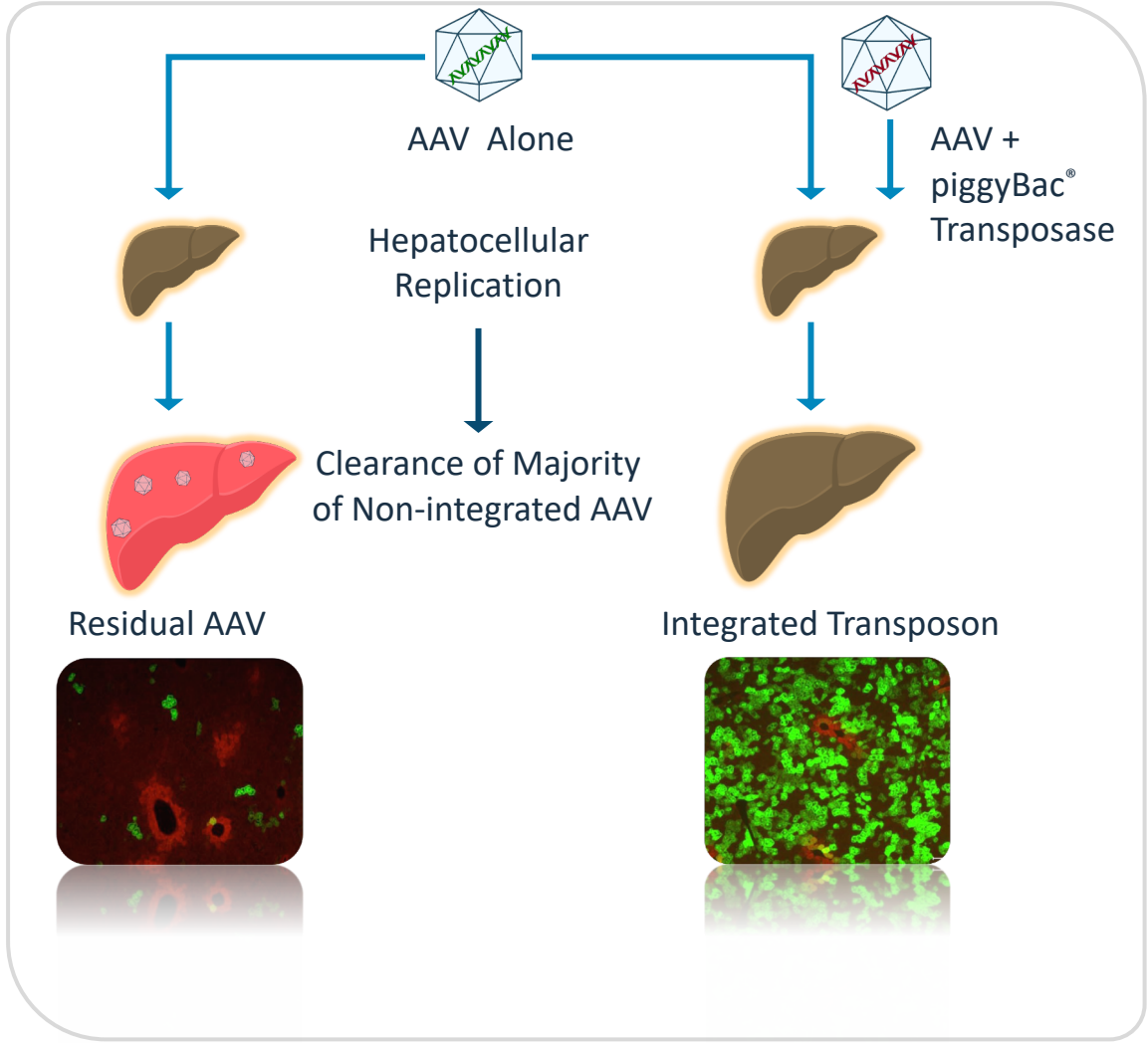


Cunningham et al., Hepatology 2015

POSEIDA THERAPEUTICS

Rationale for Dual piggyBac® AAV Therapy

- With AAV-piggyBac®, Cunningham et al.
  - Reported single injection correction of the two UCD subtypes (OTC & ASS deficiency) usually responsible for early onset illness
  - In the OTC deficient Spf^{ash} mouse model
    - Durable, high-level transgene expression
    - Rescue of lethal phenotype
    - OTC activity increased up to 100x
  - Reported single injection correction of a genetic cholestatic liver disorder affecting infants and young children (PFIC3)

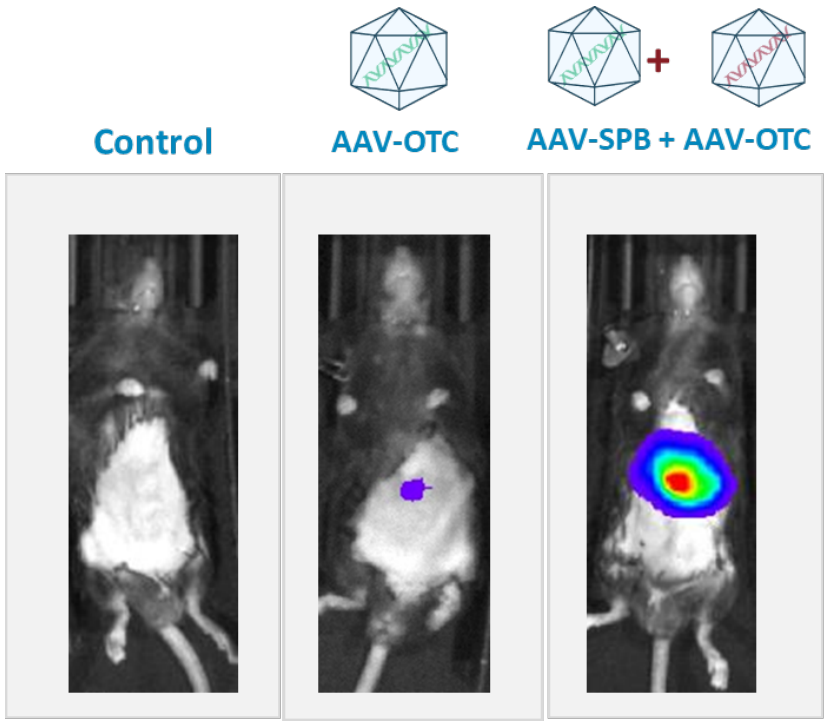


Cunningham et al., Hepatology 2015 Siew et al., Hepatology 2019

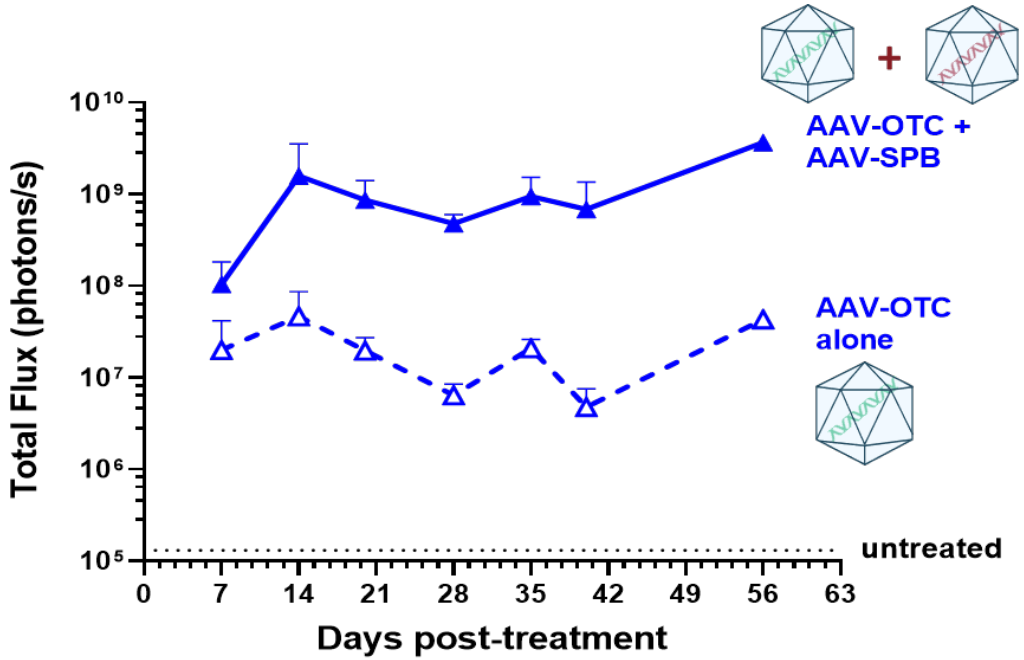
POSEIDA THERAPEUTICS

SPB Enhances Transgene Expression in Growing Liver

Liver Bioluminescence



Liver Bioluminescence



KP1 selected as lead capsid*

- Infects mouse and human hepatocytes in humanized liver mouse model
- Favorable neutralization profile

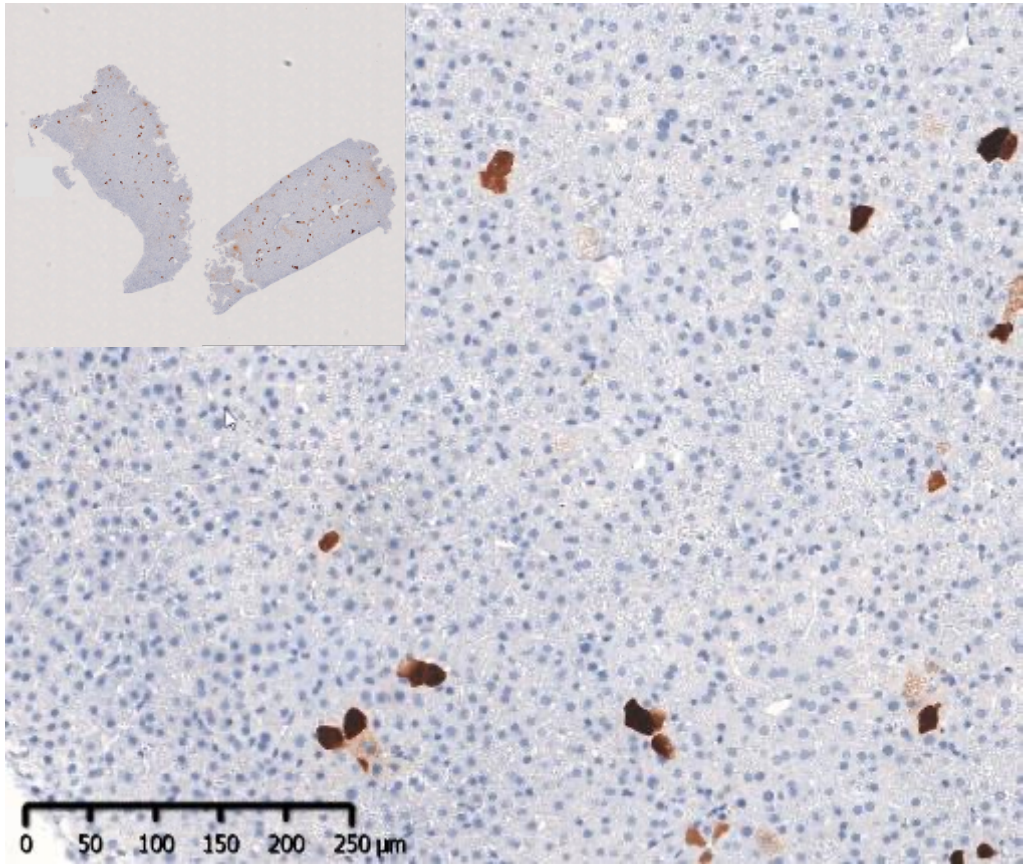
*Pekrun et al., *JCI insight* 2019

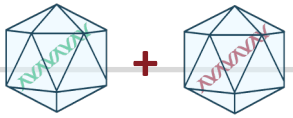
POSEIDA THERAPEUTICS

SPB Enhances Transgene Expressing Hepatocytes in Growing Liver

GFP

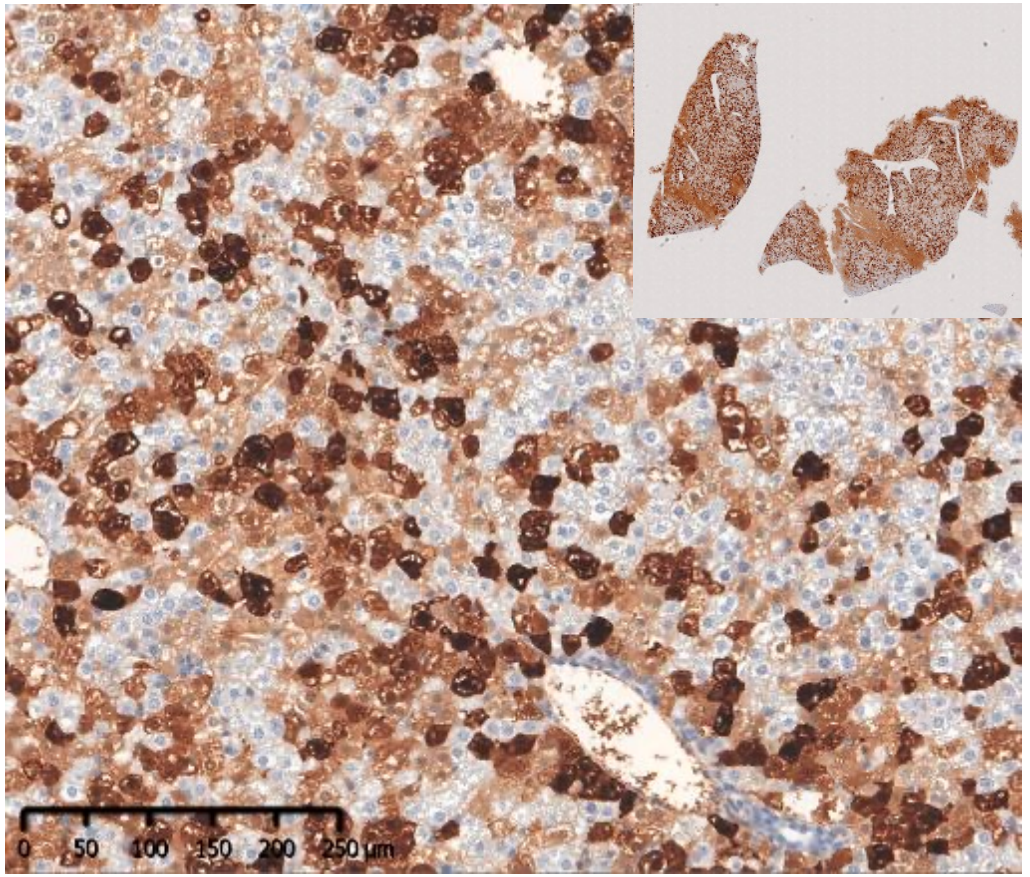
AAV-OTC reporter alone





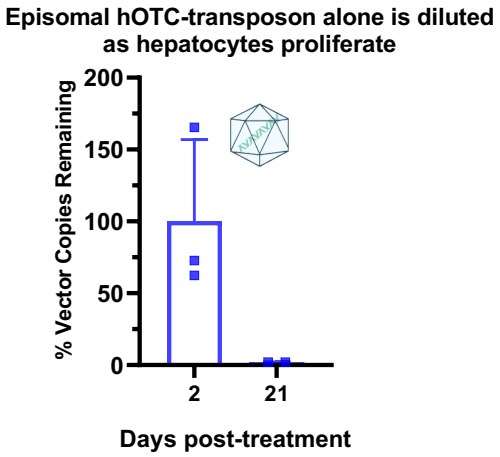
GFP

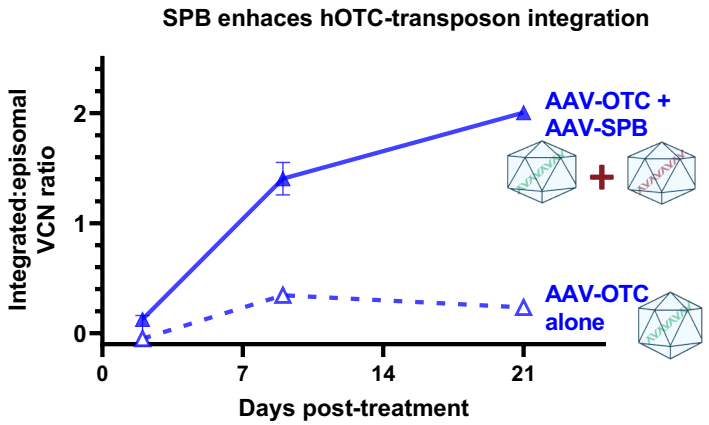
AAV-OTC reporter + AAV-SPB

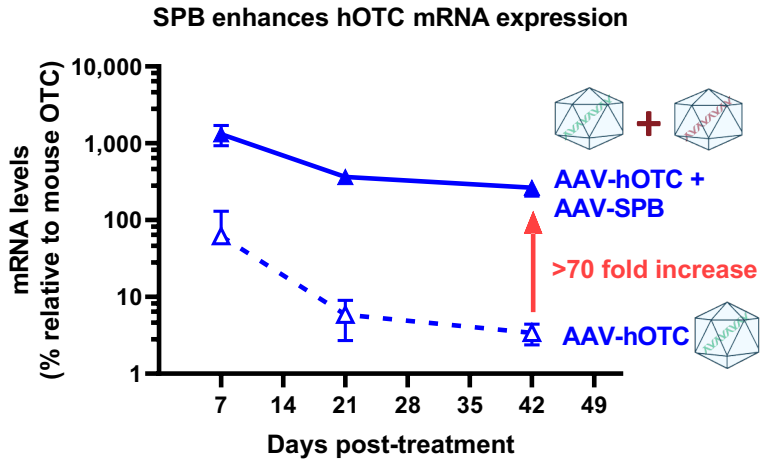


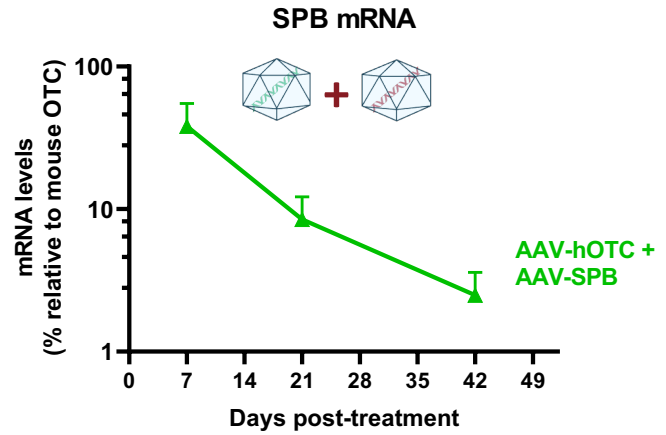
POSEIDA THERAPEUTICS

Transient Expression of SPB Enhances OTC Transgene Levels via Integration





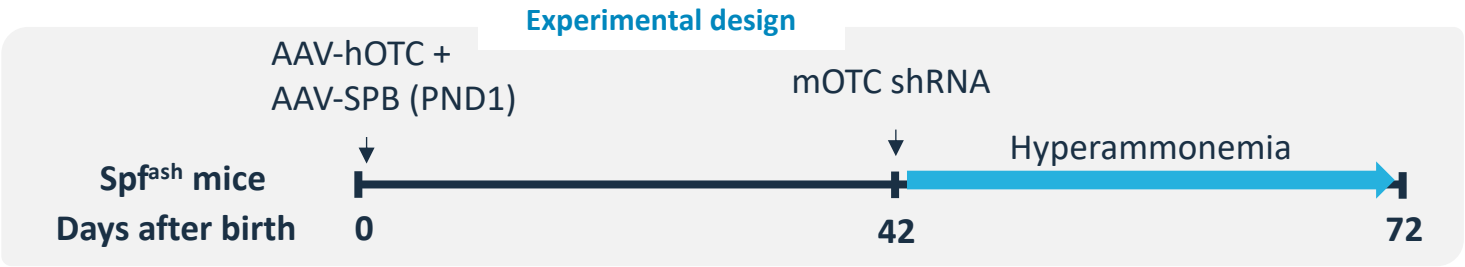


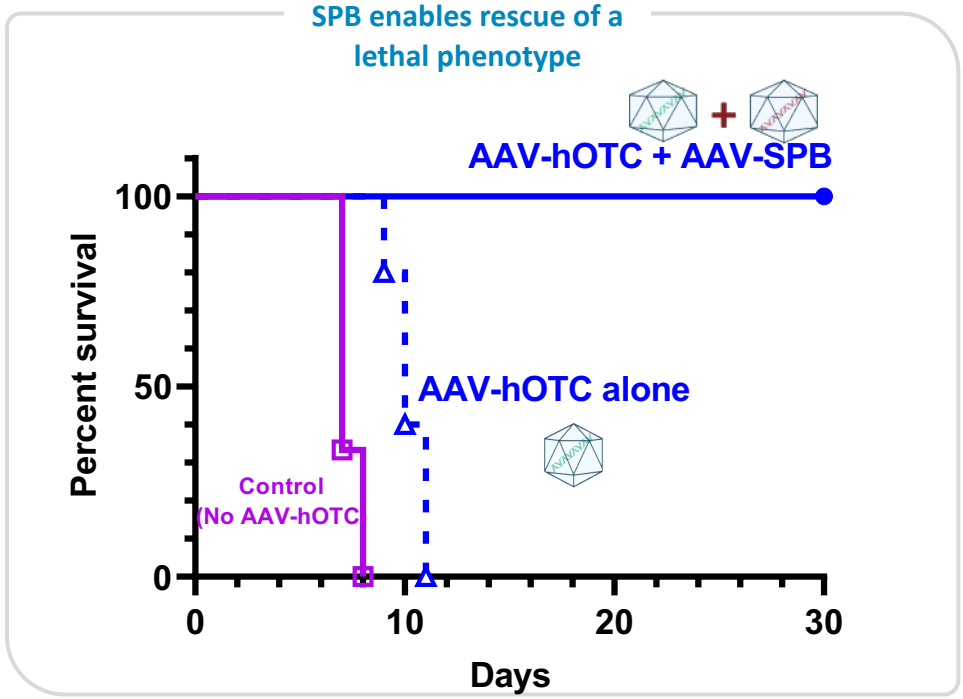


- Early, transient SPB expression enables transgene transposition
- Genomic integration confirmed by LM-PCR
  - Further bioinformatics analysis ongoing

POSEIDA THERAPEUTICS

SPB Enables Rescue of Lethal Illness in Early Onset OTCD Mouse Model

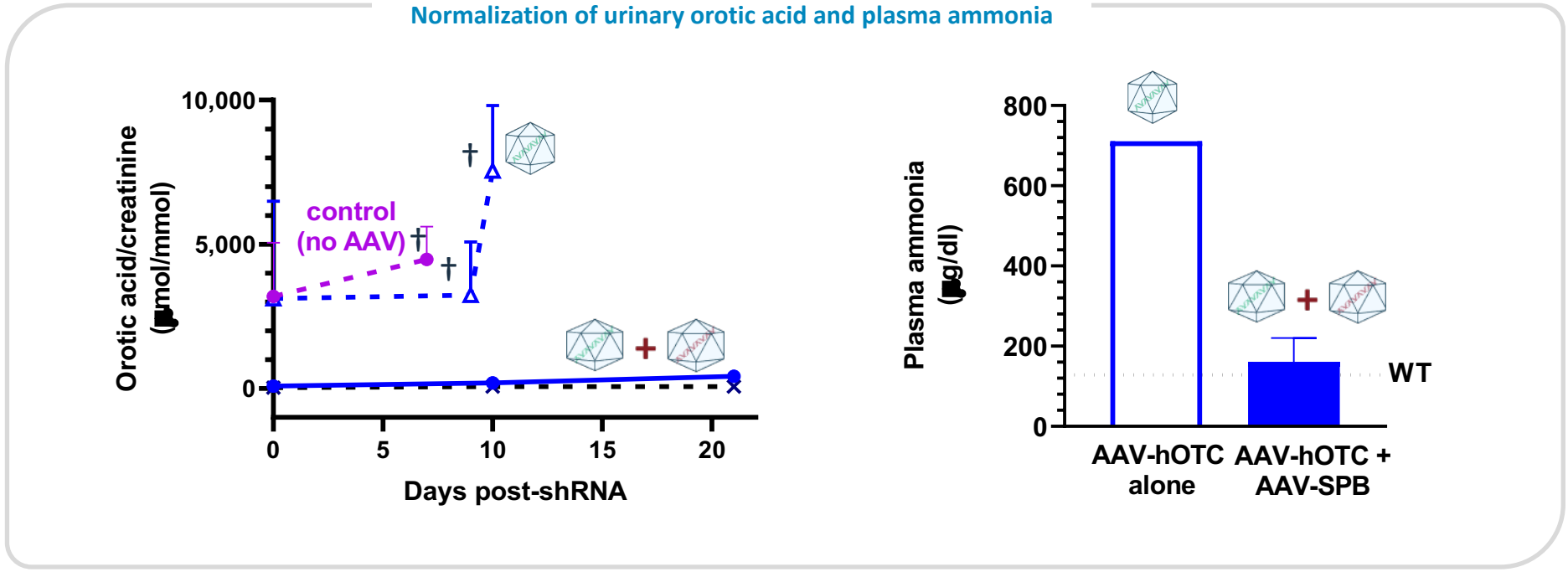




- AAV alone does not rescue OTCD mice dosed as neonates -> differentiates piggyBac from AAV alone

POSEIDA THERAPEUTICS

SPB Enables Disease Phenotype Correction in Early Onset OTCD mouse models



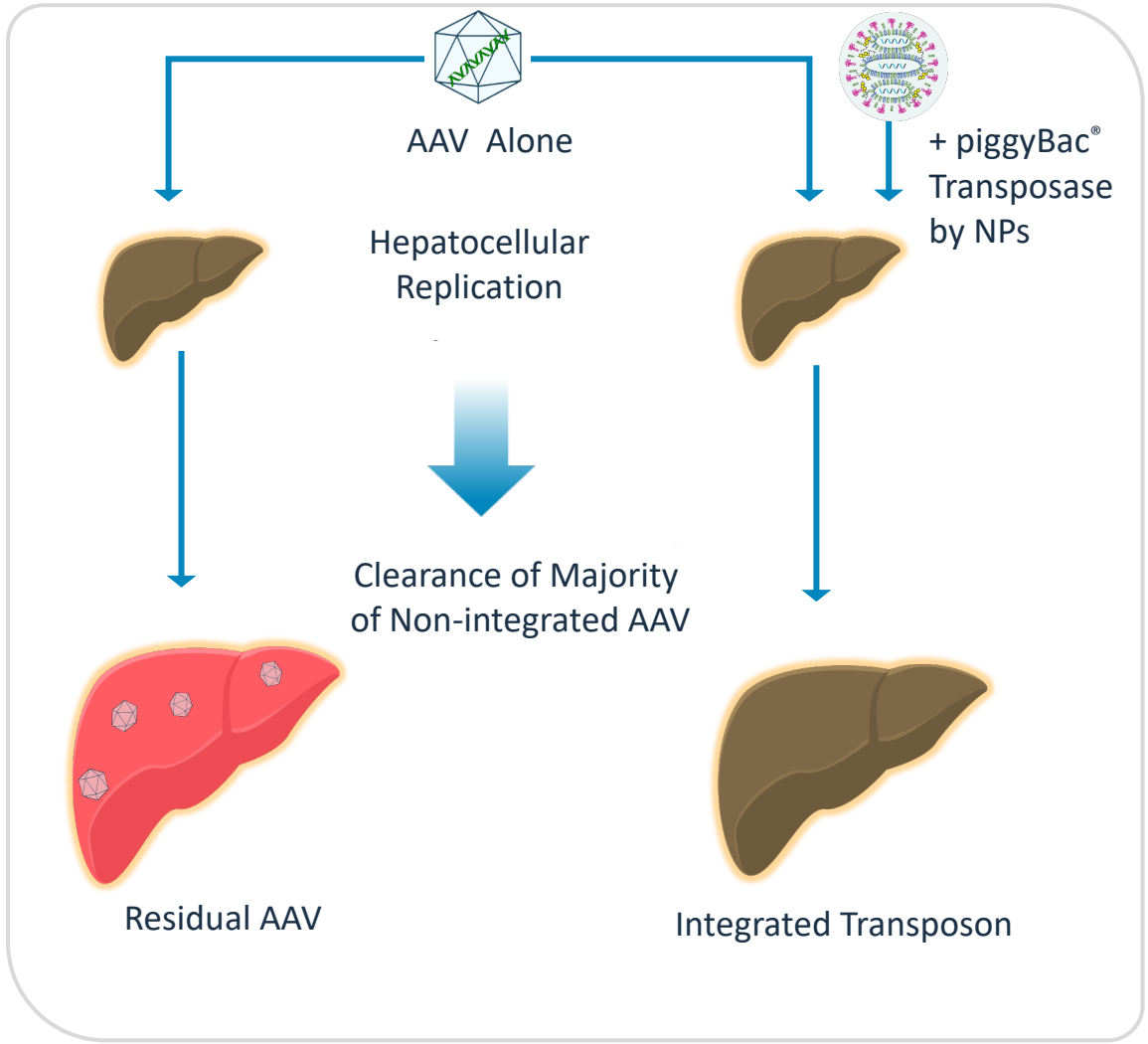
- Normal urinary orotic acid and plasma ammonia levels post shRNA challenge suggest disease phenotype correction

POSEIDA THERAPEUTICS

Rationale for Nanoparticle Delivery of piggyBac® mRNA Transposase

Novel liver-directed NP developed by Poseida for delivery of piggyBac® transposase mRNA

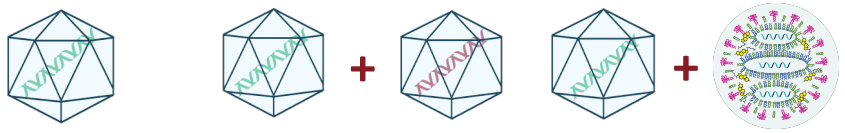
- Lower AAV dose
- No pre-existing AAV immunity
- Transient expression of transposase protein
- Ease of manufacturing

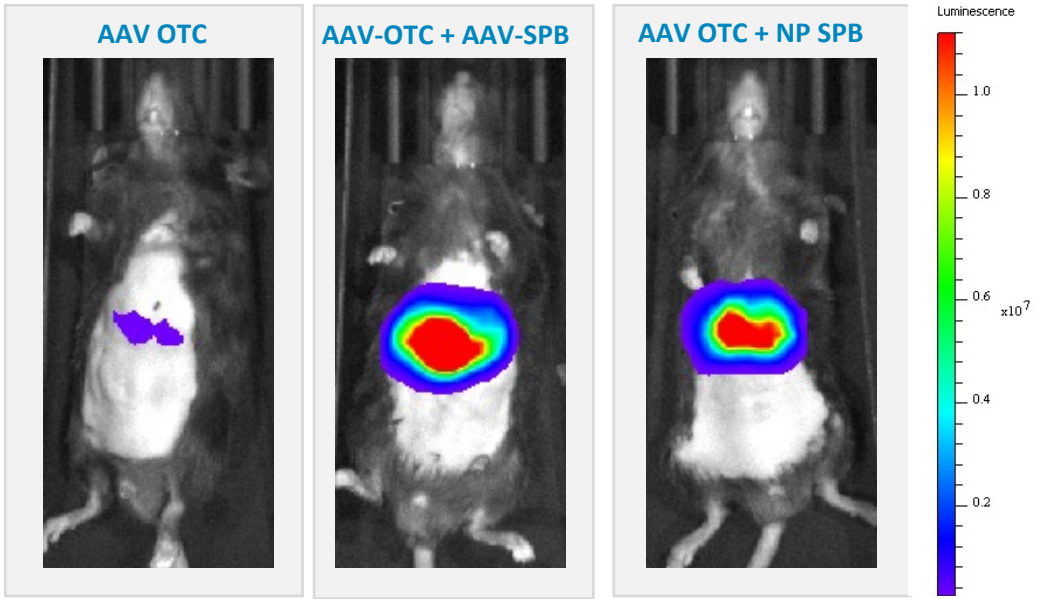


POSEIDA THERAPEUTICS

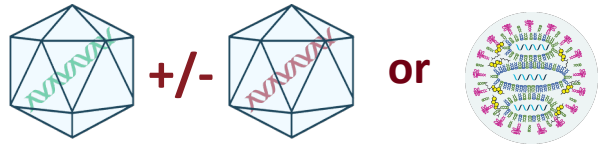
Comparable SPB Enhancement of Transgene Expression in Growing Liver with AAV or NP Delivery in Neonatal Mice

Liver Bioluminescence

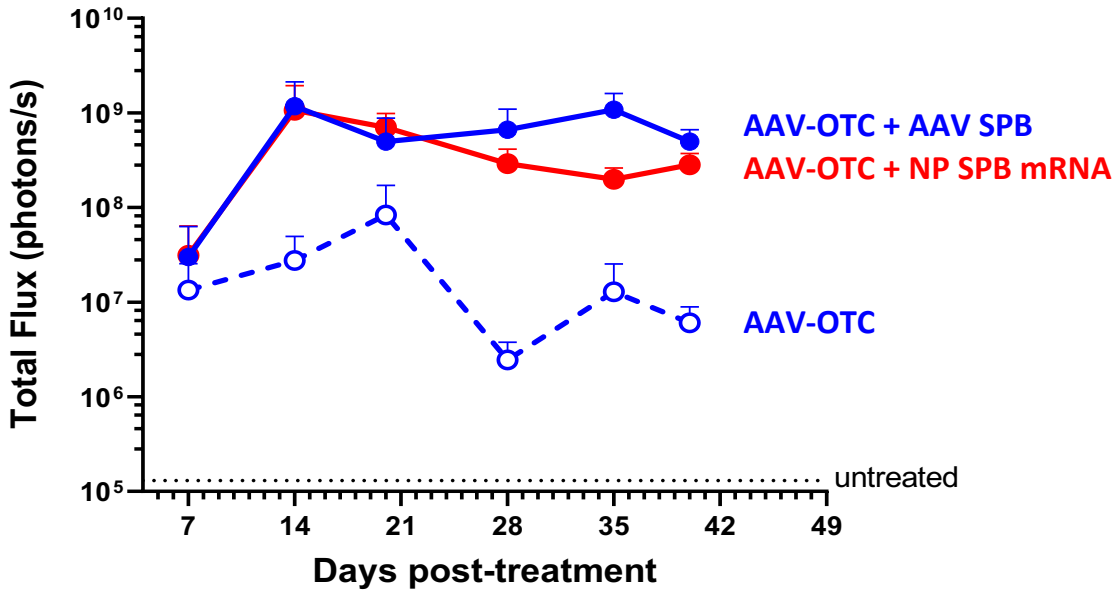




Liver Bioluminescence



AAV-OTC AAV-SPB NP-SPB

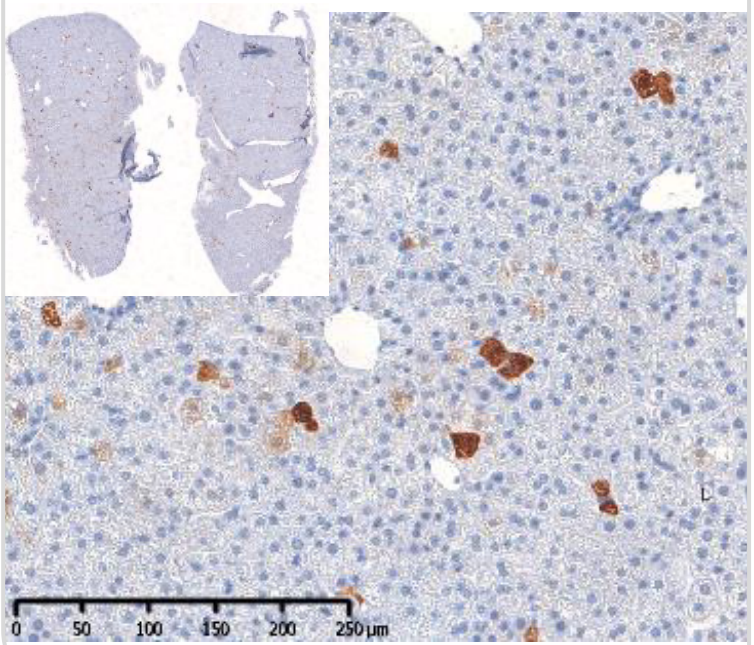


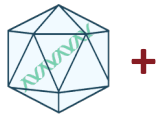
POSEIDA THERAPEUTICS

Comparable SPB Increase in Transgene Expressing Hepatocytes in Growing Liver with AAV or NP Delivery in Neonatal Mice

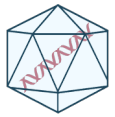
GFP

AAV-OTC-reporter alone



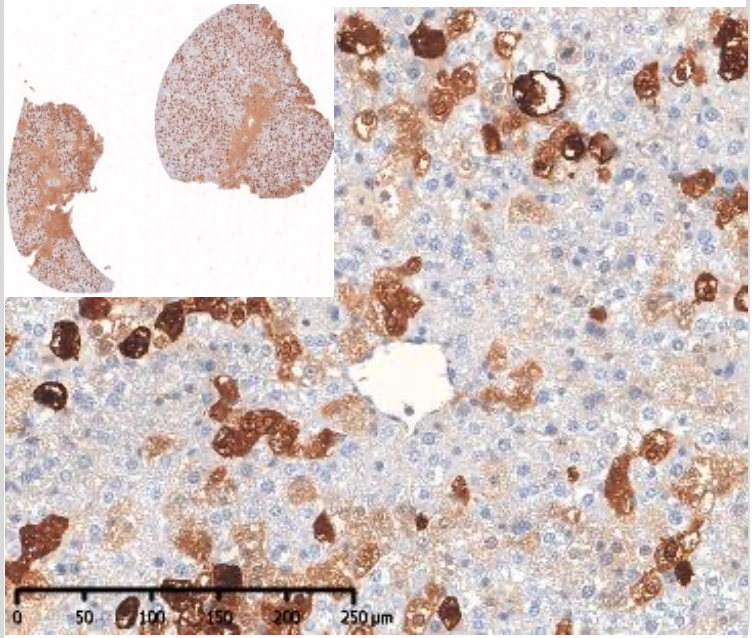


+

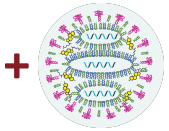


GFP

AAV-OTC-reporter + AAV-SPB

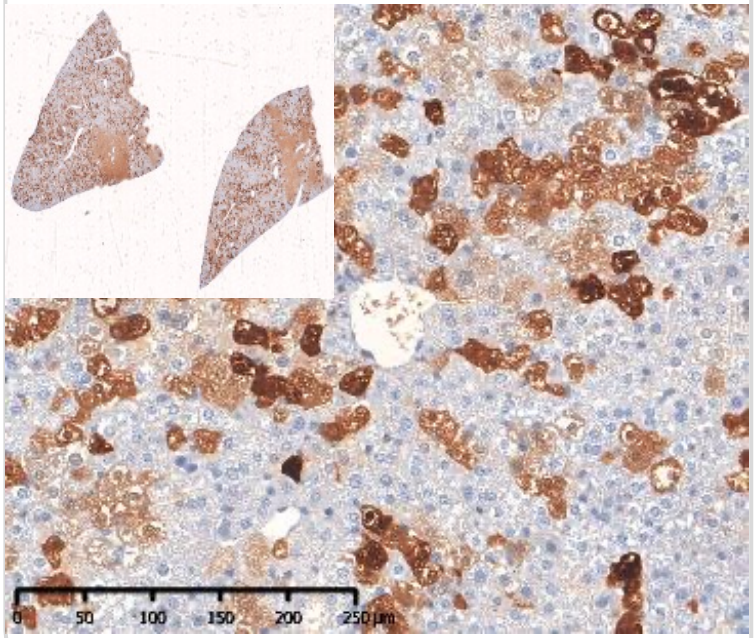


+



GFP

AAV-OTC-reporter + NP-SPB mRNA



- Transgene genomic integration confirmed by LM-PCR
- Further bioinformatics analysis ongoing

POSEIDA THERAPEUTICS

Summary

- piggyBac® DNA modification system delivered via a two AAV virion approach enables durable high level hOTC transgene expression sufficient to permit rescue of the lethal illness and disease phenotype correction
- NP may offer advantages over AAV for delivery of the transposase
- Liver-directed piggyBac® transposase mediated gene therapy delivered via AAV and/or LNP shows promise for treatment of metabolic and other genetic disorders involving the liver

Acknowledgements

- Bernard Kok
- Xinggang Liu
- Vananh Pham
- David Ebeid
- Mehul Dhanani
- Sean Essex
- Jivan Yewle
- Brian Truong
- Devon J. Shedlock
- Joshua Rychak
- Bruce F. Scharschmidt
- Eric M. Ostertag
- Julian D. Down

Thank You

POSEIDA THERAPEUTICS