Correction of Severe Ornithine Transcarbamylase Deficiency in Growing Neonatal Murine Liver using Combined Adeno-Associated Virus and Nanoparticle Delivery of piggyBac® Transposon System

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ABSTRACT
• Gene delivery via recombinant adeno-associated virus (rAAV) has proven efficacious in preclinical models and clinical trials for inherited metabolic disorders. However, current approaches are limited by rAAV toxicity and by dilution of episomal rAAV in rapidly dividing tissues, resulting in loss of therapeudeic transgene expression. These limitations have precluded durable phenotype correction in infants and young children severely affected by metabolic and other disorders involving the liver, such as early-onset ornithine transcarbamylase (OTC)-deficiency.

• To address these limitations, we explored the ability of piggyBac® delivered by rAAV and/or novel biodegradable nanoparticles (NP) to correct early-onset OTC disease phenotypes. Neonatal OTC deficient (Spfash) mice were treated using liver tropic rAAV containing a piggyBac® transposon with a human OTC expression cassette (hOTC) driven by a liver-specific promoter, with and without co-administration of Super piggyBac® transposase (SPB). As compared with AAV-delivery of hOTC transposon alone, which was ineffective, concomitant rAAV delivery of SPB resulted in stable hOTC integration into hepatocyte genomes with durable transgene expression and OTC activity sufficient to prevent death following otherwise lethal OTC mRNA knockdown, as well as to correct OTC disease biomarkers including urinary orotic acid and blood ammonia. Replacing rAAV with NP for delivery of SPB mRNA resulted in similarly high levels of transgene expression and disease correction.

• These findings collectively demonstrate the unique applicability of piggyBac® technology to in vivo liver-directed gene therapy of pediatric patients, as well as the versatility afforded by utilizing viral and/or non-viral delivery to achieve single-treatment correction of OTC deficiency and potentially other genetic diseases involving the liver.

INTRODUCTION

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 (NP) technologies
- Multiple safety benefits

Figure 1: Schematic of gene transposition using the PB system. This allows permanent DNA integration and stable expression of the therapeutic transgene with a favorable insertion profile and has demonstrated application with multiple delivery modalities including AAV.
INTRODUCTION

Poseida: piggyBac®-based In Vivo Gene Therapy from Viral to 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 Delivery System with Poseida’s proprietary gene delivery platforms.

Viral

P-OTC-101

AAV KP1 (SPB-DNA)
AAV KP1 (PB-DNA)
Dual AAV

Non-Viral

Ultimate Goal

Nanoparticle (SPB-RNA)
Nanoparticle (PB-DNA)
Dual NP

OTCD Deficiency:

• X-linked ultra-orphan metabolic disorder
• Most common urea cycle disorder subtype and most common cause of ‘early onset’ illness
• Causes hyperammonemic 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 treatment
  • Liver transplantation is the emerging standard of care

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

Rationale for piggyBac® AAV Therapy in Growing Liver

Figure. 2 Survival among patients with OTCD presenting in the first month of life (solid lavender line) vs. male (blue dashed line) or female (red dashed line) patients with OTCD presenting at ages > 1 month to 16 years (adapted from Brassier et al. 2015 Orphanet J Rare Dis).

Figure. 3 Long-term, high-level transgene expression is possible with PB technology. AAV alone results in minimal transgene expression, particularly in rapidly dividing tissues such as the juvenile liver (adapted from Cunningham et al. 2015 Hepatology).

Figure. 4 Knockdown of endogenous mOTC mRNA resulting in hyperammonemia was induced at ~6 weeks of age in Spfash mice using AAV8 shRNA-mOTC. Health was assessed daily, and mice were euthanized based on onset of morbidity and rapid weight loss.
**RESULTS**

Figure 5. Loss of Efficacy with AAV alone treatment in Early-Onset OTCD mouse model. A) Spf<sup>inh</sup> mice treated at PND1 with hOTC AAV alone showed signs of hyperammonemia while only minimally delaying morbidity-related euthanasia. Representative images of OTC (brown) hepatocytes in B) the no AAV group, and in C) mice treated with AAV hOTC alone. Glutamine synthetase (pink) as pericentral marker.

Figure 8. Molecular and biochemical analysis of Spf<sup>inh</sup> mice liver samples at study termination. Spf<sup>inh</sup> mice treated at PND1 with hOTC AAV in conjunction with AAV-SPB or NP-SPB mRNA resulted in high A) human OTC mRNA levels and B) OTC enzymatic activity. Integrated vector copy numbers were determined by ddPCR-based assays. Addition of SPB resulted in ~1 to 2.3 copies per diploid genome.

**SUMMARY**

- piggyBac® DNA Modification System delivered via AAV + NP or dual AAV approach enables durable high level hOTC transgene expression sufficient to rescue lethal illness and disease phenotype correction in an early-onset OTCD model; Biodegradable nanoparticles may offer advantages over AAV for delivery of the transposase.

- Liver-directed piggyBac® transposase-mediated gene therapy delivered via AAV and/or NP shows promise for treatment of metabolic and other genetic disorders involving the liver.