Development of a Novel Non-Viral Gene Therapy Platform

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ABSTRACT

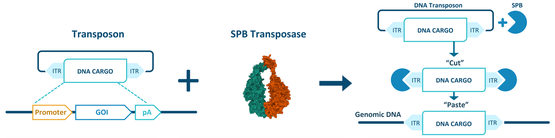
Gene therapy is a promising approach for treatment of severe bleeding disorders. AAV-based gene therapies have shown some success in this regard, though age restrictions, pre-existing capsid immunity, limited durability, and an inability to re-dose represent challenges to this platform. The piggyBac® DNA insertion system is a transposon-based gene therapy platform that enables stable integration of the therapeutic transgene into the genome, thereby offering the potential for durable and lifelong activity.

This platform requires two components: the super piggyBac transposase (SPB) and a piggyBac transposon comprising the therapeutic transgene expression cassette. The transposase functions by excising DNA cargo and inserting it into the genome and can be efficiently delivered as mRNA. One key advantage is the super piggyBac platform accommodates large cargo; this is especially advantageous for diseases such as hemophilia A in which the transgene (hFVIII) is especially large. We hypothesized that combining the piggyBac DNA insertion system with a non-viral delivery system could enable us to deploy this platform unconstrained by the cargo capacity limitations of AAV-based delivery systems, and potentially enable repeat dosing.

The lipid nanoparticle (LNP) is a mature non-viral delivery platform that has been clinically successful for liver-specific delivery of siRNA and mRNA. The implementation of the LNP platform to deliver DNA, however, has been challenging. Successful implementation of our super piggyBac system requires: 1) the development of LNPs capable of effectively and safely delivering the transposase mRNA and donor DNA template to the same target cells, 2) optimization of the transposon DNA format and sequence, and 3) optimization of the SPB mRNA to maximize transposase expression to give optimal excision and insertion of our gene of interest. With LNPs optimized using Design of Experiments (DOE), we explored (1) dual formulation, in which the transposon DNA and transposase mRNA are encapsulated in separate LNPs, and (2) co-formulation, in which the DNA and mRNA are encapsulated together within the same LNP.

We observed substantial hFVIII expression (>100% of normal) in juvenile wild-type mice following a single intravenous dose. Optimization of the transposon DNA: transposase mRNA ratio resulted in a further 8-fold increase in FVIII expression for both formulation strategies. Delivery of the hFVIII DNA alone or in concert with a control transposase (unable to perform genomic integration) resulted in significantly (>10X) lower hFVIII expression than using functional SPB. Vector copy numbers remained low, with an insertion profile consistent with previous studies. Production of hFVIII in the expected therapeutic range (10-50%) was also confirmed in a mouse model of severe hemophilia A. Contrary to conventional AAV-based gene therapies, this fully non-viral approach also supports repeat dosing. Repeated administration of the transposon/transposase nanoparticle system over 10 days resulted in dose-proportional increases in hFVIII antigen levels. In conclusion, we have demonstrated the feasibility of a non-viral approach using novel LNPs for treating severe bleeding disorders using the piggyBac DNA insertion system, with the potential for repeat dosing.

piggyBac® DNA Modification System for Integrating Gene Therapies



- Formulated as double-stranded DNA
- Cargo comprises promoter, gene(s) of interest (ORF), and regulatory elements

- Formulated as mRNA
- Transient expression is adequate for high-efficiency transposition; no concerns from persistent transposase expression

- SPB-mediated genomic insertion of genetic cargo to address early-onset genetic deficiencies
- Scarless integration into open chromatin (no INDELs)

Lipid Nanoparticles (LNPs) Use Several Lipid Types to Efficiently Encapsulate Nucleic Acids

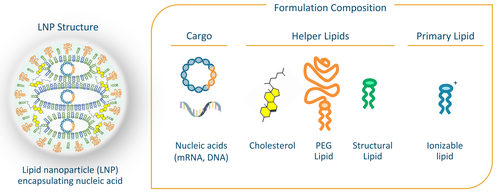


Fig. 1 Lipid nanoparticles use several lipid components, each with a unique function, to effectively encapsulate and deliver the nucleic acids required for the piggyBac platform. The ionizable lipid, also known as the primary lipid, is responsible for condensing and encapsulating the nucleic acid cargo, as well as driving the efficacy of the LNP. Cholesterol and the structural lipid are crucial for the stability of the LNP, while the PEG lipid is important for tuning the circulation time and preventing uptake by circulating immune cells.

Poseida's Proprietary LNPs Have a Favorable Liver Clearance Profile Suitable for Repeat Dosing

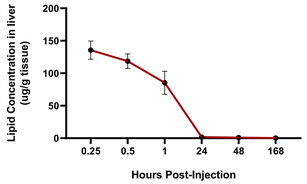


Fig. 2 Lipid concentration reaches undetectable levels by 24h post-LNP injection. LNPs were administered as an IV dose to adult BALB/c mice at a nucleic acid dose of 0.5 mg/kg. Liver lipid concentrations were determined by LC-MS at various time intervals after injection. Peak LNP liver accumulation occurred at early time points (~15 mins post-injection).

Optimization of the LNP Composition by DOE Significantly Improved the DNA Delivery Potency

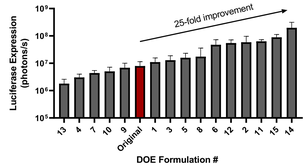


Fig. 3 Optimizing the ratio of the lipids in the LNP improved the DNA delivery performance by 25-fold relative to the same lipids in a "base composition". Design of experiments (DOE) was used to optimize the ratio of the four lipids in the formulation. LNPs containing Luciferase-encoding DNA were dosed at 0.5 mg/kg. Luciferase expression measured by whole body luminescence 48 hours post-injection.

LNP-formulated piggyBac Enables Repeat Dose Pharmacology

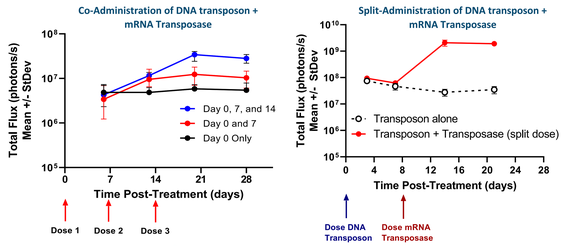


Fig. 4 Demonstration of repeat dose harmacology with non-viral piggyBac platform. Separate LNP compositions for transposon (DNA) and transposase (mRNA) were identified based on DoE-based in vivo formulation discovery campaigns. Adult wild-type mice were administered the transposon LNP (comprising a luciferase reporter gene) and the transposase LNP (comprising the SPB transposase mRNA) as an IV bolus. Left: the transposon and transposase LNPs were co-administered on day 0, 7, and 14 (repeated dose), and BLI performed serially over 28 days. Right: the transposon LNP was administered to mice on day 0 and the transposase LNP subsequently administered 8 days later (split dose), and BLI performed serially over 21 days.

Optimization of the Hemophilia A (hFVIII) Transposon Elements Resulted in Significant Improvements in Potency

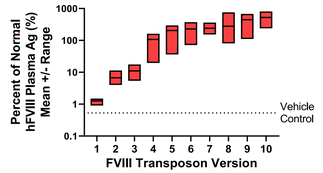


Fig. 5 Optimizing the hFVIII transposon elements had a dramatic effect on *in vivo* expression. A panel of hFVIII transposons with variable promoter, UTR, coding sequence, and other regulatory elements (>7Kb) were screened. Transposon LNPs were co-administered with SPB LNP as single dose IV to juvenile mice. hFVIII plasma levels measured by ELISA after 1 week.

Therapeutic Levels of hFVIII Detected in Juvenile Wild-type Mice Following a Single IV Dose of Functional SPB LNP

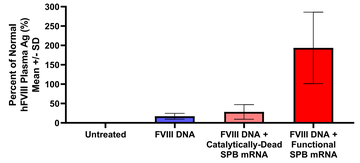


Fig. 6 Significantly greater (>10X) hFVIII antigen levels measured with integrating piggyBac system compared to non-virally delivered DNA alone. Dual-LNP co-administered as single dose IV to juvenile BALB/c mice. Transposon DNA-LNP dose was 0.25 mg/kg, transposase mRNA-LNP dose was 0.35 mg/kg. hFVIII plasma levels measured by ELISA after 1 week. Catalytically-Dead SPB is an inactive form of the transposase enzyme that is unable to mediate insertion of the genetic cargo.

Repeated Dosing of Dual LNPs in Adult Wild-type Mice Resulted in Dose-proportional Increases in hFVIII Levels

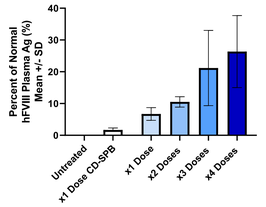


Fig. 7 A dose-proportional increase in hFVIII antigen level was observed, supporting the concept of a repeat dosing, non-viral piggyBac system. Dual-LNPs co-administered as single dose IV to adult (10wk) BALB/c mice on day 0, 3, 8, and 10. Transposon DNA-LNP dose was 0.25 mg/kg, transposase mRNA-LNP dose was 0.5 mg/kg. hFVIII plasma levels measured by ELISA on day 13.

Two Formulation Strategies for the Delivery of piggyBac

piggyBac Dual LNP Approach

hFVIII Transposon LNP + SPB Transposase LNP

- Each LNP formulation separately optimized around DNA or mRNA cargos
- ✓ Ability to optimize delivery timing of each piggyBac component enables increased pharmacological flexibility
- X Relies on co-transduction of the same cell to deliver both piggyBac components to enable genomic integration

piggyBac Co-encapsulated LNP Approach

hFVIII Transposon + SPB Transposase LNP

- Single complex LNP formulation required to encapsulate both DNA and mRNA cargos
- X Co-encapsulated piggyBac components cannot be delivered separately
- ✓ Guaranteed co-delivery of both piggyBac components to enable genomic integration

Co-encapsulated LNP Outperforms Dual LNPs

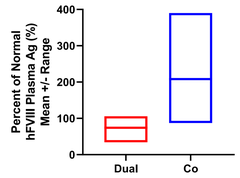


Fig. 8 Co-encapsulated LNP demonstrated superior FVIII expression over Dual-LNPs. Co-encapsulated LNPs also maintained physical characteristics within optimal range. Co-encapsulated LNP or Dual-LNP administered as single IV dose to juvenile (day 13-15 of life) BALB/c mice. The total LNP Dose: 0.75 mg/kg (0.15 mg/kg transposon DNA, 0.6 mg/kg transposase mRNA).

Optimization of the Transposon DNA : Transposase mRNA Ratio Resulted in Increased FVIII Expression

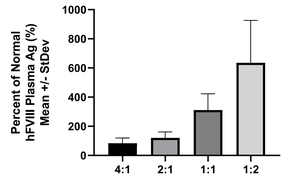


Fig. 9 Increasing the amount of transposon DNA relative to transposase mRNA significantly improved hFVIII expression. Co-encapsulated LNPs were administered as an IV dose to juvenile BALB/c mice. All formulations had the equivalent amount of TOTAL nucleic acid (0.5 mg/kg). hFVIII plasma levels measured by ELISA on day 7.

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