Enhanced delivery and expression of mRNA to T-cells using CD8-targeted lipid nanoparticles with Athebody® Designed Ankyrin Repeat Proteins

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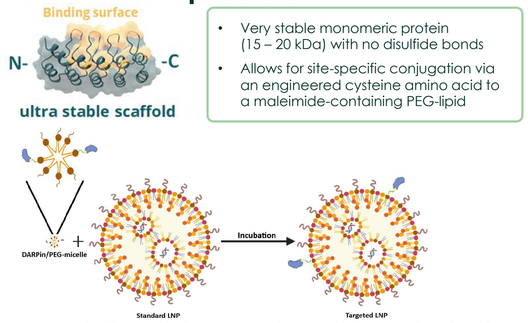
ACUITAS THERAPEUTICS

Abstract

The use of lipid nanoparticles (LNP) for nucleic acid delivery to the liver by intravenous (IV) administration is well established with approval of Onpattro®, an LNP-based medicine that contains an siRNA payload, and clinical trials with mRNA-LNP for gene editing. Here we show that IV-administered LNP can also be used for effective delivery to extrahepatic (eh) tissues and target cells, such as T cells in circulation, by enabling cellular uptake through the inclusion of active targeting ligands and optimizing the LNP composition to achieve long circulation time in the blood and reduced uptake in the liver. Cellular uptake of the LNP is enabled by Athebody® DARPins (designed ankyrin repeat proteins), which are antibody mimetics, to target cell membrane proteins such as CD8 on T cells. In mice, we showed that the LNP plasma half-life was increased from 25 minutes for a standard LNP optimized for liver delivery to 2 h for an ehLNP; further, the circulation half-life was not affected by the conjugation of an anti-mouse CD8⁺ (mCD8⁺) DARPin onto the LNP. In the spleen, the mCD8⁺ DARPin-conjugated ehLNP was delivered to >95% of splenic CD8⁺ T cells and an expression of >80% when using mCherry as a reporter at a 0.5 mg/kg dose. When compared to a standard LNP, the change in composition resulted in an 11- and 7.5-fold increase in uptake and expression, respectively. In addition, there was "de-targeting" of the liver marked by >7-fold reduction in the level of mCherry expression with the ehLNP, compared to standard LNP. In NHP, when ehLNP encapsulating a CD20 CAR mRNA and functionalized with a hCD8⁺ DARPin was infused IV, a complete depletion of B cells was observed in the peripheral blood and lymphoid tissue at 0.25 and 0.75mg/kg doses. These findings have supported advancement of the targeted-ehLNP platform to GMP manufacturing for clinical development.

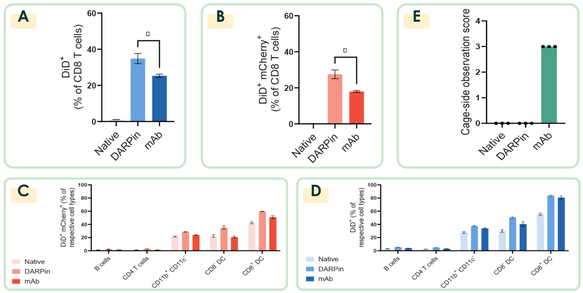
1 Athebody® DARPin & LNP association process



- Very stable monomeric protein (15 – 20 kDa) with no disulfide bonds
- Allows for site-specific conjugation via an engineered cysteine amino acid to a maleimide-containing PEG-lipid

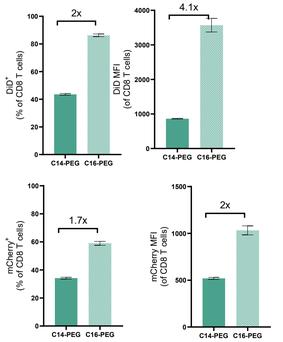
DARPins are a class of antibody mimetics composed of two caps (N-cap and C-cap) and 2-4 library modules that contain the variable (target binding) region (A). The LNP used in these studies are composed of a cationic lipid, distearoylphosphocholine (DSPC), cholesterol and a PEG-lipid. DARPin-LNP were made by insertion of a DARPin-PEG-lipid conjugate to the LNP (B). DARPin-conjugated LNP developed in collaboration with Athebio AG. Created with BioRender.com

2 Evaluation of ligand formats on targeted LNP activity



A commercially available mCD8⁺ mAb (clone 53-6.7) was conjugated to LNP and compared side-by-side with DARPin-LNP. Both mAb and DARPin-LNP can elicit targeted binding (A) and expression (B) in CD8 T cells. The overall activity of DARPin-LNP was significantly better than mAb-LNP while the viability (data not shown) and activity in other cells types were not significantly affected (C & D). No cage-side observations were found for DARPin-LNP, but reduce activity and piloerection were seen with mAb-LNP animals at 0.5 mg/kg (E).

3 Improved targeted activity by altering PEG-acyl chain length to make ehLNP

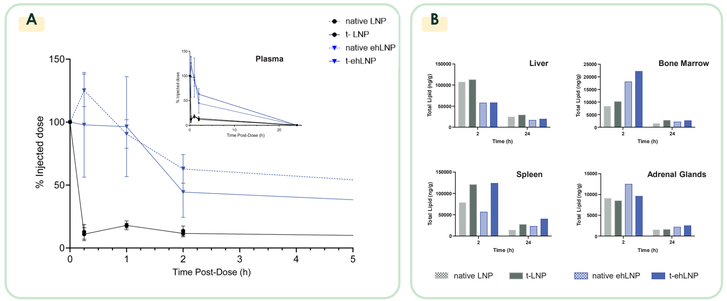


A known LNP change to increase circulation time in vivo has been to exchange the PEG-lipid species to have a longer acyl chain which allows for it to remain associated with the LNP longer. Here the PEG-C14 lipid in the standard LNP was replaced with a PEG-lipid containing C16 diacyl chains. An mCD8⁺ DARPin was conjugated to the above LNP and injected IV into C57/Bl6 mice at 0.5 mg/kg.

The PEG-C16 LNP showed increased uptake, (measured using DiD, a lipophilic fluorescent marker) in splenic CD8+ T cells compared to the standard tLNP. A resulting 4x and 2x fold increase in uptake and mCherry payload expression, respectively, were seen.

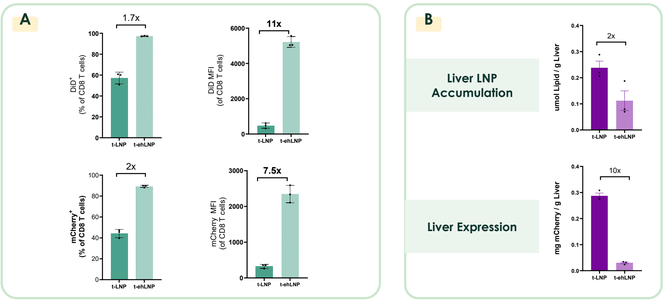
However, use of PEG-C16 is not preferred as it can inhibit activity and can contribute to an adaptive immune response.

4 Alternative compositional changes to generate ehLNP



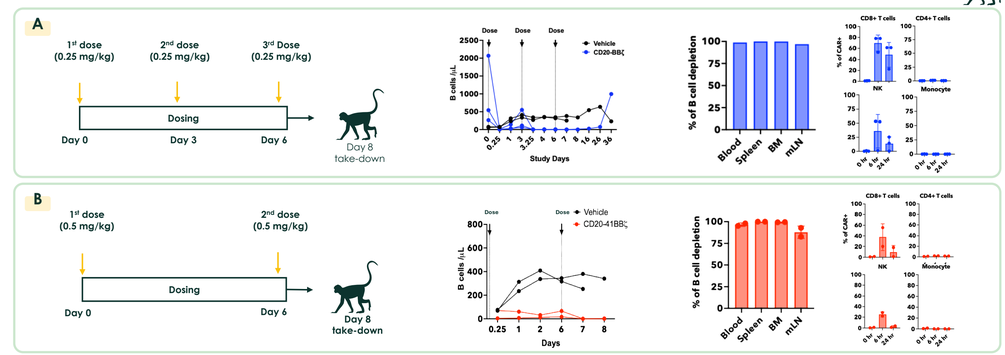
Non-PEG-lipid compositional changes were made to increase LNP circulation time and de-target the liver. A standard LNP and an ehLNP were conjugated with a mCD8⁺ DARPin and the cationic lipid distribution was measured by LC/MS in mice 24 h after a 0.5 mg/kg IV injection. The compositional changes led to an increased plasma half-life from 15min to 2hrs (A). The addition of the DARPin increased the lipid accumulation in T-cell rich tissue, while avoiding the liver (B).

5 Improved targeted activity with alternative ehLNP



A standard LNP and an ehLNP made through compositional changes were conjugated to a mCD8⁺ DARPin. LNP delivery (DiD) and reporter mCherry expression was measured in spleen cells (FACS) and liver tissue of C57/BL6 mice 24h after a 0.5 mg/kg IV injection. In splenic CD8 T cells, a 11 and 7.5x fold increase in DiD and mCherry MFI's, respectively, were seen (A). In the liver, a 2x and 10x fold lower LNP accumulation and mCherry expression, respectively, were seen (B). No significant changes in CD8- uptake and expression were observed (not shown).

6 Complete B cell depletion using targeted-ehLNP (t-ehLNP)



t-ehLNP encapsulating a CD20 CAR mRNA targeted with a hCD8⁺ DARPin was infused IV into non-human primates (NHP) at a dose of 0.25mg/kg x 3 (A) or 0.75mg/kg x 2 (B). Complete B cell depletion was observed in the peripheral blood following each infusion and a durable reduction was observed >2 days after the final dose. Similarly, complete B cell depletion was observed in the spleen, bone marrow (BM), and mesenteric lymph nodes (mLN). Target specificity of t-ehLNP resulted in >60% CAR expression only on CD8⁺ leukocytes . Data generated in collaboration with Create Medicines.

Summary

1. LNP can be effectively delivered to cells outside the liver by changing the LNP composition to increase its circulation time and incorporating a targeting ligand to facilitate cell uptake.
2. This was demonstrated in mice in vivo using a mCD8⁺ Athebody® DARPin-conjugated to an optimized ehLNP. At 0.5 mg/kg, LNP was delivered to >90% CD8 T cells while mCherry expression was detected in >80%.
3. A hCD8⁺ Athebody® DARPin has been used with our ehLNP in PoC NHP studies leading to complete depletion of B cells through expression of anti-CD20 CAR RNAs by our partners. Scale-up and GMP production of the tLNP are underway.

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