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

**Grayson Wong[1], Fan Yan[1], Joon Song[1], Ghania Chikh[1], Nora Guidotti[2], Richard Woods[2], Ying K. Tam[1], Barbara Mui[1]**

[1]Acuitas Therapeutics, Vancouver, BC, Canada; [2]Athebio AG, Zurich, Switzerland

ACUITAS THERAPEUTICS

## Abstract

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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 non-liver target cells, such as T cells in circulation, by enabling cellular uptake through inclusion of active targeting ligands and optimizing the LNP composition to achieve long circulation (lc) time in the blood and reduce uptake in the liver. Cellular uptake of the LNP is enabled using Athebody® DARPins (designed ankyrin repeat proteins), which are antibody mimetics, to target cell membrane proteins such as CD8 on T cells. In mice, we show that the LNP plasma half-life is increased from 25 minutes for a standard LNP optimized for liver delivery to 2 h for a lcLNP; further, the circulation half-life is not affected by conjugation of an anti-mouse CD8 (mCD8) DARPin onto the LNP. In the spleen, the mCD8 DARPin-conjugated lcLNP was delivered to >95% of splenic CD8+ T cells, with expression of an mCherry reporter seen in >80% at a 0.5 mg/kg dose. For targeted lcLNP, this is a 2-fold improvement in the number of cells that bind and subsequently take up the LNP and express the encapsulated mRNA, compared to a targeted standard LNP. Similarly, an 11 and 7.5-fold enhancement in the level of LNP uptake and mRNA expression was seen for the lcLNP compared to standard LNP. Conversely, no enhanced delivery/expression is seen in splenic CD8- cells, compared to untargeted LNP. In addition, there was "de-targeting" of the liver where a >7-fold reduction in the level of mCherry expression was observed with the lcLNP, compared to standard LNP. These findings have supported advancement of the targeted-lcLNP platform to GMP manufacturing for clinical development.

## DARPin & conjugation to LNP

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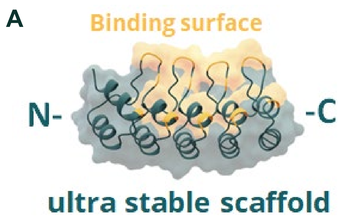
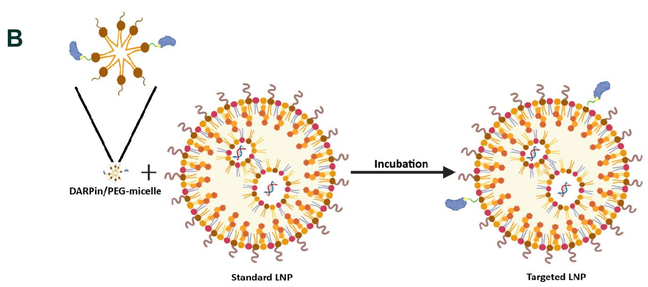



- Very stable monomeric protein (15 – 20 kDa) with no disulfide bonds
- Allows for site-specific conjugation via an engineered cysteine amino acid to a maleimide-containing PEG-lipid




(A) 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.

(B) DARPin-LNP were made by insertion of a DARPin-PEG-lipid conjugate to the LNP. The LNP used in these studies are composed of a cationic lipid, distearoylphosphocholine (DSPC), cholesterol and a PEG-lipid.

## Activity comparison with mAb-LNP

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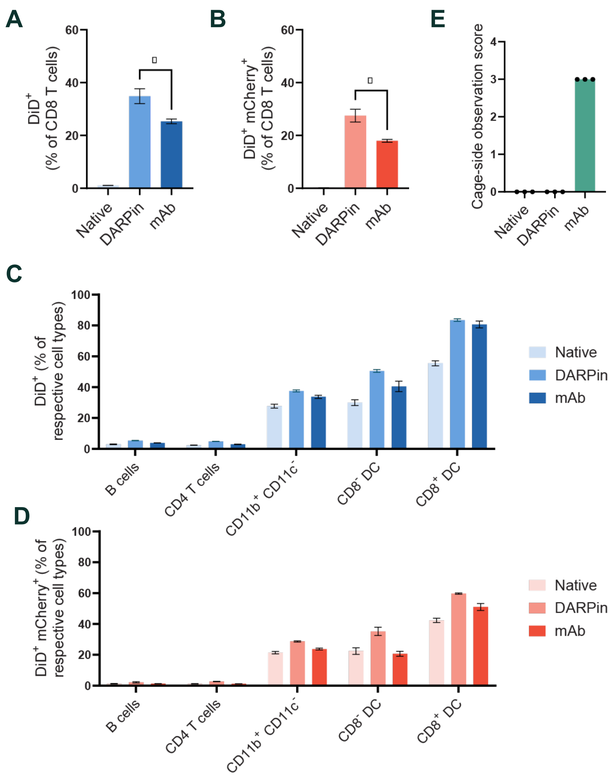


A commercially available CD8 mAb (clone 53-6.7) was conjugated to LNP and compared side-by-side with DARPin-LNP. Both mAb and DARPin-LNP can elicit targeted binding (A) and expression (B) in CD8 T cells. The overall activity of DARPin-LNP was significantly better than mAb-LNP while the viability (data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

## Improved targeted activity by changing PEG-acyl chain length to generate a lc-tLNP

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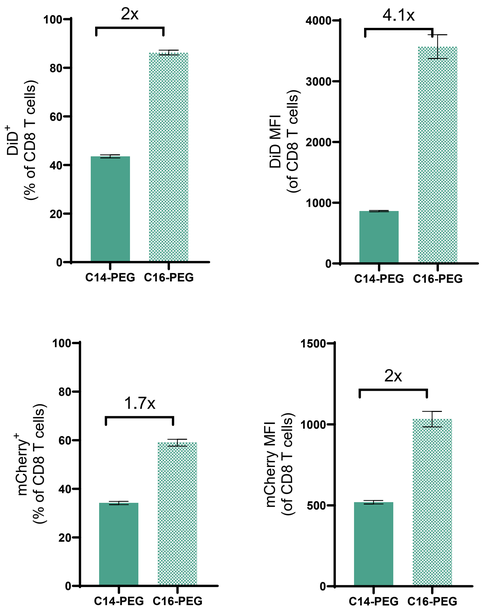


A LNP compositional change known to increase its circulation time *in vivo* is by using a PEG-lipid with a longer acyl chain length so that it remains associated with the LNP. Here the 1.8 % PEG-C14 lipid in the standard LNP is replaced with a PEG-lipid containing C16 diacyl chains. An mCD8 DARPin was then conjugated to the LNP and the tLNP injected IV into C57/Bl6 mice at 0.5 mg/kg.

As expected, LNP uptake (measured using DiD, a lipophilic fluorescent marker) in splenic CD8+ T cells was increased with the PEG-C16 containing tLNP, compared to the standard tLNP. A resulting 2x increase in mCherry payload expression was seen.

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

## Other compositional changes to generate lcLNP

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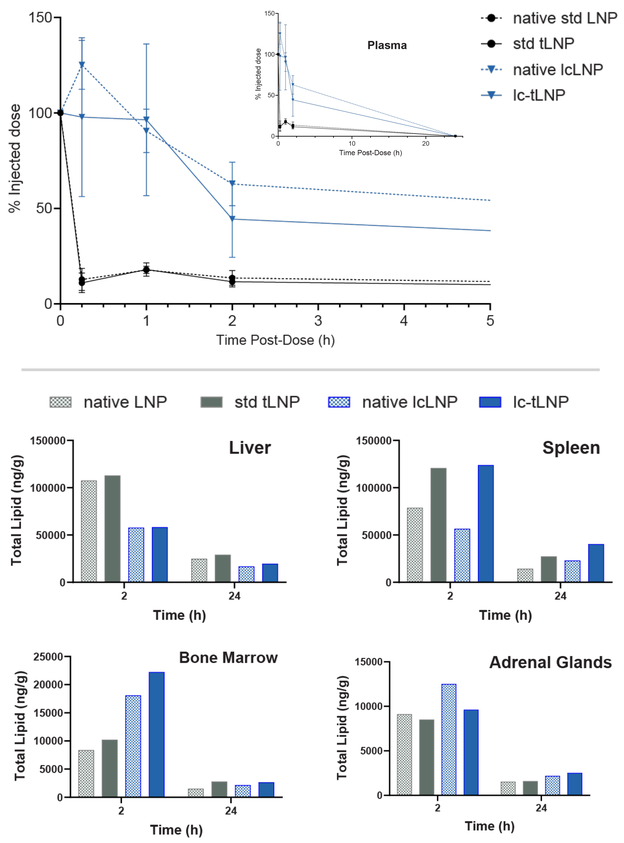


Other non-PEG-lipid compositional changes were made to increase LNP circulation time and de-target the liver. A standard LNP and a lcLNP with compositional changes were conjugated to mCD8 DARPin and the cationic lipid distribution measured by LS/MS in mice 24 h after a 0.5 mg/kg i.v. injection.

## Improved targeted activity with above optimized lc-tLNP

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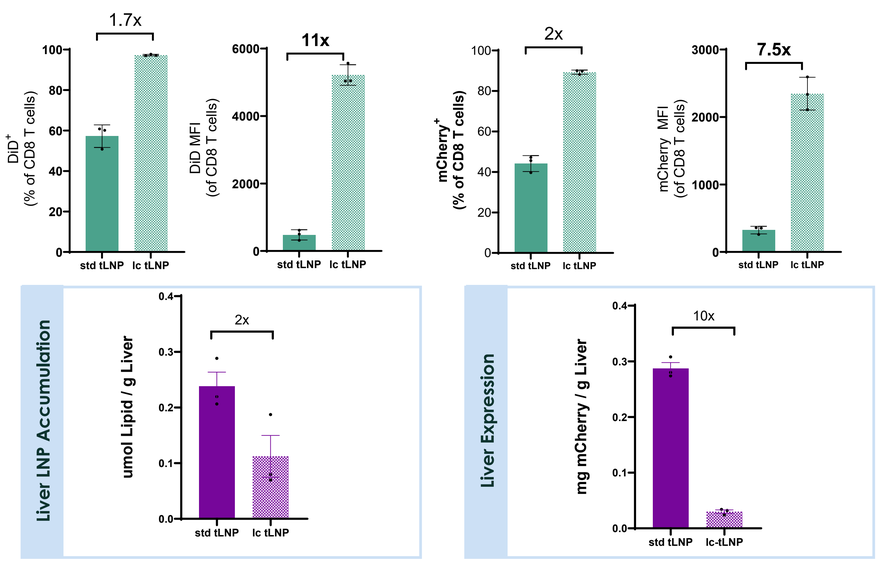


A standard LNP and lcLNP made through compositional changes were conjugated to a mCD8 DARPin and LNP delivery (DiD) and reporter mCherry expression was measured in spleen cells by FACS and in liver tissue of C57/BL6 mice 24 h after a 0.5 mg/kg i.v. injection.

- In splenic CD8 T cells, a 11 and 7.5x fold increase in DiD and mCherry MFI's, respectively, were seen
- In the liver, a 2x and 10x lower LNP accumulation and mCherry expression, respectively, were seen

## DARPin to target hCD8 for clinical use

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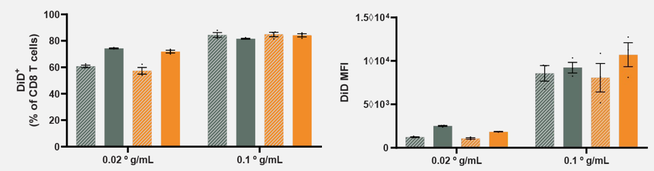
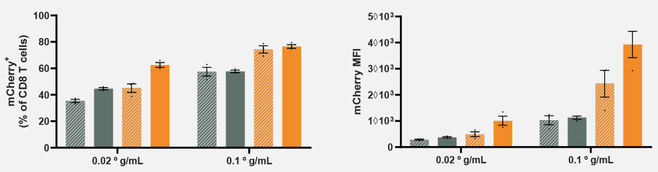
### LNP Delivery




### RNA Expression




| Group | Tested in NHP |
|---|---|
| DARPin1 | yes |
| DARPin2 | pending |

Studies conducted by our partners with DARPin1, an anti-human CD8 DARPin, conjugated to our lcLNP have shown promising results in NHP (e.g. Create Medicines, Keystone-Emerging Cell Therapy, January 2026).

Further improvement to DARPin1 was made, identifying DARPin2. In the Figures above, DARPin-LNP made at 4 or 8 DARPins per LNP were incubated with human PBMCs from 3 different donors. LNP delivery to CD8 T cells with DARPin2 was similar to DARPin1 but reporter mCherry expression was significantly higher.

## 8 *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 DARPin-conjugated to an optimized lcLNP. At 0.5 mg/kg, LNP was delivered to >90% CD8 T cells while mCherry expression was detected in >80%.
3. A hCD8 DARPin has been used with our lcLNP in PoC NHP studies for 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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