

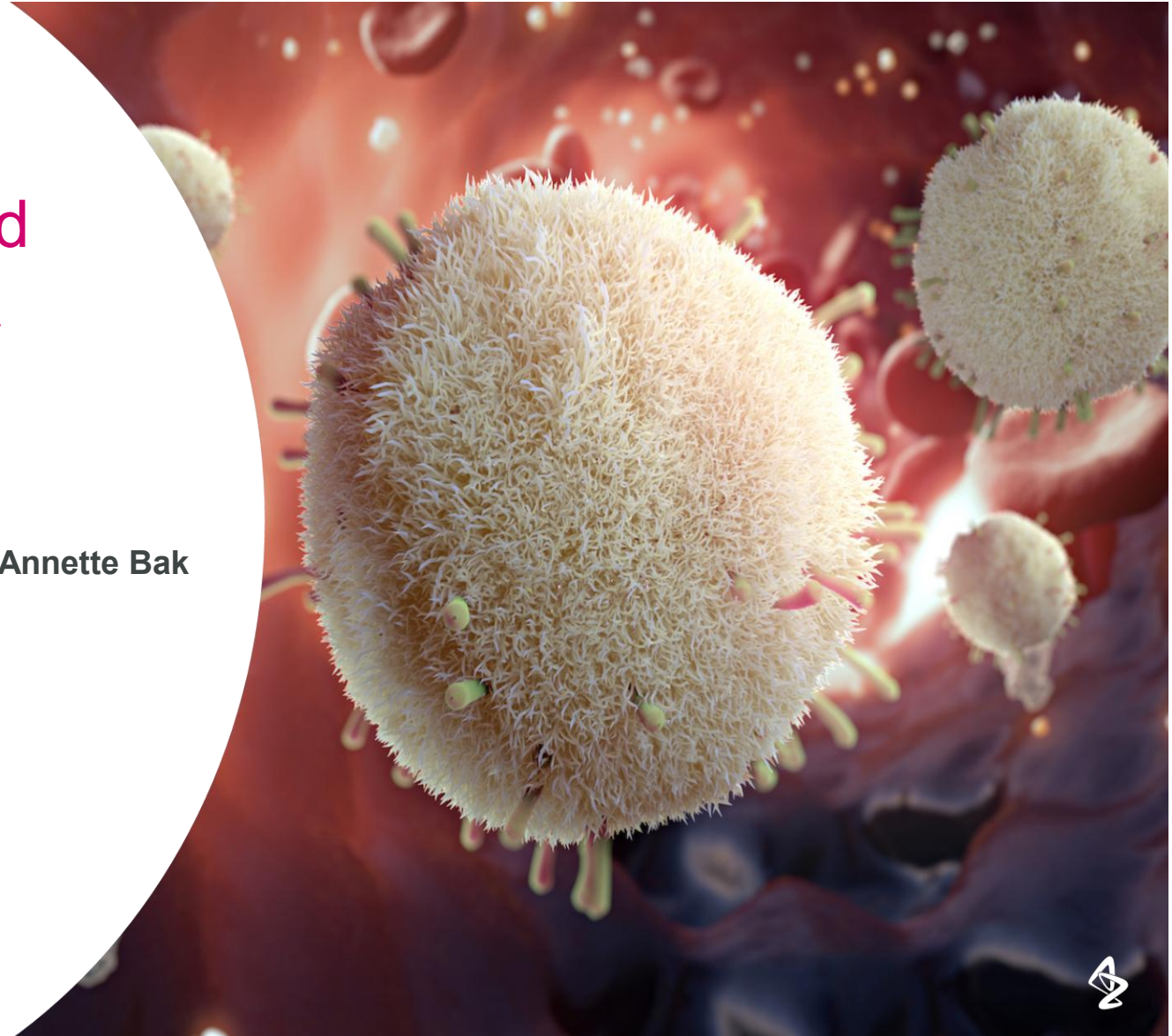
In Vivo Nucleic Acid Targeted Delivery in Bone-Marrow via Antibody Conjugated Lipid Nanoparticles

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LNPs – Versatile Platform for Advancing mRNA Modalities in Therapeutics

Vaccines

Delivering mRNA encoded antigen to muscle or immune cells for expression of antigen

Gene Editing

Delivering mRNA encoding CAS9/gRNA or other nucleases to tissues of interest for gene deletion or correction

Protein Replacement Therapy

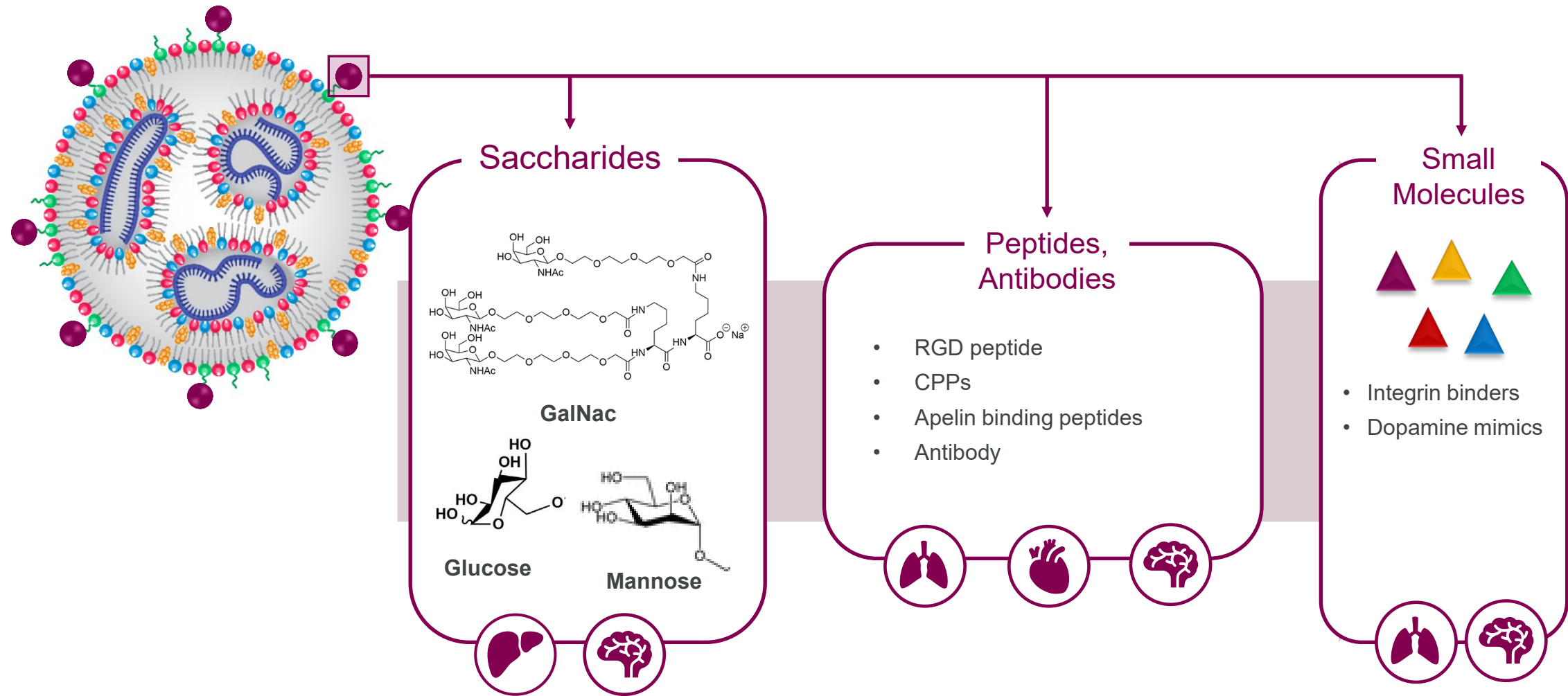
Delivering mRNA encoding for a disease modifying protein

Cell Therapy

Using mRNA loaded LNPs to deliver CRISPR or other agents to cell therapies *in* or *ex vivo*

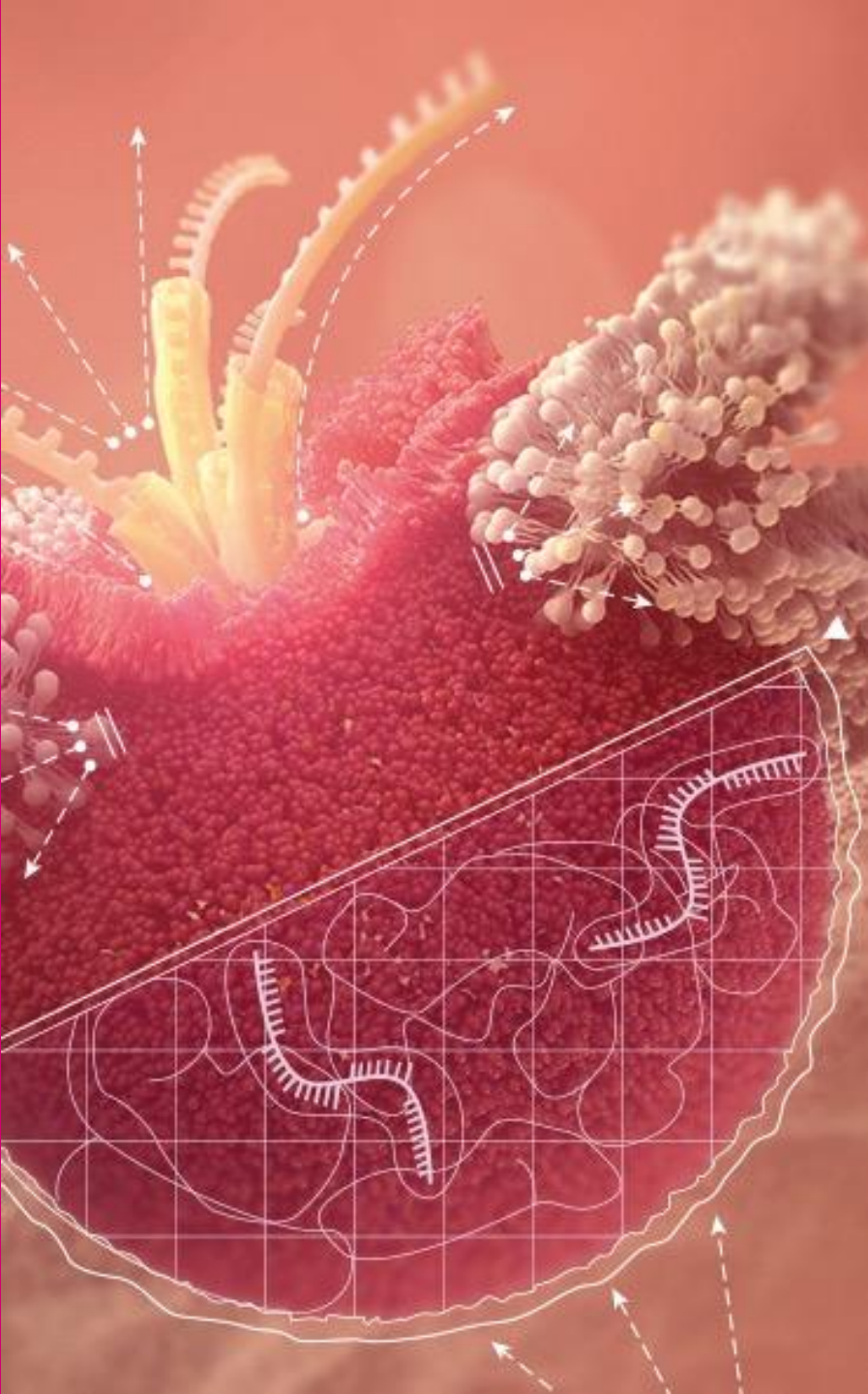


Active Targeting with LNPs: Shaping the Future of Precision Delivery



Bioconjugation allows for placement of variety of ligands on LNP surfaces.
Enabling delivery in extrahepatic tissues and providing a wide range to treat variety of disease targets.



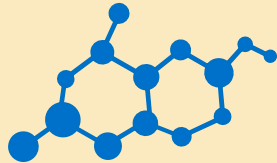
A 3D visualization of a cell membrane. The membrane is a reddish, textured surface. On the left, there are yellow, finger-like projections (microvilli) with small white dots (receptors) on their surface. Dashed white arrows point away from these projections. To the right, there is a cluster of small, light-colored spherical particles. Below the membrane, a white grid is overlaid on a semi-circular area. Inside this grid, there are white, wavy lines representing a network or pathway. Dashed white arrows point towards and away from this grid area.

Platform case study with tool LNP and antibody

Part 2

Developing Anti-CD117 tLNPs for Editing HSCs

Bone-marrow active ionizable lipid (IL)

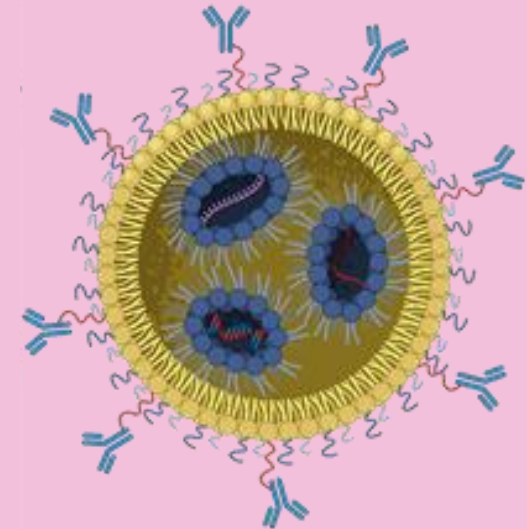


HSC targeting via antibody



mAb (150 kDa)

Enhance potency in HSCs and improve liver de-targeting via surface modification of LNP



Phys-Chem Properties of LNPs

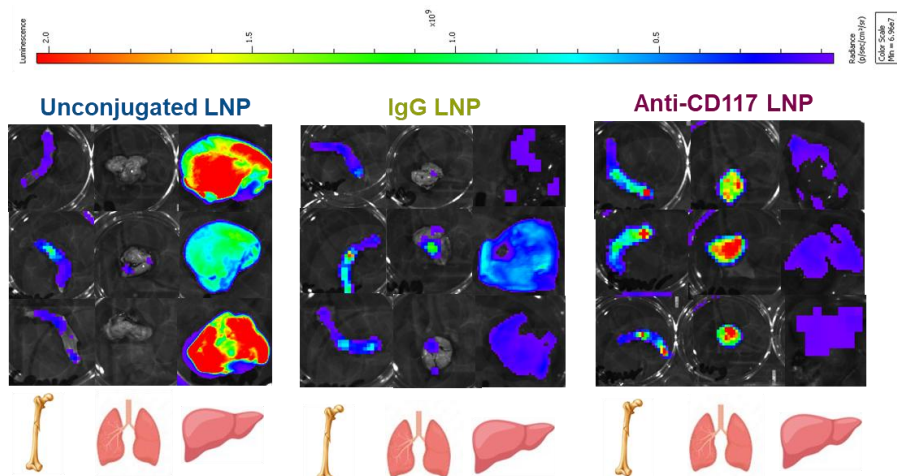
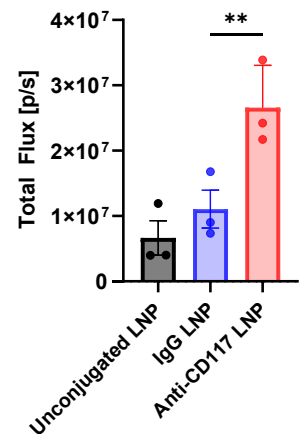
LNP groups	Size (nm)	PDI	EE (%)
Unconjugated LNP	77	0.09	99
IgG LNP (isotype control)	96.8	0.11	93.8
aCD117 LNP (tLNP)	98.9	0.12	93.3



Anti-CD117 tLNPs Enhanced Luciferase mRNA Expression in Bone Marrow

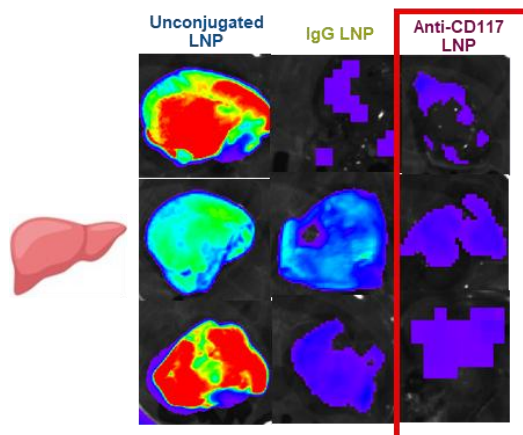
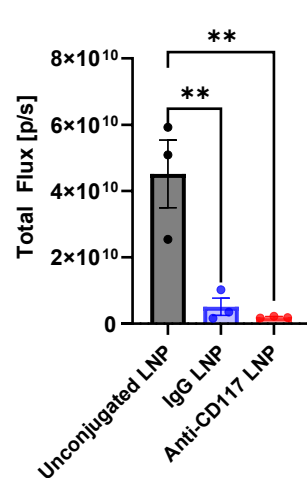
Anti-CD117 tLNPs significantly enhanced mRNA expression in BM compared to unconjugated/IgG LNPs

24hr Total Radiance in Bone Marrow



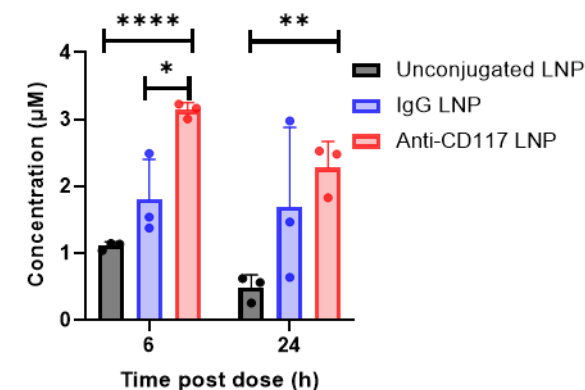
Reduced mRNA expression in liver

24hr Total Radiance in Liver



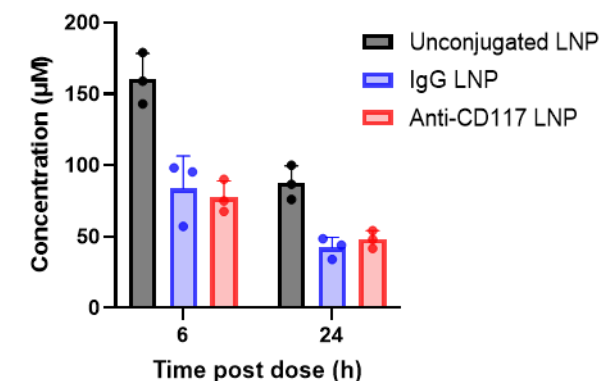
Anti-CD117 tLNPs significantly enhanced LNP delivery to BM compared to unconjugated/IgG LNPs

Bone Marrow



Reduced LNP delivery to liver

Liver

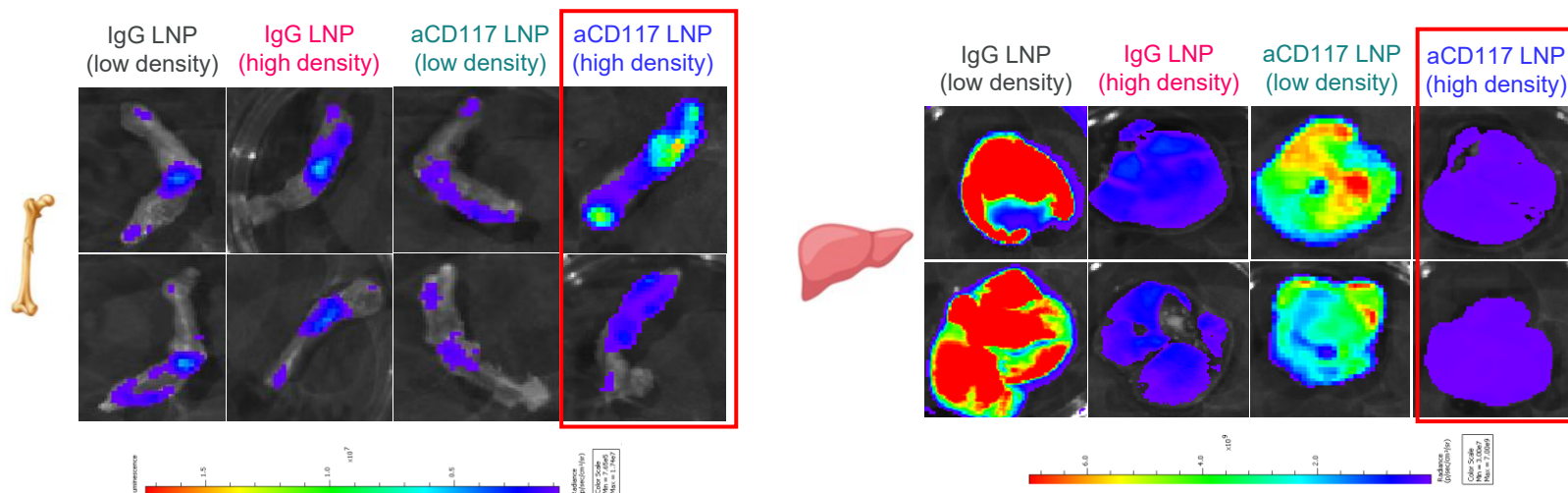
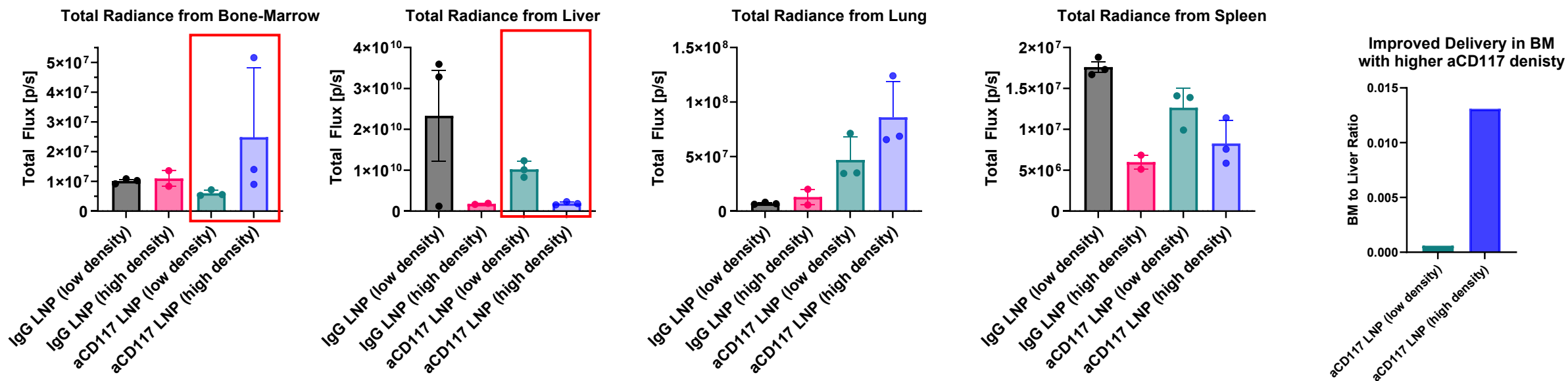


Ionizable lipid quantification in tissue homogenates by LC-MS/MS

LC-MS/MS: Eric Gosselin



tLNPs with Higher aCD117 Density Improved Luciferase Activity in Bone Marrow



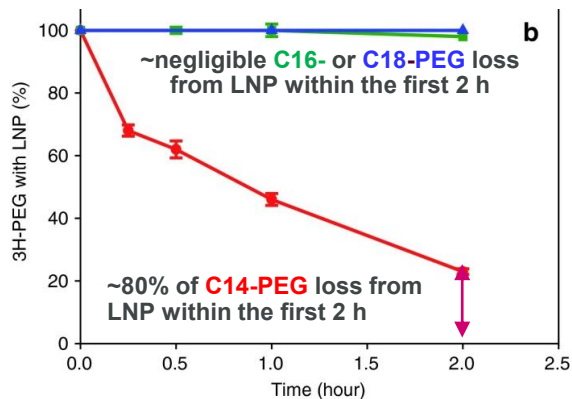
Phys-Chem Properties of LNPs

LNP groups	Size (nm)	PDI	EE (%)
IgG LNP (low density)	95.5	0.08	97.2
IgG LNP (high density)	99.3	0.12	97.1
aCD117 LNP (low density)	92.9	0.07	97.1
aCD117 LNP (high density)	99.9	0.12	97.1



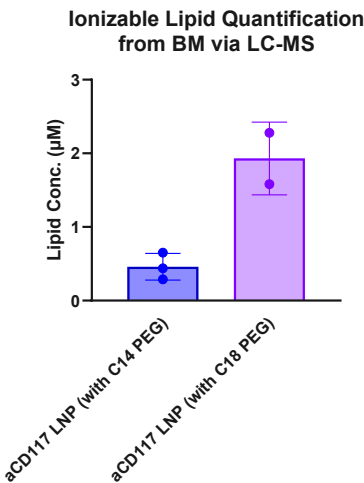
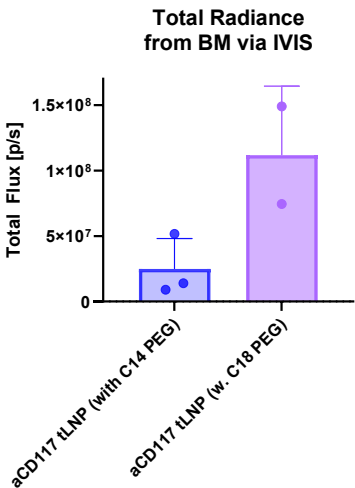
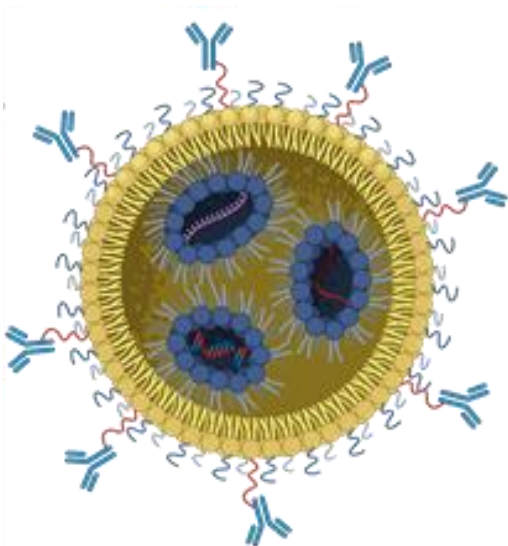
C18 PEG Further Enhances Targeting Efficiency of tLNPs in Bone-Marrow

Loss of C14-PEG Lipid from LNP in Circulation



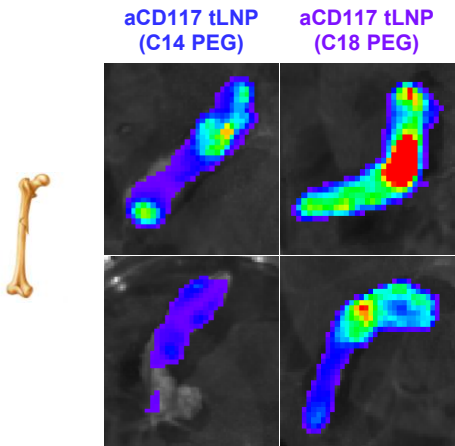
(Barbara et al. Molecular Therapy Nucleic Acids, 2, 2013, e139)

(Waggoner et al. Biomater Sci., 2023)



Phys-Chem Properties of LNPs

LNP groups	Size (nm)	PDI	EE (%)
aCD117 LNP (C14 PEG)	99.9	0.12	97.1
aCD117 LNP (C18 PEG)	120.2	0.12	97.4

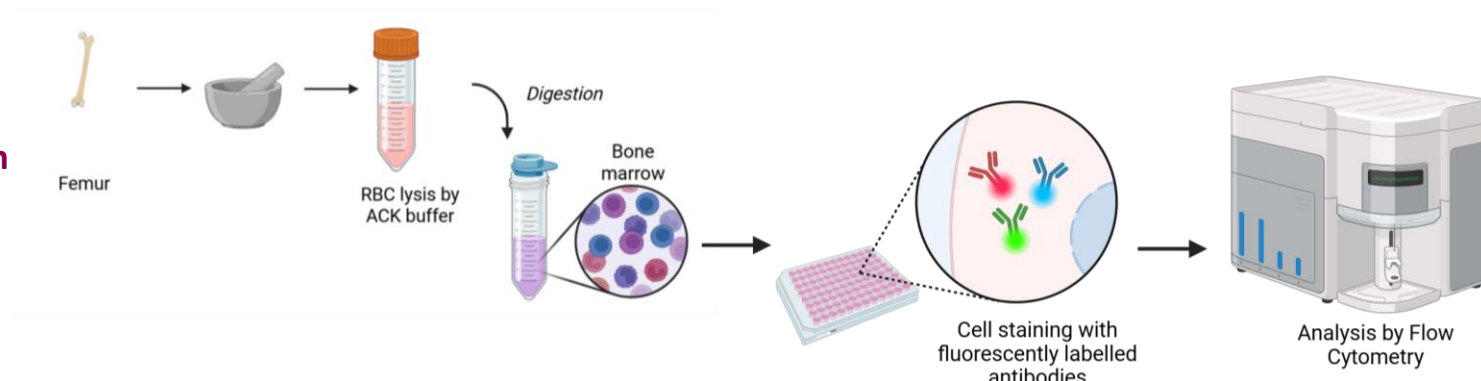


Tissue biodistribution analysis of luciferase expression, combined with LC-MS/MS assessment of ionizable lipid, indicated that C18-PEG enhanced mRNA expression and LNP distribution in bone marrow

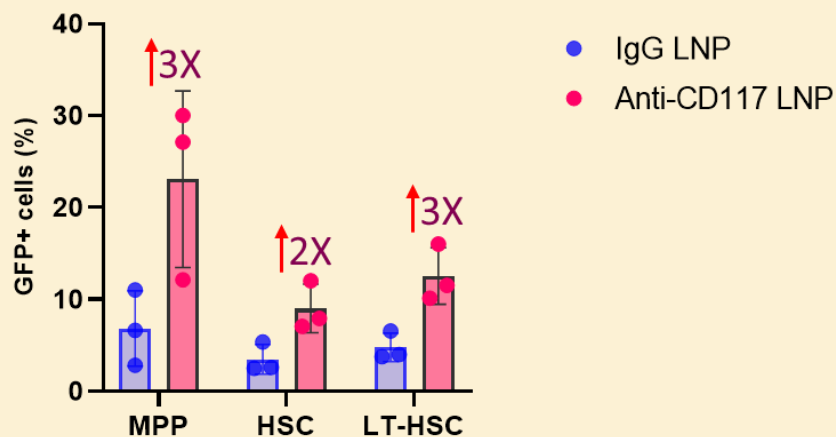


Anti-CD117 tLNPs Enhanced eGFP mRNA Expression in CD117⁺ Sub cells

Workflow to obtain CD117⁺ sub cells from BM and analysis by flow cytometry

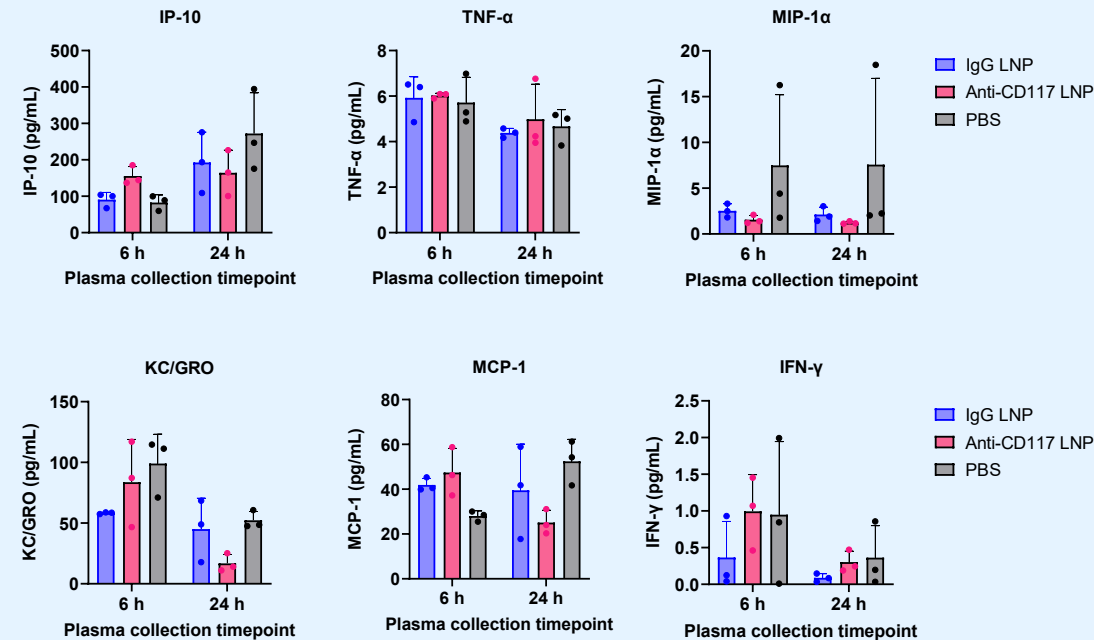


Anti-CD117 tLNPs enhanced eGFP-mRNA expression in CD117⁺ cells



MPP: Multipotent hematopoietic progenitor cells
HSCs: Hematopoietic stem cells (LSK)
LT-HSCs: Long-term hematopoietic stem cells

Anti-CD117 tLNPs didn't trigger acute effects



Summary and Next Steps

Case Study: Anti-CD117 tLNP

- **Functional Delivery *In Vivo***

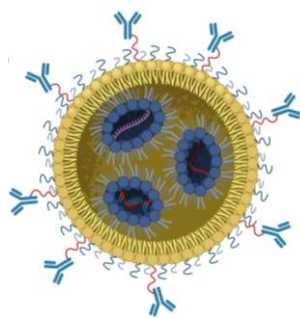
Single IV injection of tLNP in mouse can deliver mRNA and express protein in mouse bone-marrow.

- **Improved Delivery in BM and liver de-targeting**

With higher ligand density and C18-PEG, tLNPs demonstrated improved protein expression in bone-marrow.

- **Functional Delivery *In Vivo* HSPCs**

tLNPs in mouse delivered mRNA and expressed protein in CD117+ HSPCs without triggering inflammations.



mAb-tLNP

Further Optimization of the Platform

- **tLNP Optimization**

- Evaluate **antibody fragments** for improving homogeneity and manufacturability of tLNP batches and reduce potential immunogenicity.

- Identify optimum **ligand density** for achieving selective delivery in HSCs.

- Assess editing of BM cells with functional cargo.

- Scale up for functional delivery in non-human primates.



Thank You!
Any Questions?

