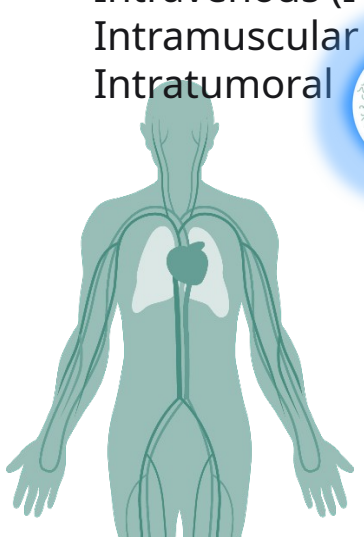
An ionizable LNP platform for scalable *in vivo* CAR T cell engineering

Martin Rabel, BPS Business Development & Licensing Manager

The challenges of cell-specific LNP delivery *in vivo*

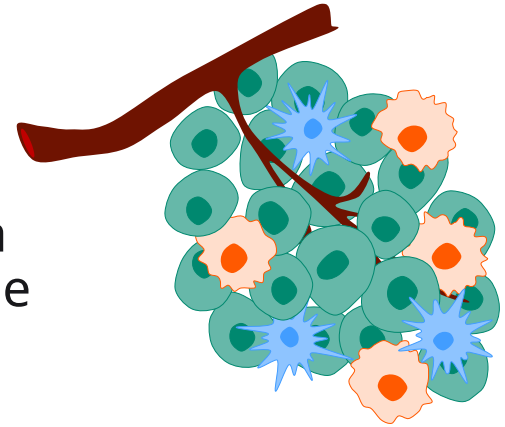
1 Route of administration
Intravenous (IV)
Intramuscular (IM)
Intratumoral



2 Protein corona on nanoparticle (NP) surface

3 Biodistribution

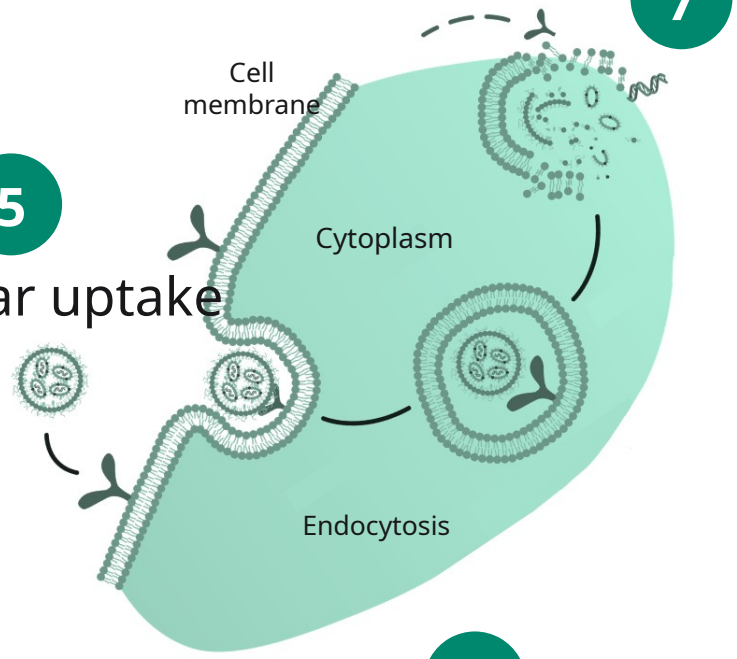
4 Transport in, out and through tissue



5 Cellular uptake

6 Endosomal escape

7 Drug activity

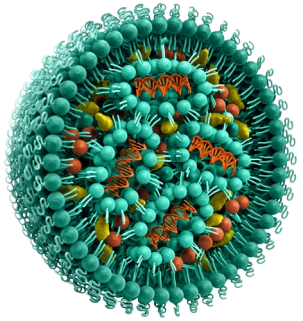


LNP – Lipid nanoparticle

Strategies for targeting

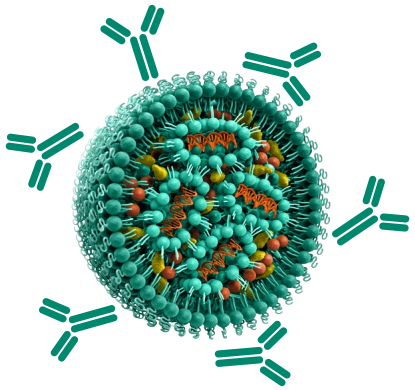
Passive

- Use particle size, surface chemistry, and route of administration to influence biodistribution



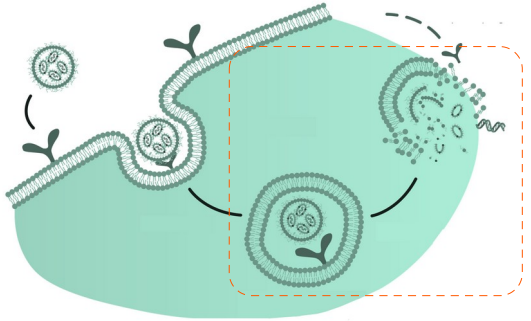
Ligand conjugation

- Add molecules to the particle surface that bind to a specific molecular target



Cell-specific uptake and release

- Tune the formulation to be more effective at delivering the payload to specific cells



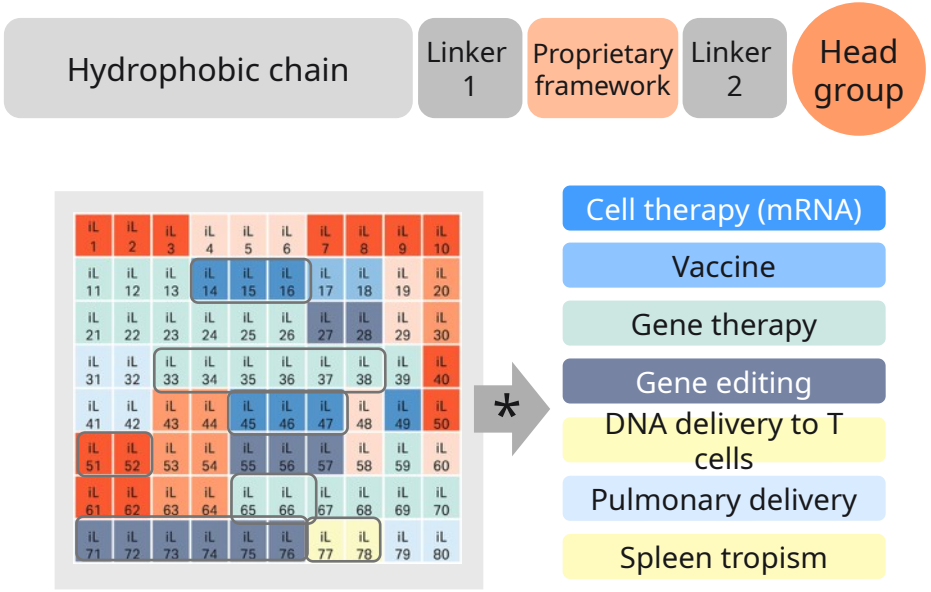
Cell-specific drug activity

- Make the payload active only in specific cells

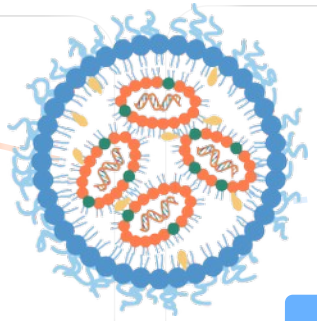
Targeting is much more than antibody conjugation.

Programmable LNP components: ionizable lipids + stabilizers

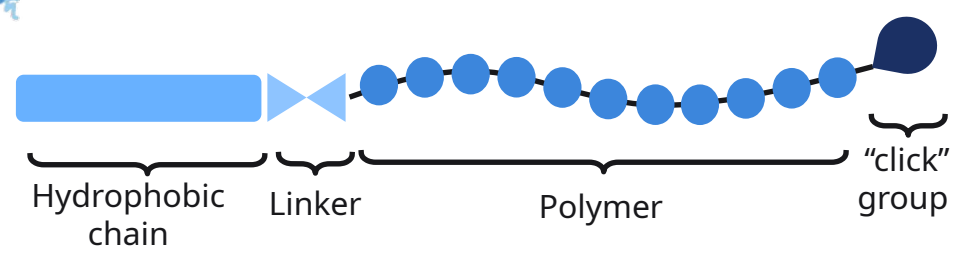
Cytiva proprietary **ionizable lipids**



Broad ionizable lipid library enabling precise LNP tuning for cell and gene therapy applications



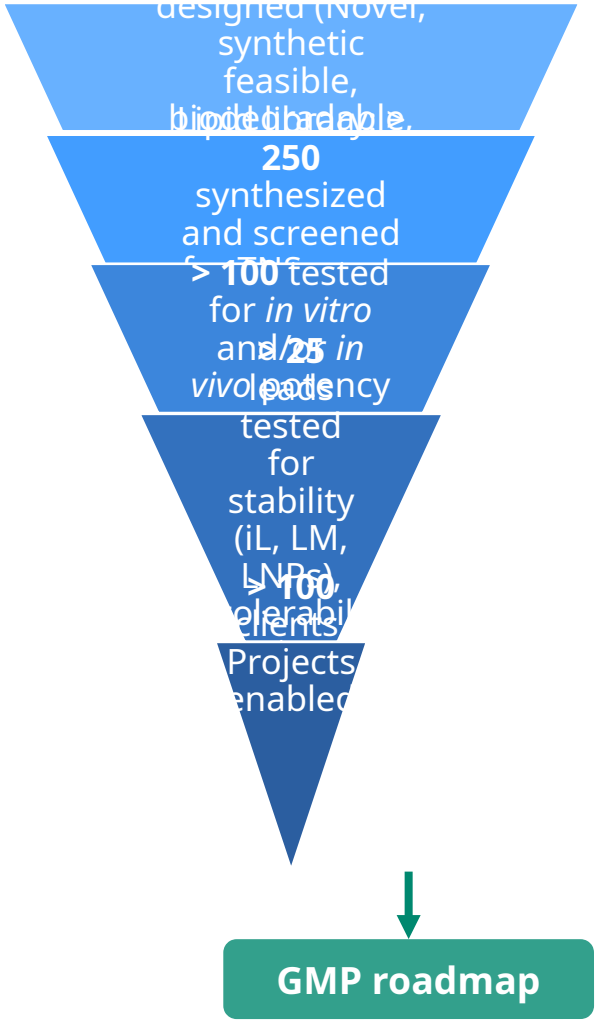
Cytiva proprietary **stabilizers**



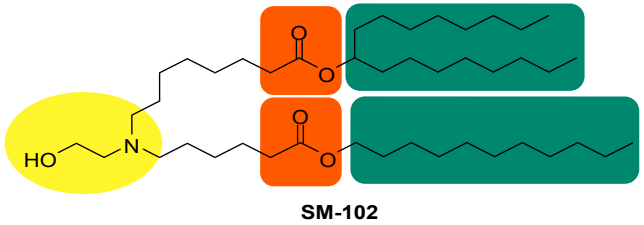
- **Beyond polyethylene glycol-conjugated lipids (PEG)**: Non-PEG lipid-polymer hybrids with excellent performance
- **Programmable design**: Control polymer architecture and amphiphilicity
- **Click-enabled targeting**: Terminal groups for rapid conjugation of ligands or antibodies

*Application mapping is for illustration only, actual applications for ionizable lipid may differ

Cytiva ionizable lipid funnel and differentiators



Other commercial lipids



Head Linker Hydrophobic chain

Cytiva proprietary lipids

The proprietary framework allows for:

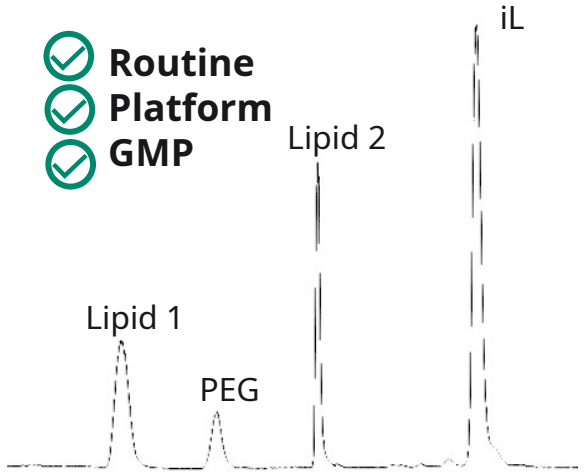
- ✓ Multiple tails for cell-specific, high activity
- ✓ Tunability of chemical features, like biodegradability

LM = Lipid mix; TNS = 6-(p-toluidino)-2-naphthalenesulfonic acid sodium salt; iL = Ionizable lipid

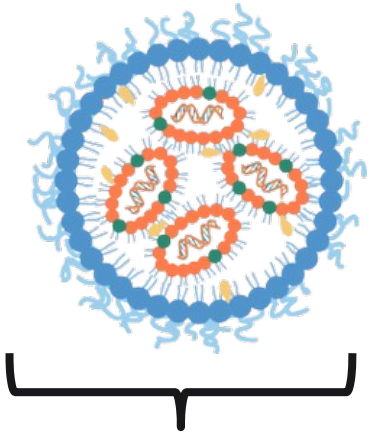
Analytical readiness - Standard analytics required for all LNPs

Lipid ID and purity by HPLC-CA

- Routine
- Platform
- GMP

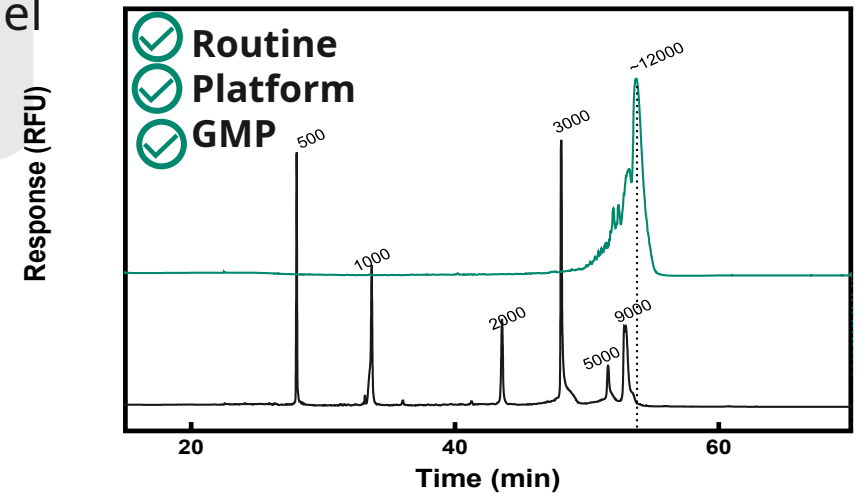


Manufacturing of a 'traditional' mRNA-LNP uses a release panel of at least 14 critical quality attribute (CQA) assays



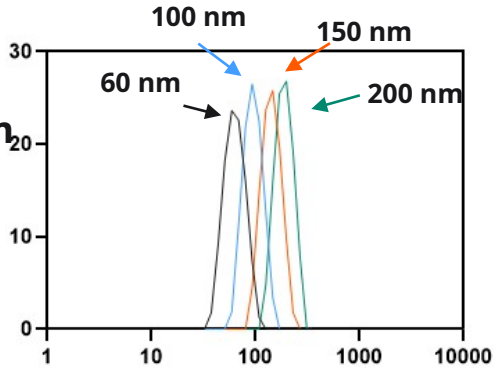
Payload characterization by capillary gel electrophoresis-laser induced fluorescence (CGE-LIF)

- Routine
- Platform
- GMP



Particle size and polydispersit

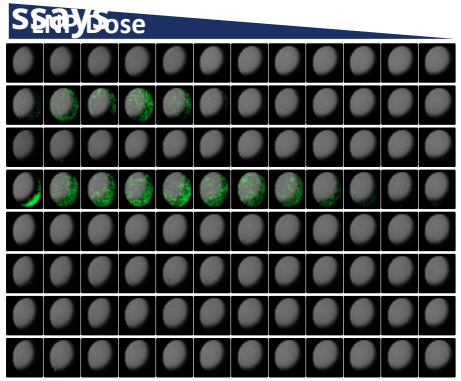
- Routine
- Platform
- GMP



Compendial and safety testin

- Routine
- Platform
- GMP

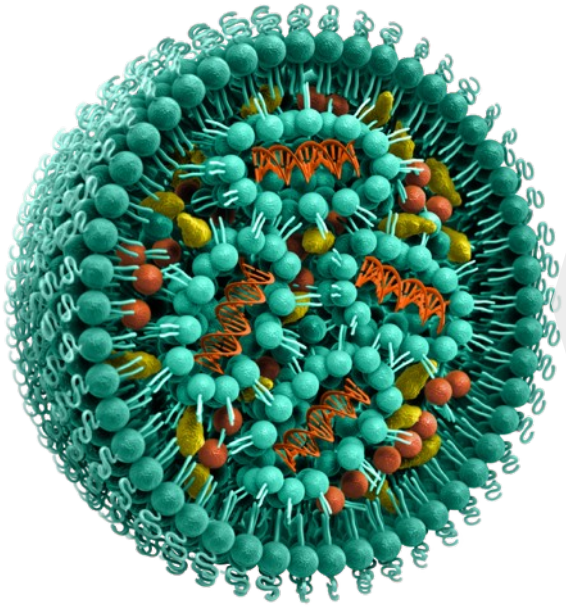
In vitro cell potency



- Routine
- Platform
- GMP

HPLC-CAD – High-performance liquid chromatography-charged aerosol detection

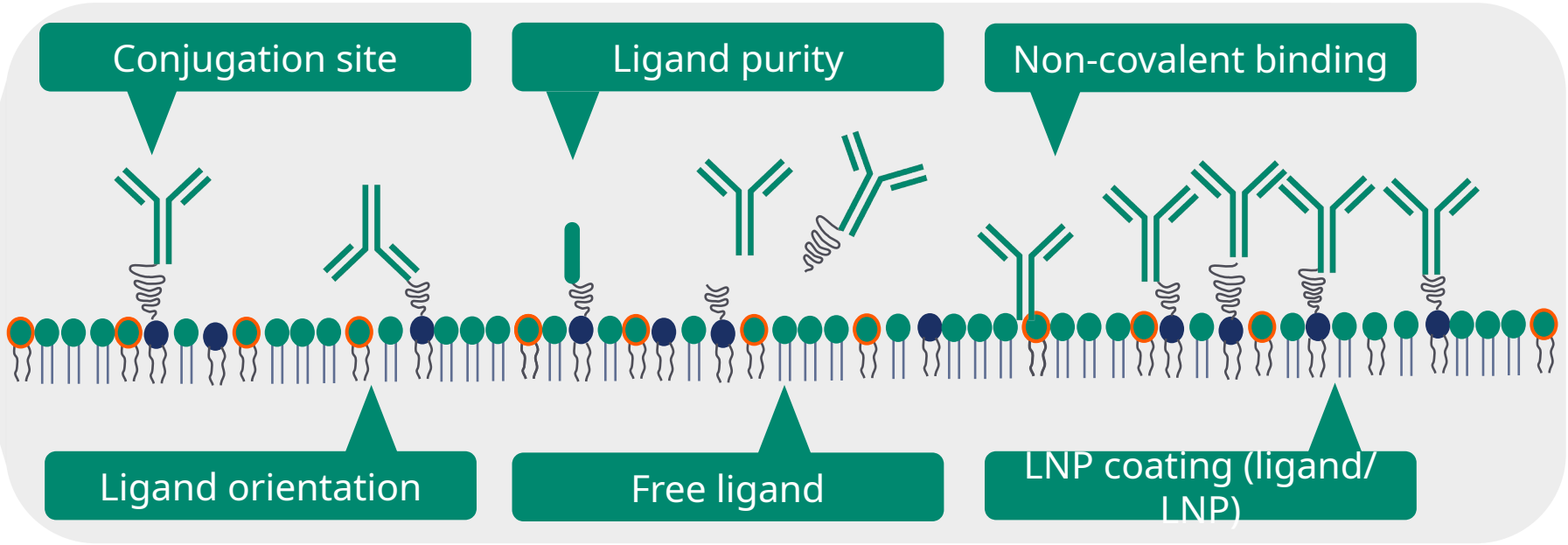
tLNPs require advanced analytics – Addressing key challenges



- Multiple Cytiva analytical assays to measure the number and location of events
- Heterogeneity control

- Ligand suitability assessment
- Integrity, aggregates, and variants may indicate downstream conjugation issues

- Cell type and culture conditions matter when assessing potency
- Ensure non-covalent controls are included



- Antigen binding site (ABS) must orient outwards from the LNP

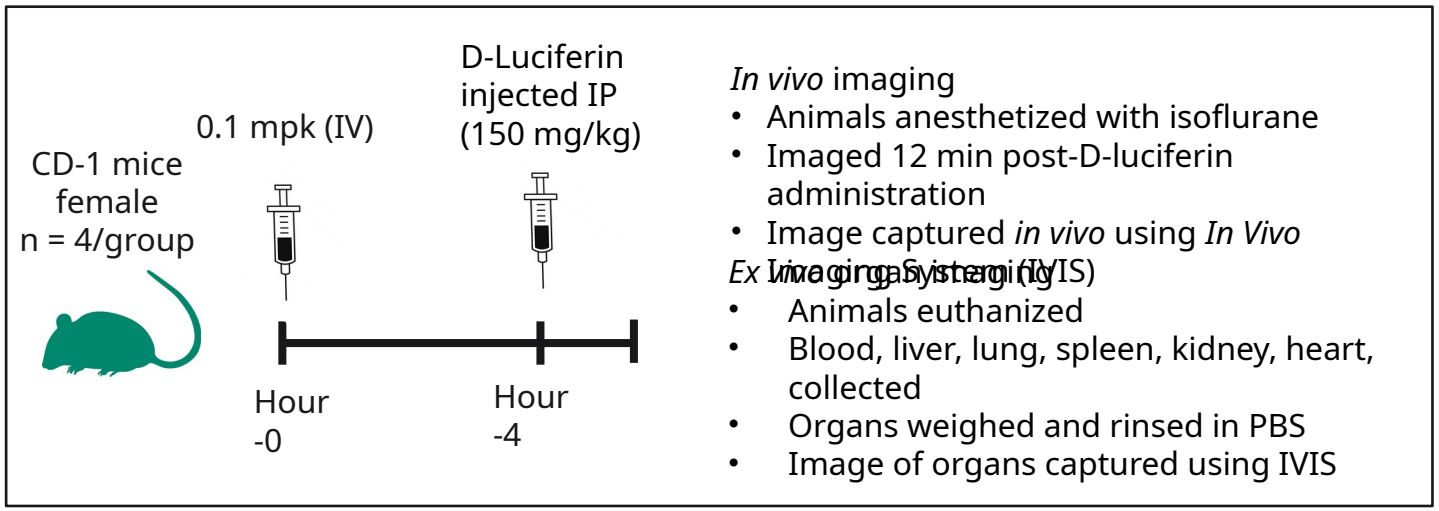
- Free ligand overestimates total ligand content and can reduce potency
- Size exclusion chromatography (SEC) resins and fractionation enable free ligand removal

- Density of ligand on LNP surface impacts interaction with target
- Changes in ligand coating impact surface properties of LNP

tLNPs – Targeted LNPs

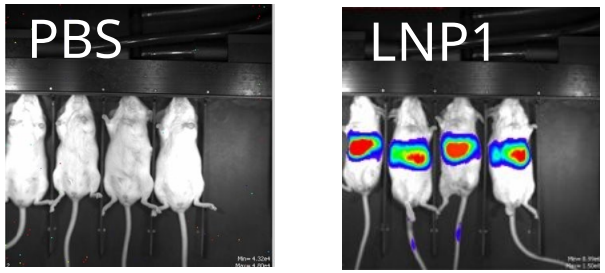
Engineering LNPs for spleen and lymph node targeting

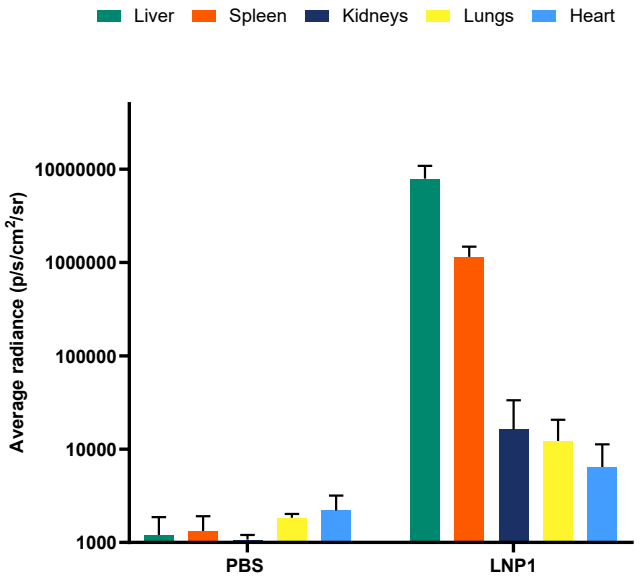
Cytiva LNPs demonstrate protein expression and liver delivery



In vivo imaging
- Animals anesthetized with isoflurane
- Imaged 12 min post-D-luciferin administration
- Image captured *in vivo* using *In Vivo* Imaging System (IVIS)

Ex vivo organ imaging
- Animals euthanized
- Blood, liver, lung, spleen, kidney, heart, collected
- Organs weighed and rinsed in PBS
- Image of organs captured using IVIS

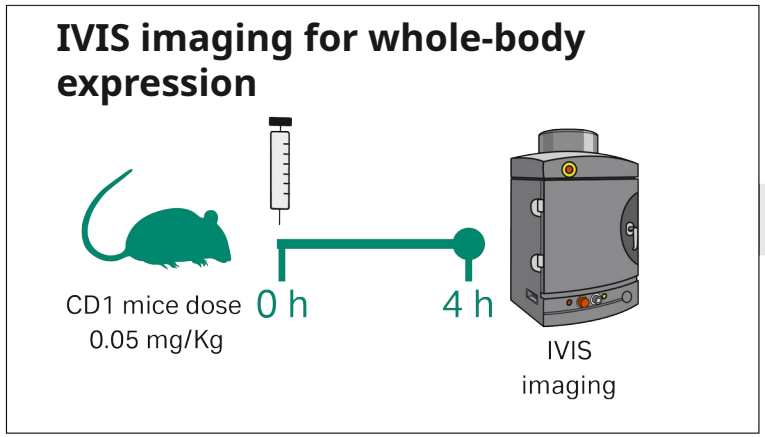


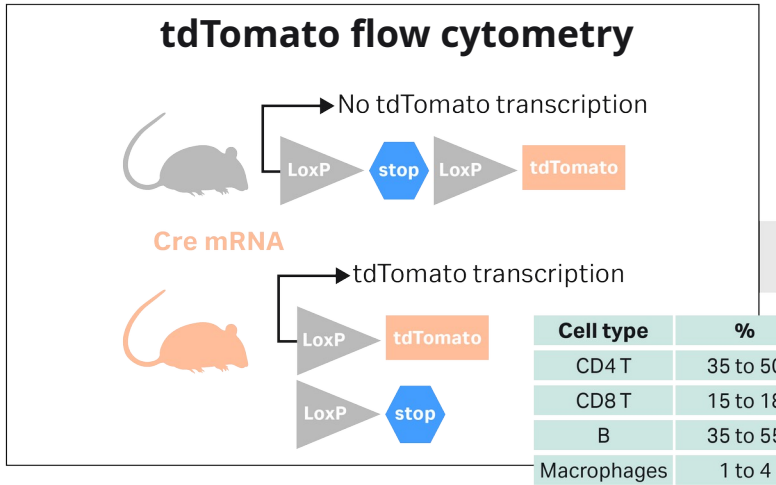


- The majority of systemic translation and translation concentration was in the liver and spleen when LNP1 is injected by intravenous (IV) administration.

PBS – Phosphate buffered saline

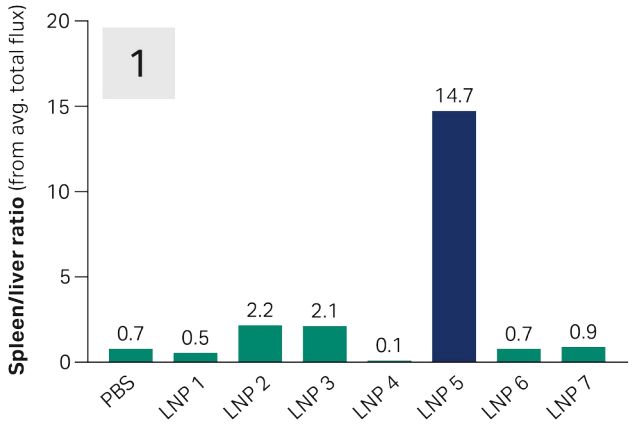
Novel LNPs designed for extra-hepatic delivery to spleen



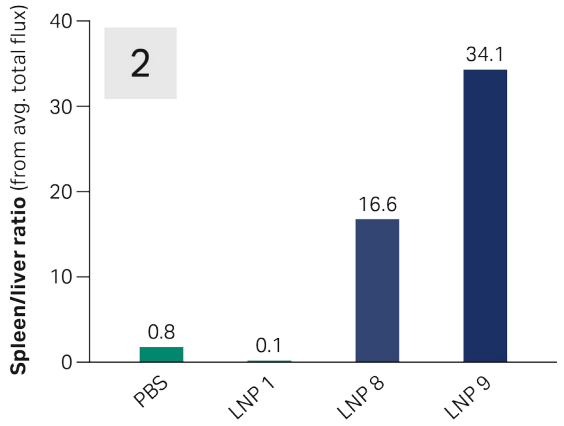


Cell type	%
CD4 T	35 to 50
CD8 T	15 to 18
B	35 to 55
Macrophages	1 to 4
Dendritic	2 to 5
NK	0.5 to 3

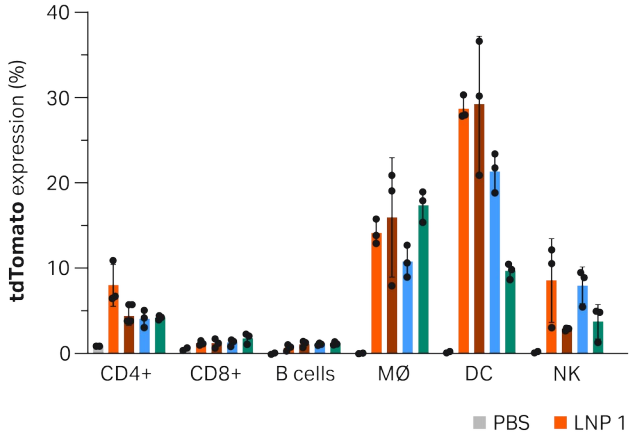
~15-fold spleen expression over liver



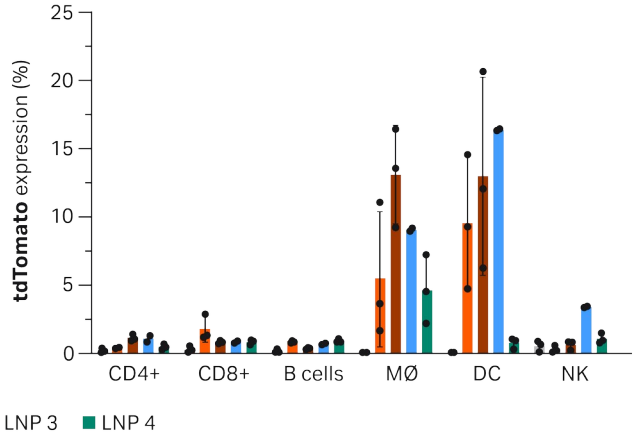
~34-fold spleen expression over liver



Spleen analysis



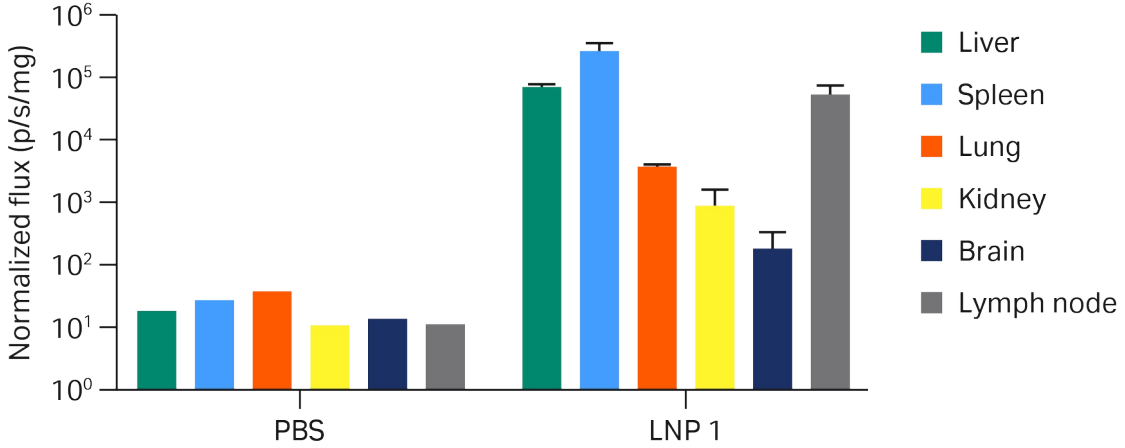
Lymph node analysis



Selected lipids demonstrate enhanced lymphoid tropism

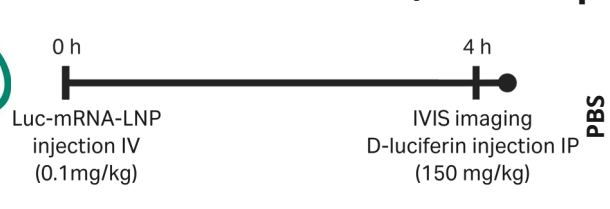
Large animal biodistribution demonstrates spleen enrichment and liver detuning

Lymphoid tropism is amplified relative to liver in non-human primates



*Data courtesy of collaborator

CD8-directed targeting increases lymphoid accumulation *in vivo* (mouse-specific binder)



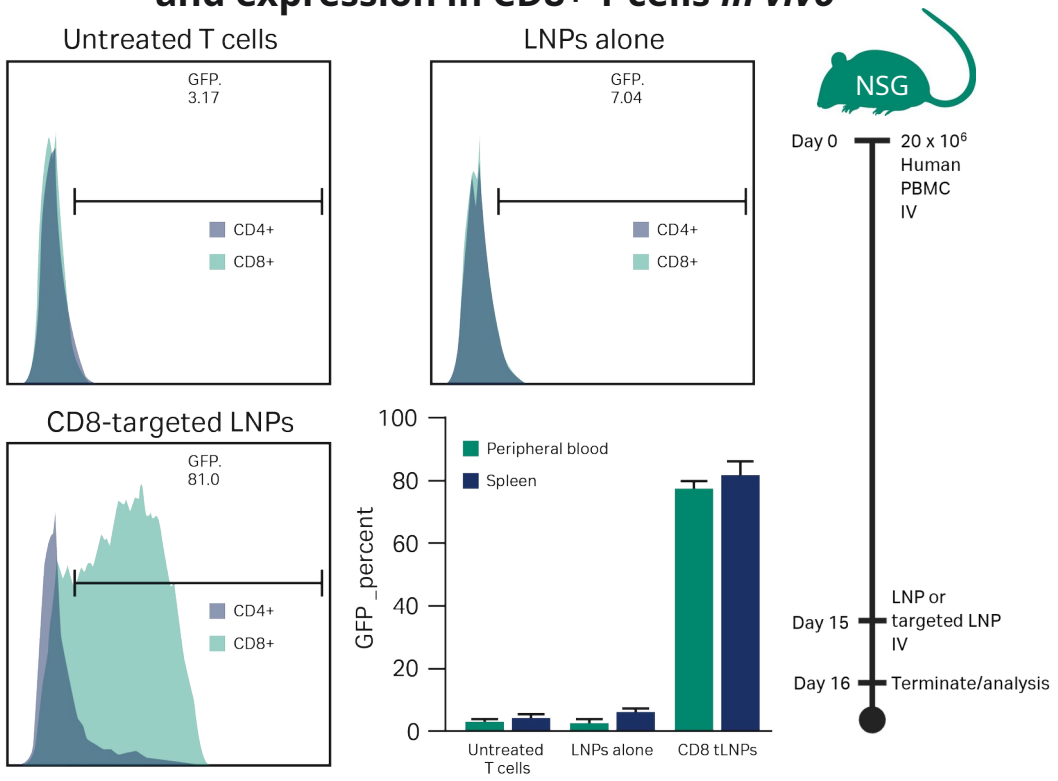
LNP composition alone enables lymphoid targeting, establishing a foundation for efficient T cell delivery without reliance on active targeting

Enhancing *in vivo* efficacy through targeted delivery

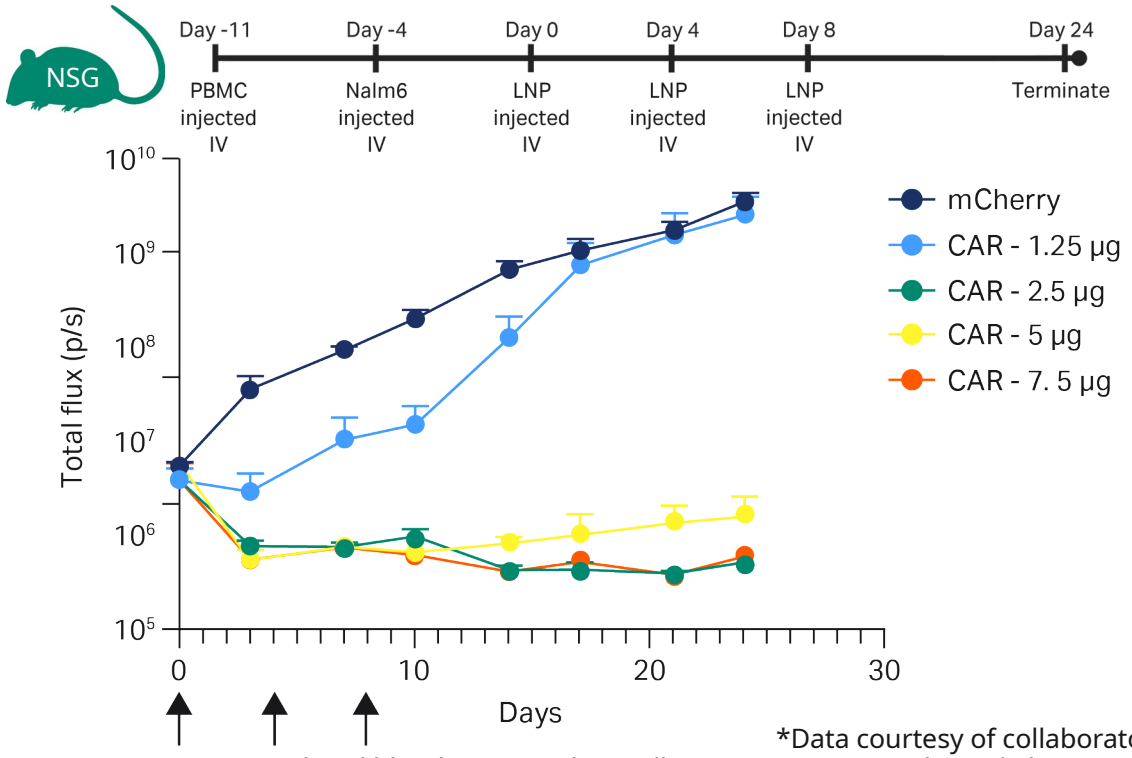
In vivo evaluation of Cytiva LNPs

CD8 targeting (marker) and tumor cell killing (CAR mRNA and CD8 targeting moiety)

CD8-targeted LNPs drive selective uptake and expression in CD8+ T cells *in vivo*



Potent CAR activity at low LNP doses



*Data courtesy of collaborator

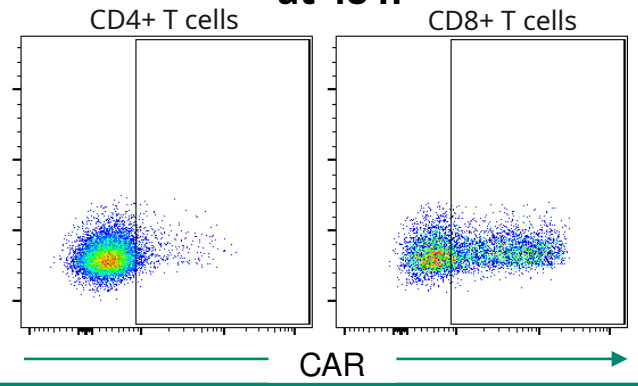
PBMC – Peripheral blood mononuclear cells; NSG – NOD (non-obese diabetic) scid gamma mouse

Active targeting enables selective CD8+ T cell delivery and potent CAR activity at low LNP doses

Durable, redosable CAR expression is achieved in NHPs

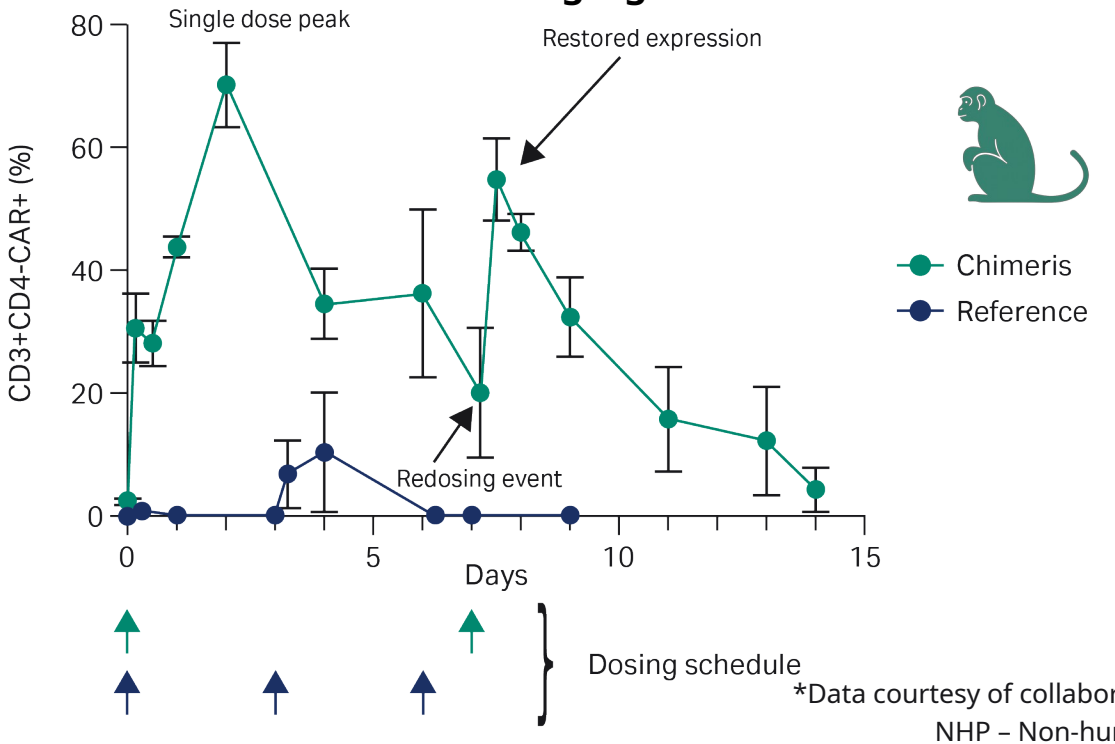
A human CD19 CAR lacking cynomolgus target cross-reactivity used to isolate expression kinetics

Expression is enriched in CD8+ over CD4+ T cells at 48 h



- Non-cross-reactive human CD19 CAR used to assess *in vivo* expression independently of depletion
- Peak expression exceeded comparator levels and remained detectable for ~7 days after a single dose
- [illegible] evidence of cytokine elevation

CAR expression remains detectable for ~7 days at 0.1 mg/kg



*Data courtesy of collaborator

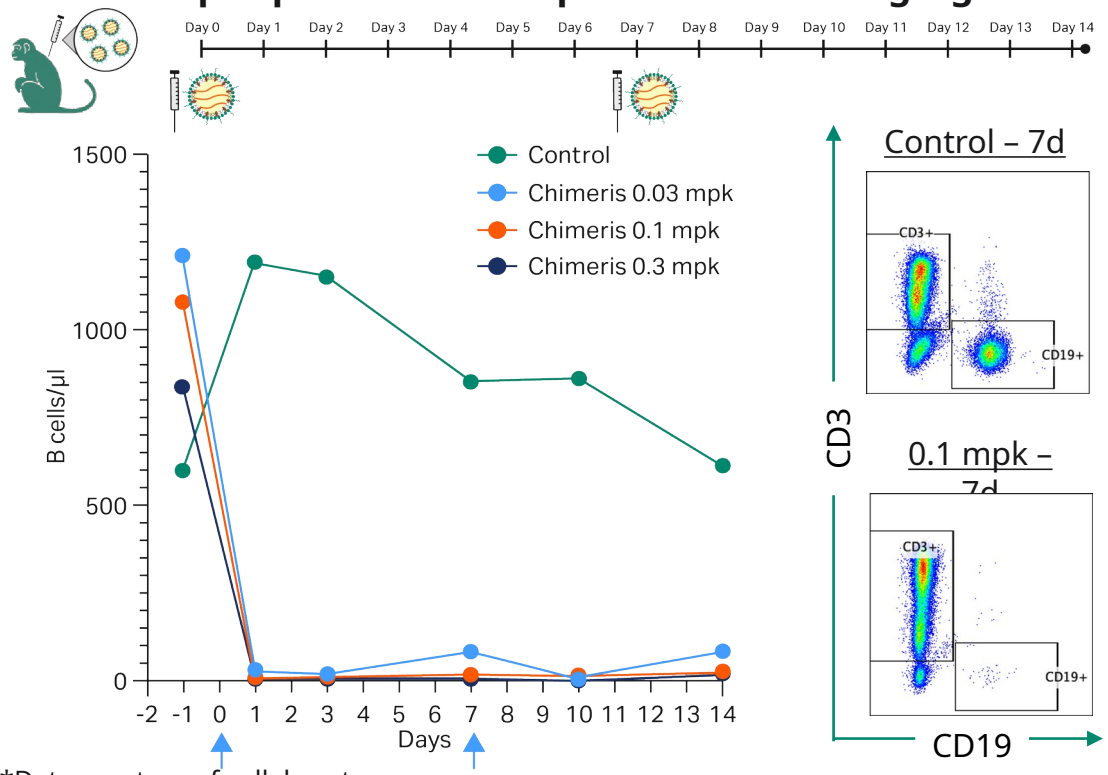
NHP – Non-human primate

- **Longitudinal expression compared with a current LNP CAR benchmark**
- **Durable, repeatable CAR expression in NHP supports clinical translation of transient *in vivo* CAR T**

In vivo CAR efficacy in NHPs

A CD20 surrogate CAR enables evaluation of efficacy in cynomolgus macaques

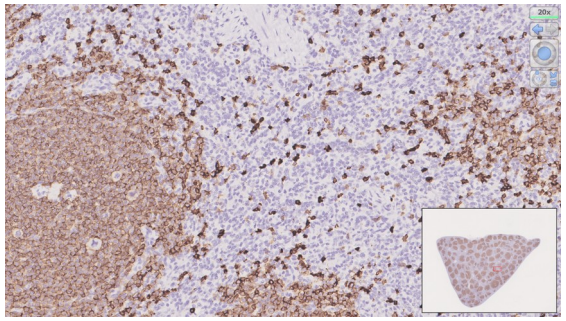
CD20 surrogate CAR enables rapid and sustained peripheral B cell depletion at ≤ 0.1 mg/kg



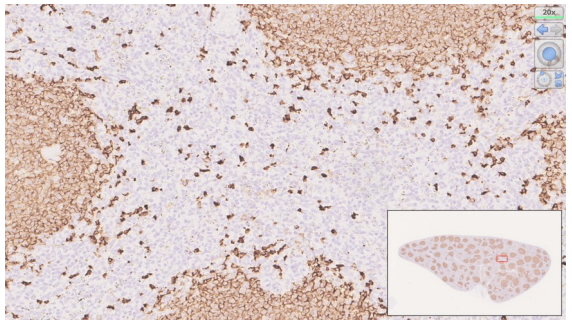
*Data courtesy of collaborator

Deep tissue B cell depletion in spleen at day 14

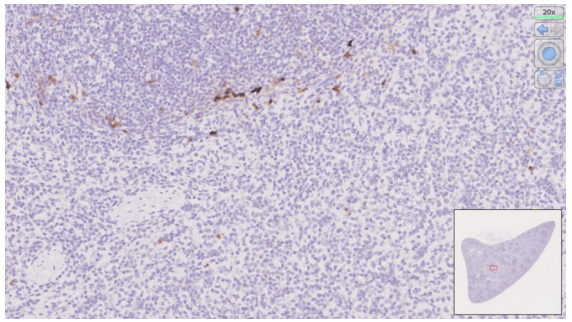
Control (saline)



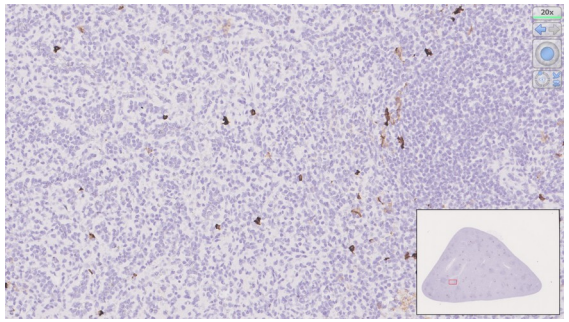
CD20 CAR - 0.03 mpk



CD20 CAR – 0.1mpk



CD20 CAR 0.3 mpk



Low-dose CAR establishes a measurable efficacy baseline in NHPs, which can be further enhanced through cytokine armoring

Conclusions

Advanced Analytics
Comprehensive evaluation of tLNP composition and *in vivo* performance

Lymphoid Targeting
Novel ionizable LNPs deliver preferentially to lymphoid tissues without active targeting, with GMP-ready scalability

Selective CAR Expression
Targeted RNA delivery achieves T cell-specific expression with sustained functional potency

In Vivo Efficacy
Low-dose efficacy demonstrated across murine and NHP models with B cell depletion

Novel ionizable LNPs, advanced analytics, and integrated control of targeting, and expression, enable potent *in vivo* CAR T cell therapy with scalable, GMP-ready manufacturing

Thank you

Martin Rabel, Cytiva
martin.rabel@cytiva.com

www.cytiva.com

For local office contact information, visit cytiva.com/contact
CY60345-26MAY26-PP

cytiva.com