



Engineered Bio-Reducible Lipid Nanoparticles Enable Highly Efficient Delivery

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5th Annual mRNA-based Therapeutics Summit
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etherna's mission is to be
a **technology leader** in the development of
nucleic acid-based medicines
which enables our **partners** to create new
therapeutics and improve patients' lives.



mRNA design and synthesis including GMP manufacturing



LNP formulation and optimization using our proprietary lipid libraries



Integrated CMC process development and analytics



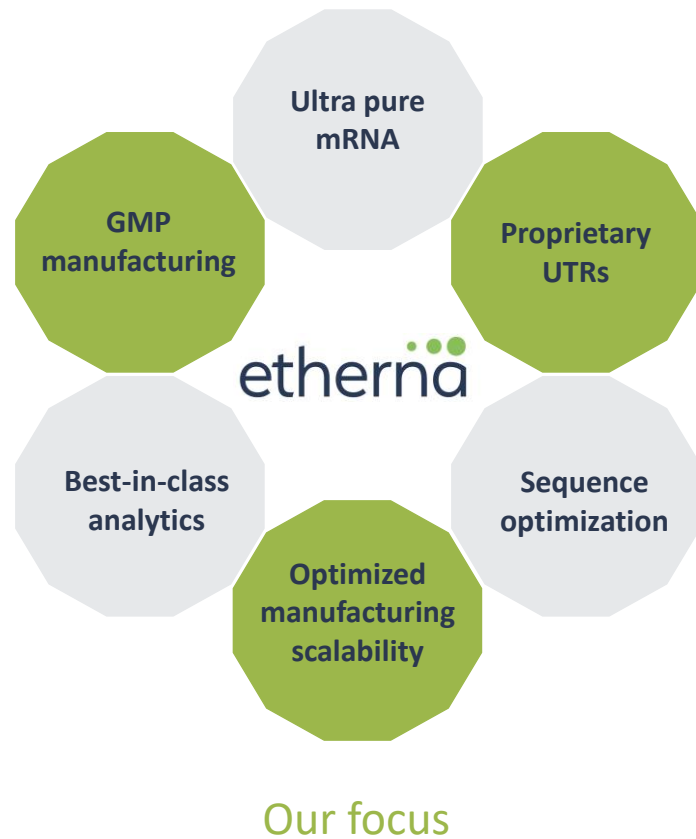
Cell biology and pharmacology expertise



Clinical and regulatory expertise related to mRNA/LNP development

Proprietary mRNA chemistry and manufacturing solution

mRNA chemistry focused on achieving robust protein expression is validated across multiple pre-clinical & clinical studies

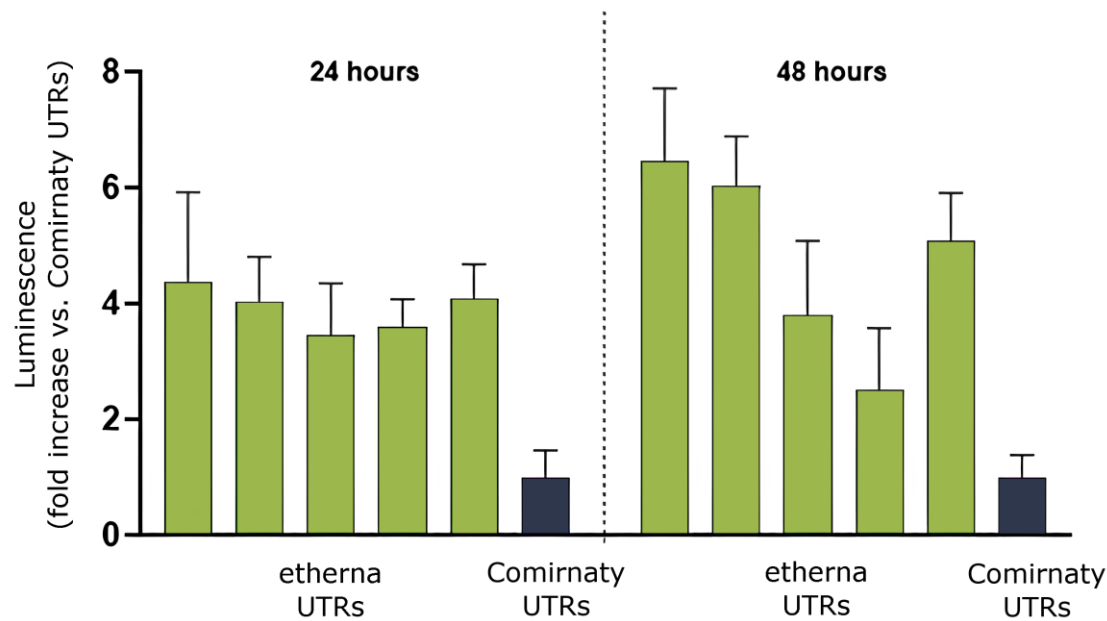


- mRNA sequence engineering yielding optimal protein translation and mRNA stability that outperforms BioNTech/Moderna clinical benchmark designs by >5-fold
- Optimization for tissue specific expression
- Manufacturing & design of mRNA for immune silent or immune stimulatory applications
- Purity of manufactured mRNA that is world leading and significantly better than other suppliers
- Ultra-low levels of double-stranded RNA (dsRNA) – a critical factor in ensuring safety and efficacy in therapeutic applications

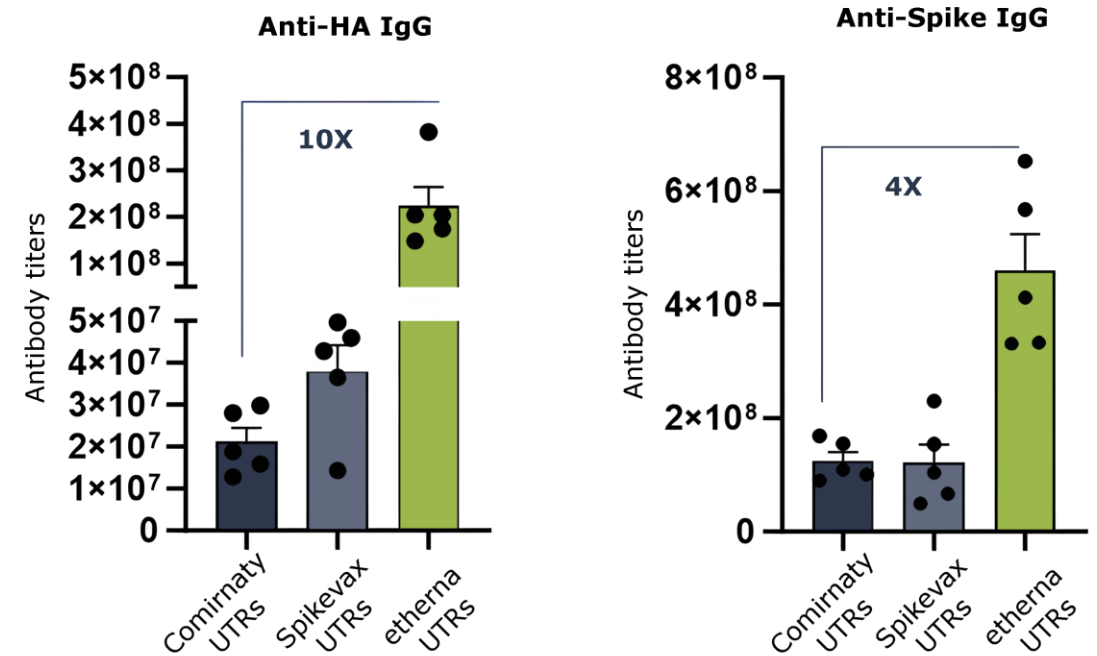
etherna's proprietary UTRs boost *in vivo* expression and antibody titers beyond benchmark standards

Enhanced expression after IM administration

fLuc mRNA-LNPs



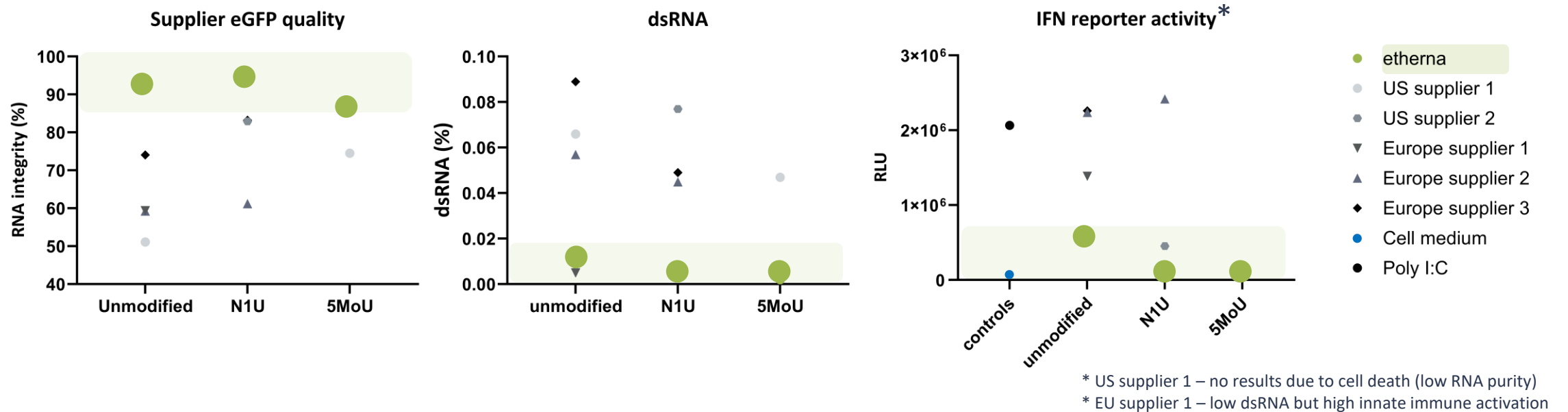
HA mRNA or Spike mRNA-LNPs



Our optimized UTR combinations produce up to 5-fold increase in expression and antibody titers compared to Moderna (SpikeVax™) & BioNTech (Comirnaty™)

etherna mRNA exhibits unmatched purity, quality and safety when tested against top industry comparators

etherna's proprietary mRNA production processes significantly enhance mRNA quality thereby abrogating inflammatory responses



Our mRNA demonstrated the highest purity, lowest dsRNA levels / innate immune system activation

Our mRNA provides a safer, more effective research and therapeutic development option

Our commitment to quality ensures that partners can confidently advance their projects with the highest-quality material

State-of-the-art customized LNP platforms

etherna has built proprietary world class customized LNP (cLNP) expertise with cLNPs for multiple specific therapeutic applications



Customized LNP composition

- Proprietary ionizable lipid libraries with a total of >2500 different lipids with wide chemical diversity
- Fit-for-purpose LNPs: smart and customized LNPs tailored to the intended application ensure maximized activity and safety, customized to payload
- *In vivo* activity that outperforms benchmark LNPs including by >5-10-fold versus ALC-0315 and SM-102 IM-LNPs and Intellia LP01 IV-LNP
- *Ex vivo* transfection LNPs with high editing and superior cell viability to electroporation
- LNPs that allow improved targeted extrahepatic biodistribution (Bone marrow, T-cells, ...)
- Ability to lyophilize mRNA/LNP formulations to achieve stability for up to 12 months

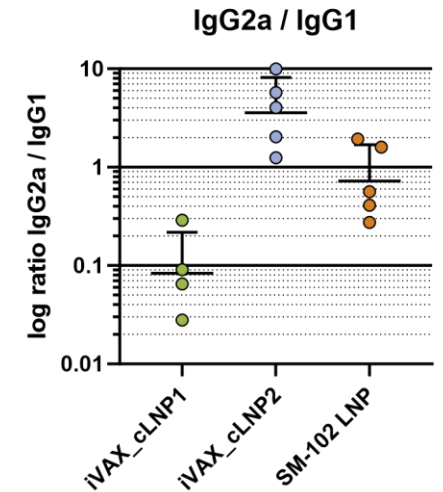
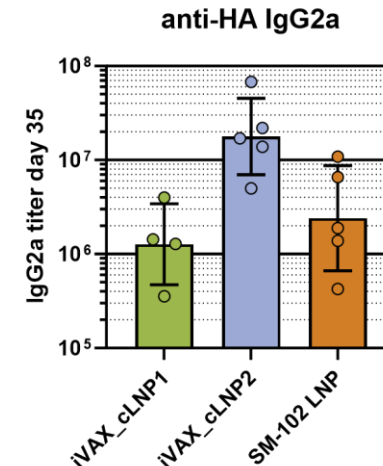
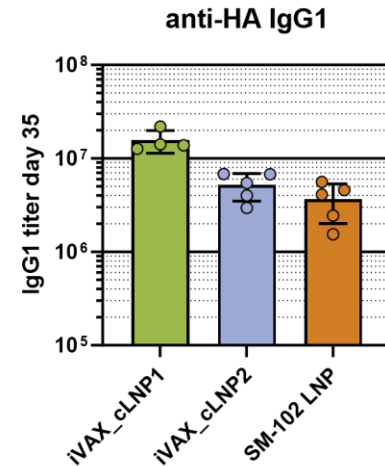
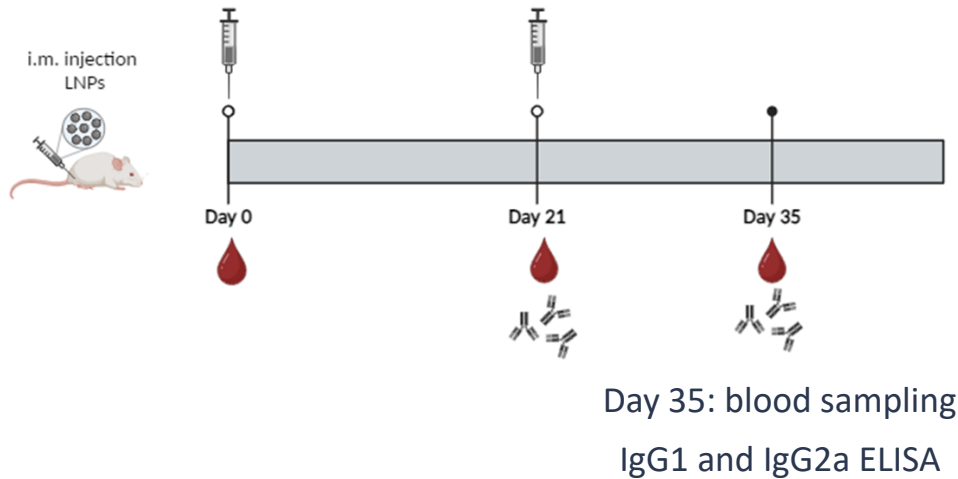
Vaccination LNP platforms



Superior efficacy of etherna's optimized vaccine platform

Best-in-class immune response against influenza HA

Antigen: influenza viral Hemagglutinin (HA)

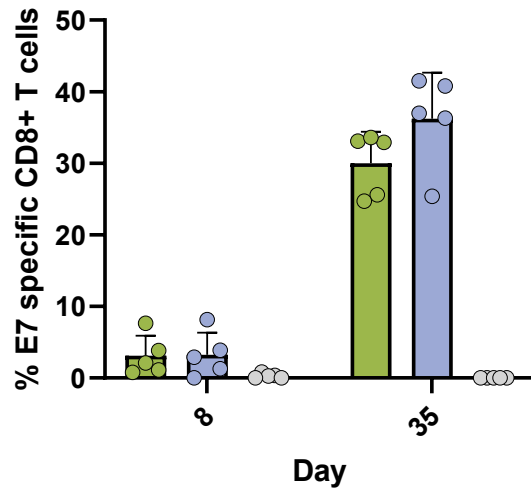


etherna vaccine platforms: iVax-cLNP1 & iVax-cLNP2

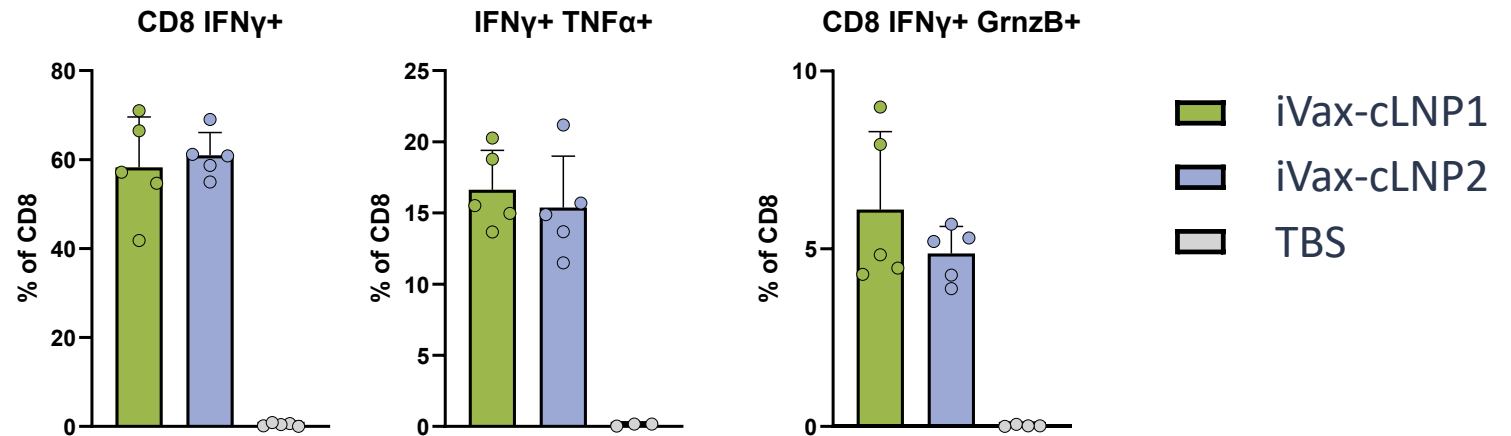
Increased antibody titers compared to Moderna SM-102 LNPs (Spikevax™)
Different IgG1 (iVax-cLNP1) vs IgG2a bias (iVax-cLNP2)

etherna's iVax-cLNPs induce robust CD8 T cell responses

Blood: % E7-specific CD8 T-cells



Spleen: % cytokine + CD8 T-cells

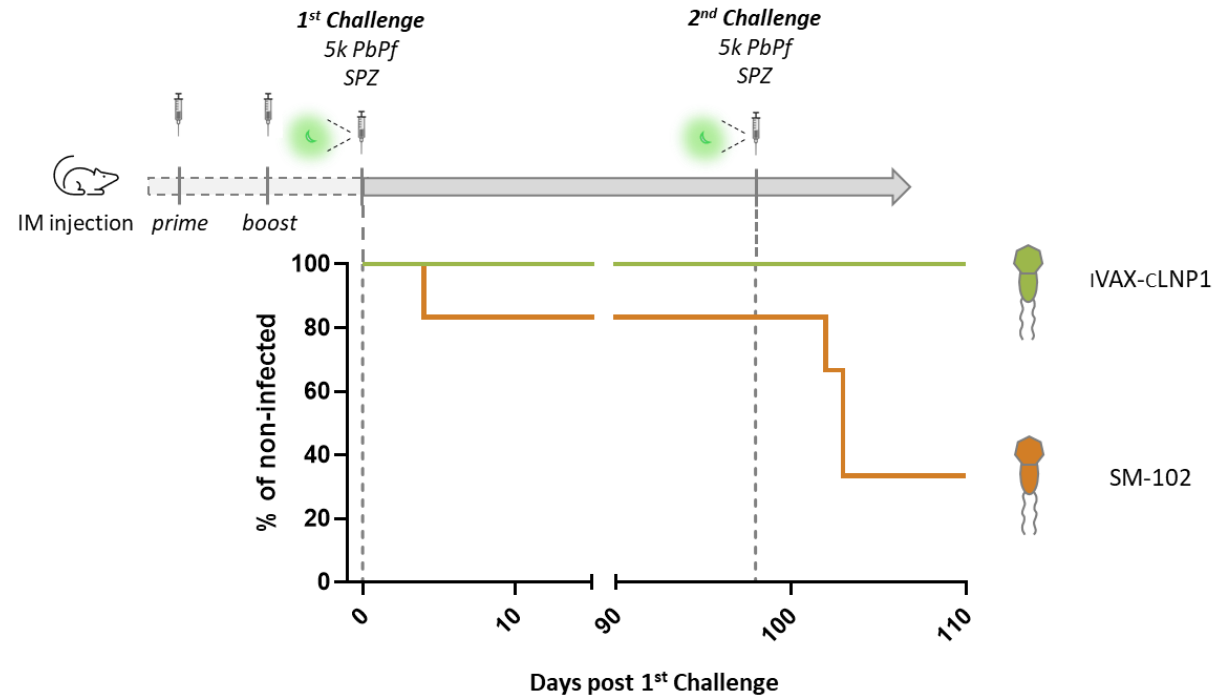
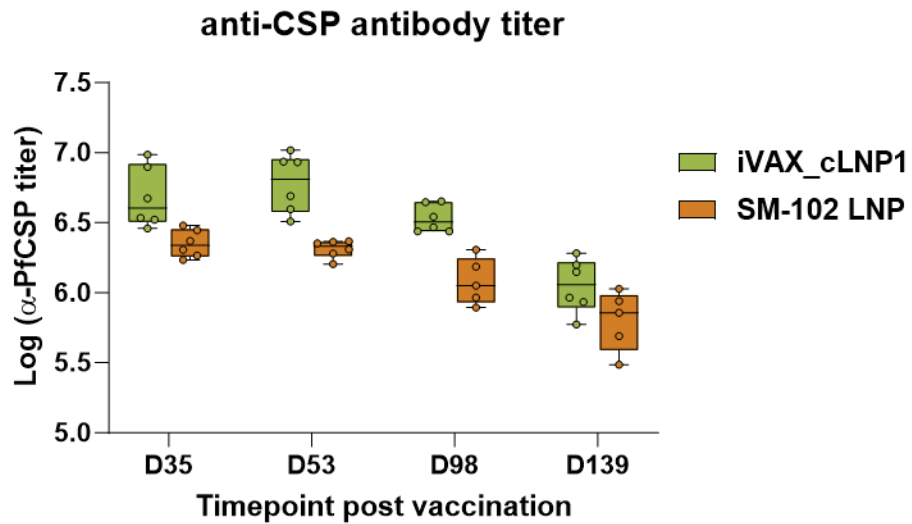


Activity: HPV16 E7 oncoprotein encoding mRNA

iVax-cLNPs induce robust CD8 T cell responses

etherna mRNA-LNP based malaria vaccine

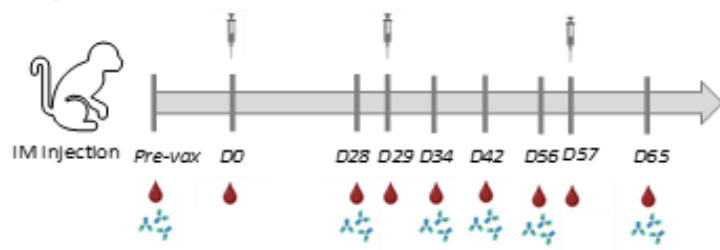
Enhanced immune responses translates into superior protection



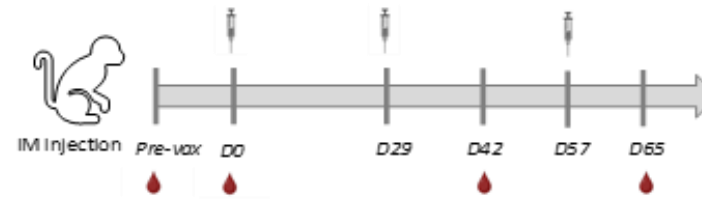
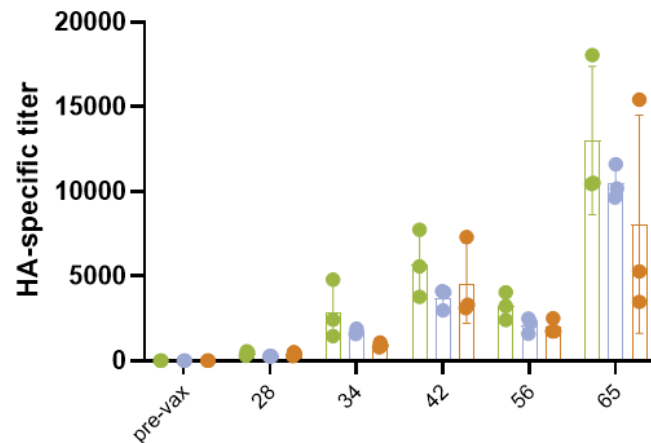
etherna mRNA-LNP malaria vaccine

Superior antibody responses against CSP compared to identical mRNA formulated in SpikeVax' SM-102 LNPs
Superior and longer lasting protection against Plasmodium challenge

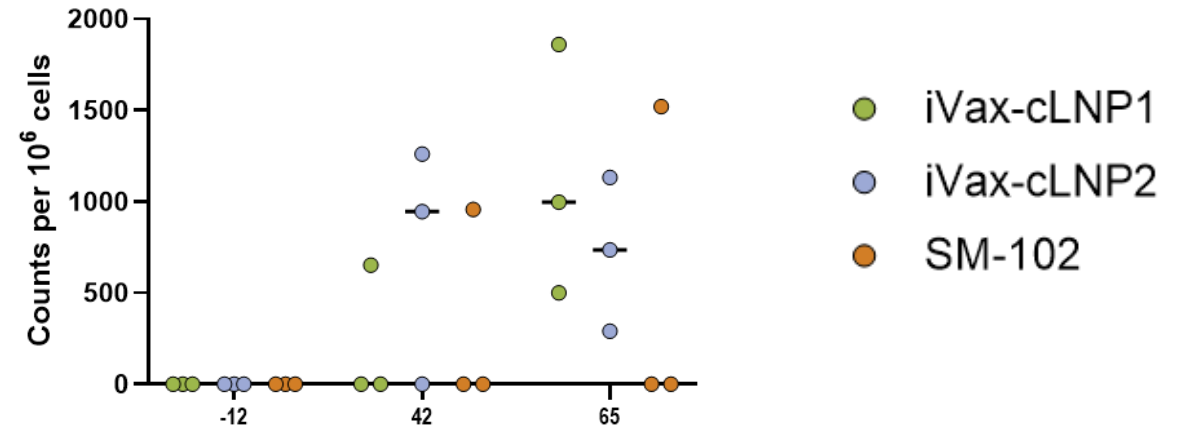
iVax-cLNP based mRNA vaccines are efficacious and safe in NHPs



**Influenza HA mRNA:
Antibody response**



**Influenza NP mRNA:
CD8 T cell response**



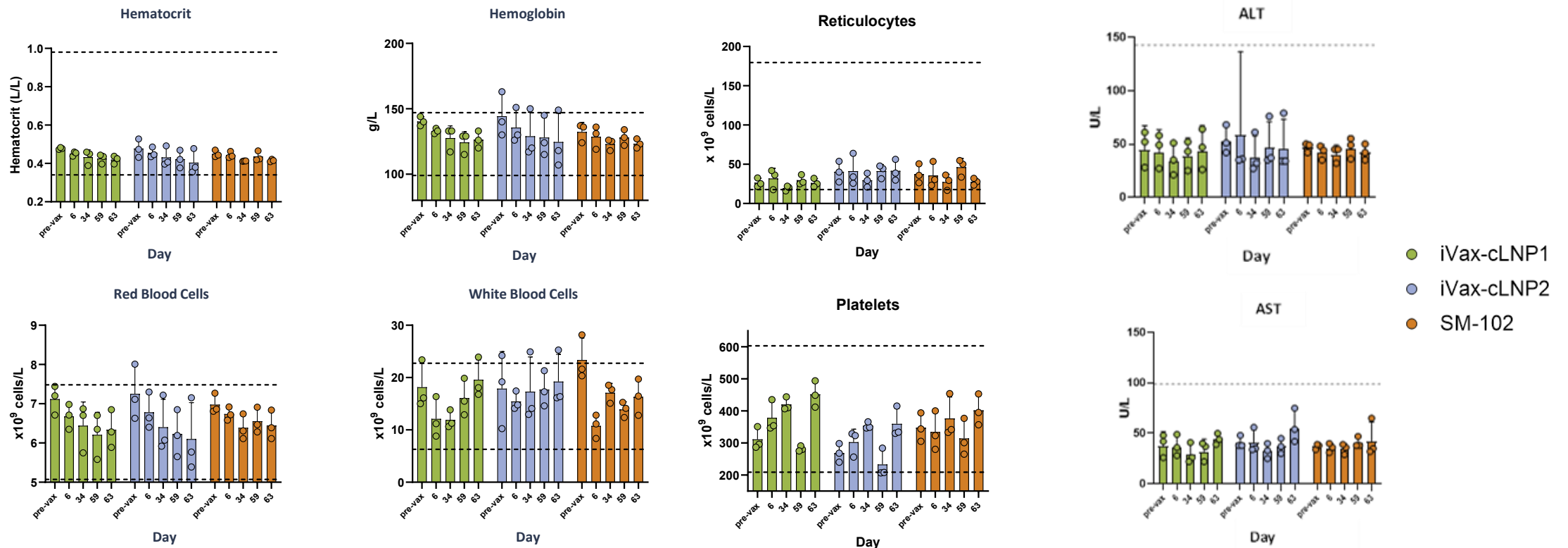
- iVax-cLNP1
- iVax-cLNP2
- SM-102

Activity etherna prophylactic vaccine platform:

iVax-cLNPs induce antibody titers outperforming SM-102

iVax-cLNPs induce strong CD8 T cell responses against NP

iVax-cLNP based mRNA vaccines are efficacious and safe in NHPs



Safety etherna prophylactic vaccine platform:

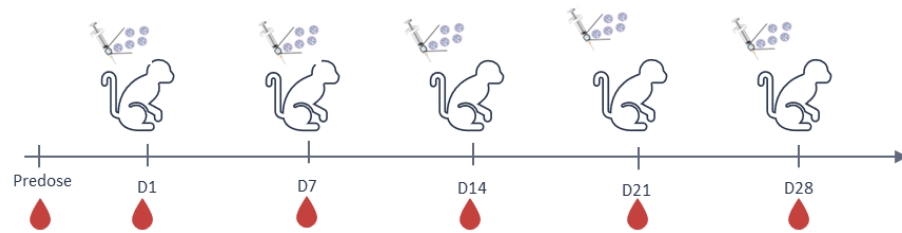
No test item related changes in body weight, temperature, clinical chemistry and hematology, transient local grade 1 reactions after 3rd dosing

Liver targeted cLNPs



etherna liver-cLNPs enable high protein expression in NHP

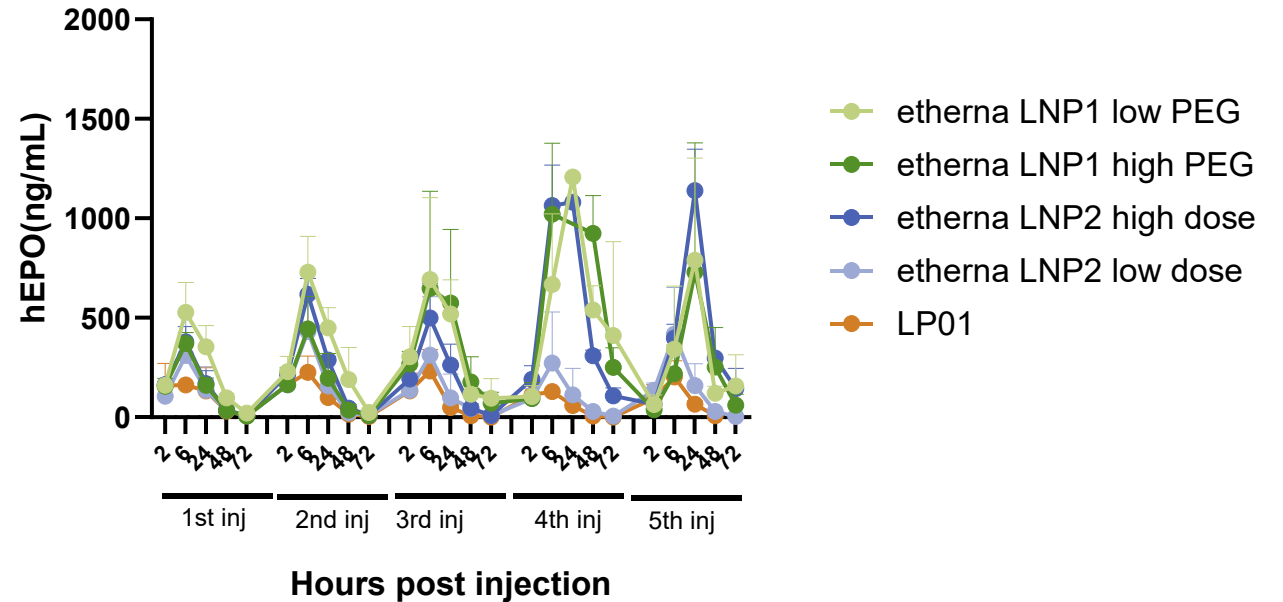
huEPO mRNA – protein expression levels



huEPO mRNA: 5 times, weekly dosing

- Quantification in blood
- ALT; AST, hematology and clinical chemistry

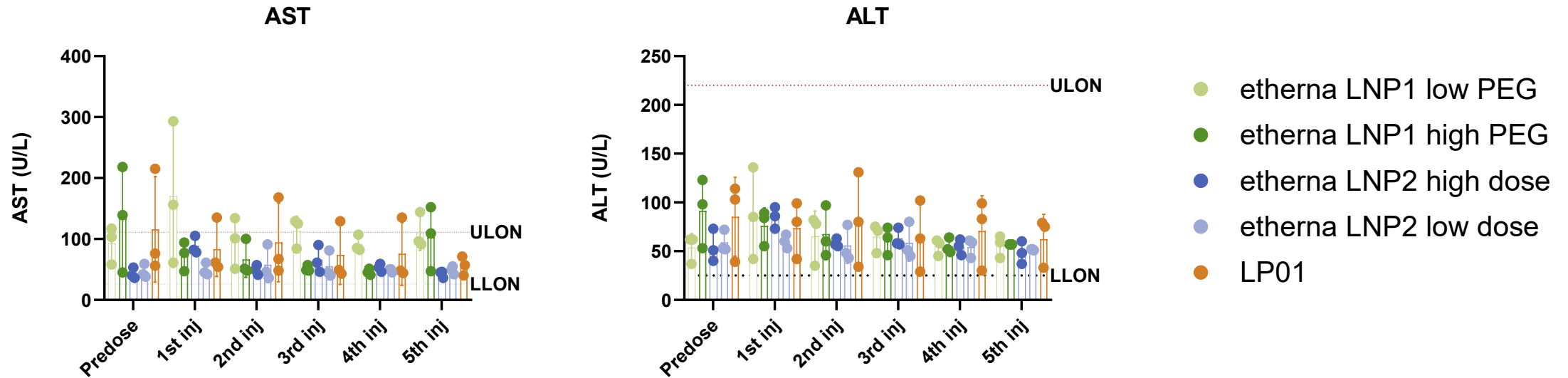
hEPO expression in NHP



- etherna liver-LNPs drive **higher huEPO expression levels** than an equivalent dose of LP01 LNP
- **4-fold lower dose of etherna LNPs show comparable protein expression** to high dose LP01
- No observed loss of expression upon repeated weekly administration (up to 5x)

etherna liver cLNPs are well-tolerated in NHP

Safety – Liver enzymes



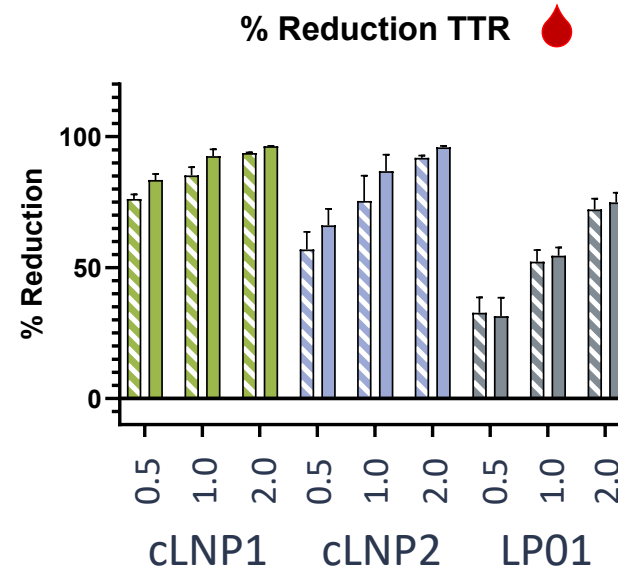
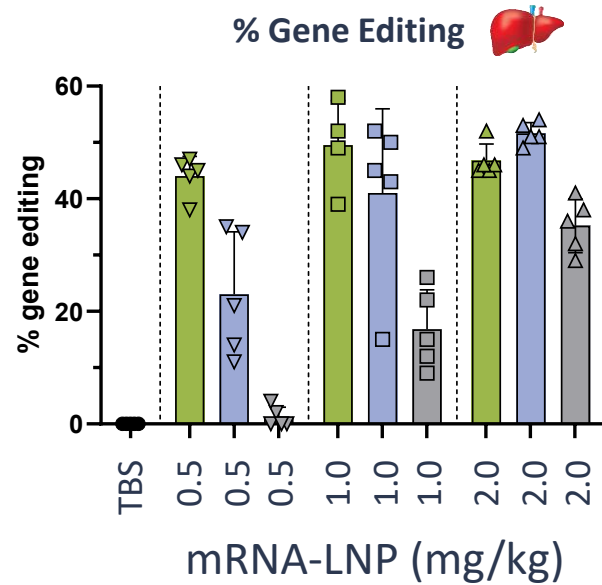
ALT levels within physiological range

Episodic and transient AST elevations not coupled to other parameters of toxicity and not considered LNP related

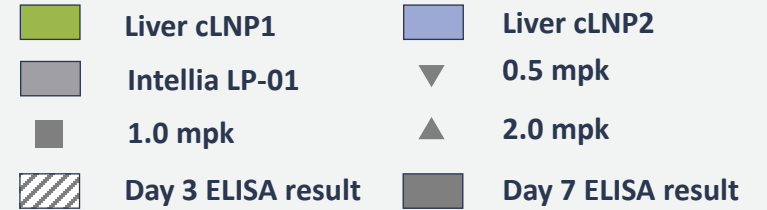
etherna liver cLNPs outperform Intellia LP01 for gene editing

Rodent data – TTR editing

Results:



Legend:



	Etherna performed experiment		
	Liver cLNP1 2 mpk	Liver cLNP2 2 mpk	LP-01 2 mpk
% editing	46.8 %	51.6 %	35.2 %
% reduction TTR	96.3 %	95.8 %	74.9 %

**etherna cLNPs outperform Intellia LP-01 formulations at all dose levels
in terms of % editing and serum TTR levels**

Extrahepatic targeting

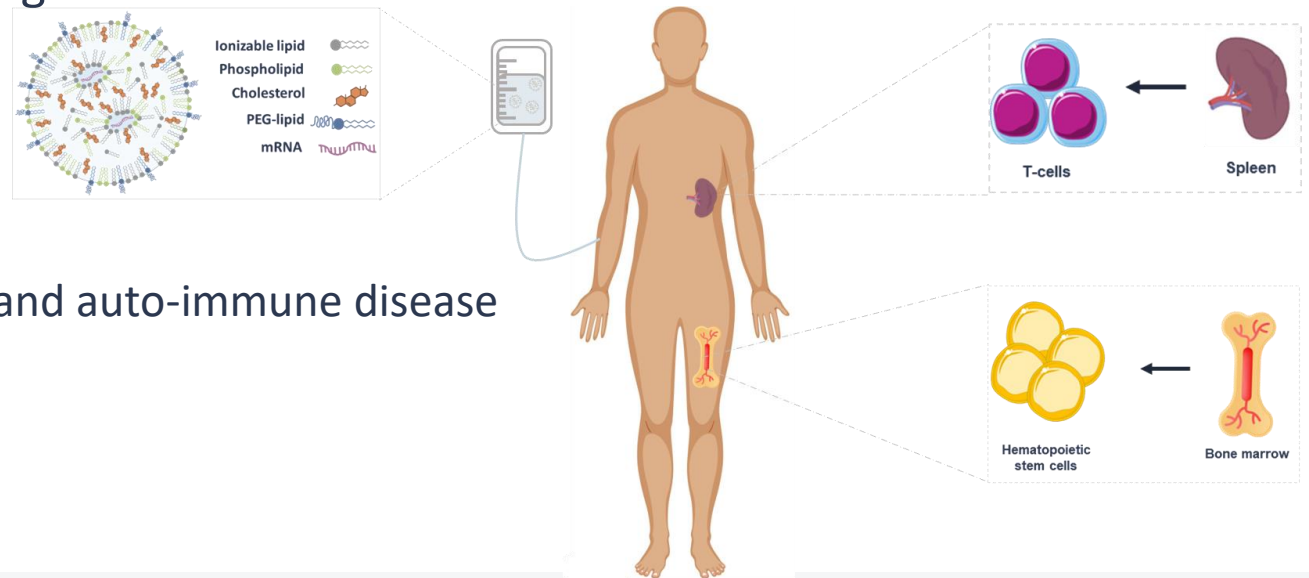


Extrahepatic delivery: scope

- Develop cLNP platform for extrahepatic delivery to HSC's and T-cells
 - **Passive targeting:** improved expression/specificity in target cells imposed by lipid composition (no surface conjugation of ligands)
 - **Active targeting:** improved specificity in desired cells imposed by conjugation of targeted antibody to LNP surface

- Potential therapeutic applications are huge

- **HSCs:** genetic hematological disorders (sickle cell, beta-thalassemia, X-linked CGD, erythropoietic porphyria...)
- **T cells:** CAR/TCR therapies in oncology and auto-immune disease



Several strategies are being employed to enable extra-hepatic targeting

- Use of a **non-sheddable polymer-lipid (diC18-PEG)** to extend circulation time by formation of a stable hydrophilic shell to reduce protein binding (approach discarded due to low activity and other drawbacks)
- Addition of a charged, **fifth lipid component: (SORT) LNP** to alter the surface charge/pKa to modify serum protein interactions, which subsequently influences organ distribution
- **Increase ratio of bilayer lipids (chol and phospholipid) while lowering ionizable lipid** to reduce adsorption of serum proteins and result in longer circulation and broader tissue distribution
- Addition of **ligands onto the surface of the LNP** to direct distribution towards cell type(s) of interest

etherna - extrahepatic approach



Hypothesis: reduction of cholesterol would lower APO-E driven LNP delivery to the liver and enable increased uptake in other organs with fenestrated endothelium (spleen, bone marrow)



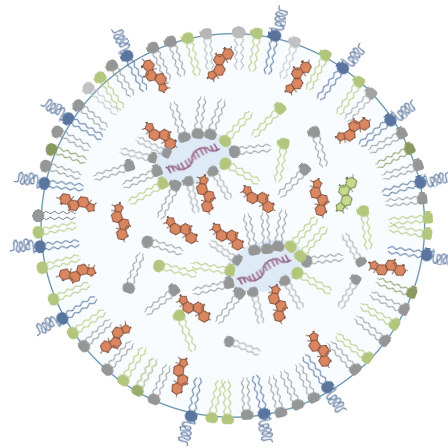
Strategy: assess the biodistribution of LNPs formulated with variable ratios of cholesterol to DSPC



Outcome: Lower chol/DSPC ratio promotes liver de-targeting and enhances delivery to bone marrow HSCs & splenic T cells



Applicability: confirmed for all (etherna) ionizable lipid libraries with distinct chemistries



Chol



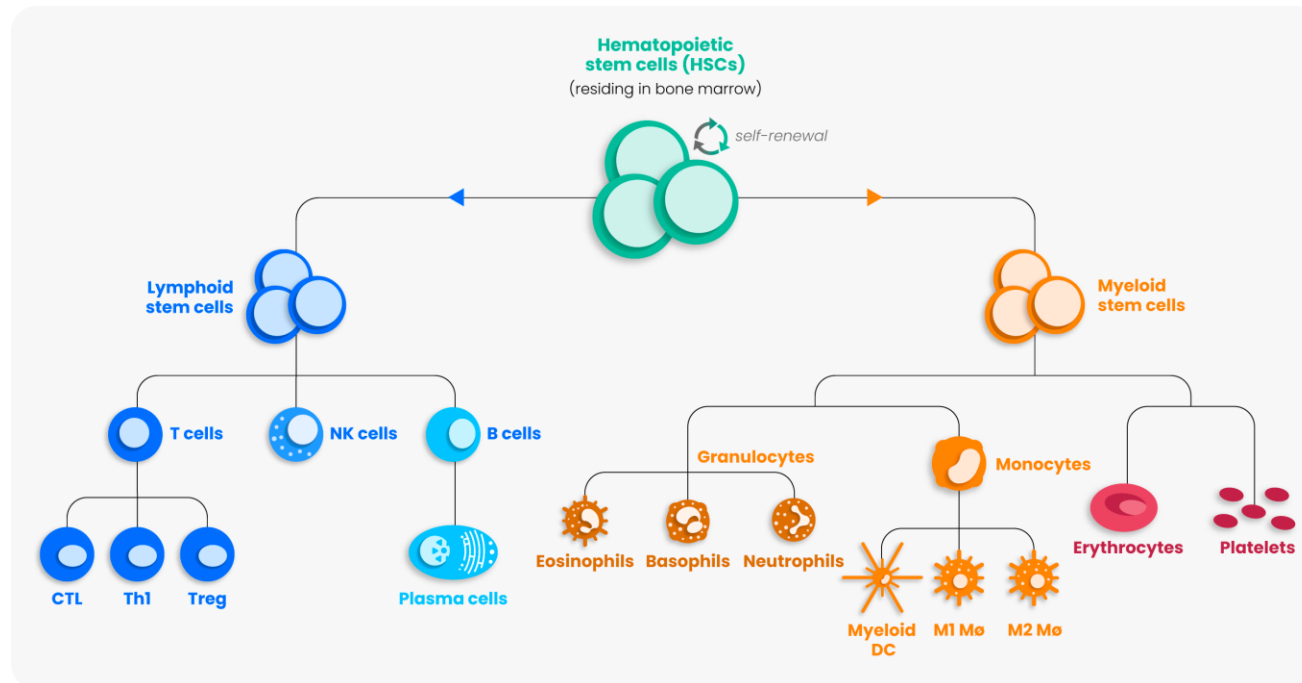
Phospholipid



Chol/HL ratio

Targeting HSCs – redesigning the hematopoietic system

Extrahepatic delivery to HSCs



HSC engineering:

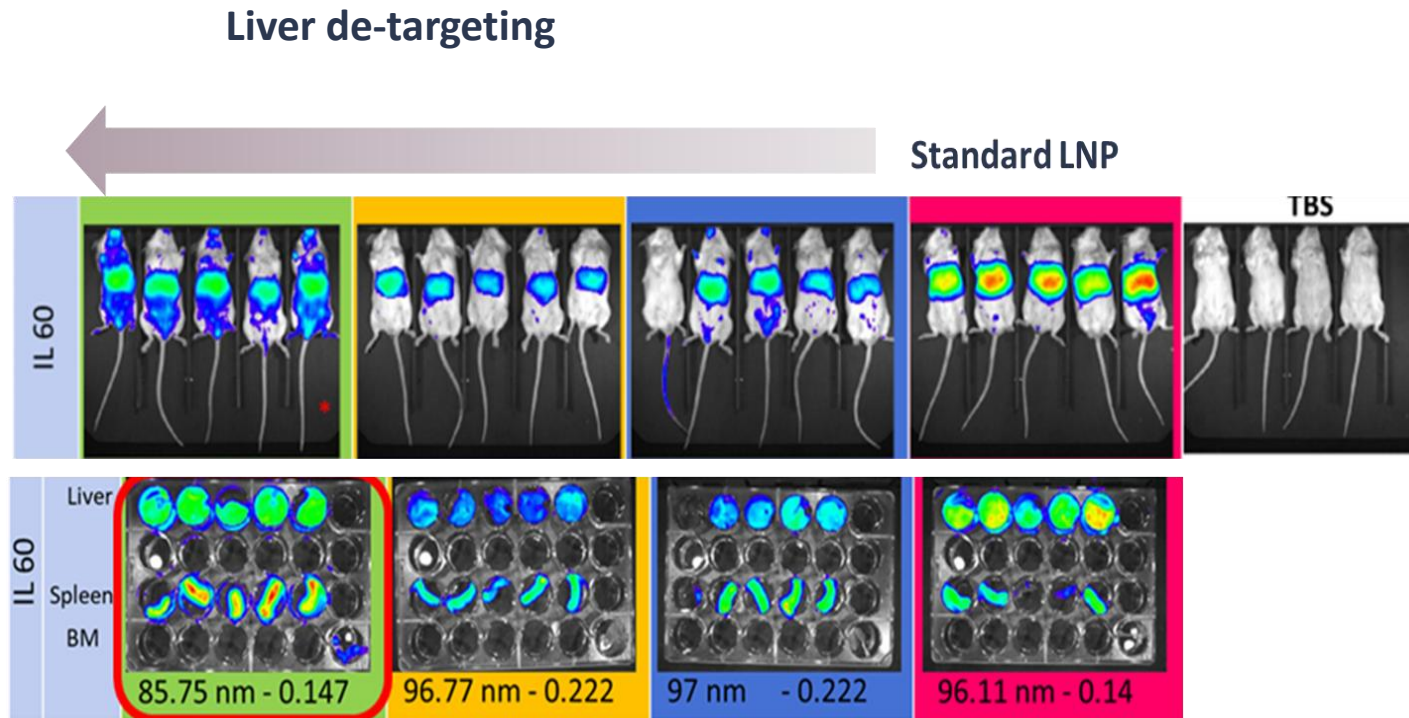
- Sickle cell disease
- B-thalassemia
- X-linked chronic granulomatous disease (X-CGD)
- ...

In situ HSC-targeting approaches

Goal: direct *in vivo* engineering of HSCs to avoid long, multi-step, burdensome *ex vivo* processes and toxic conditioning regimen

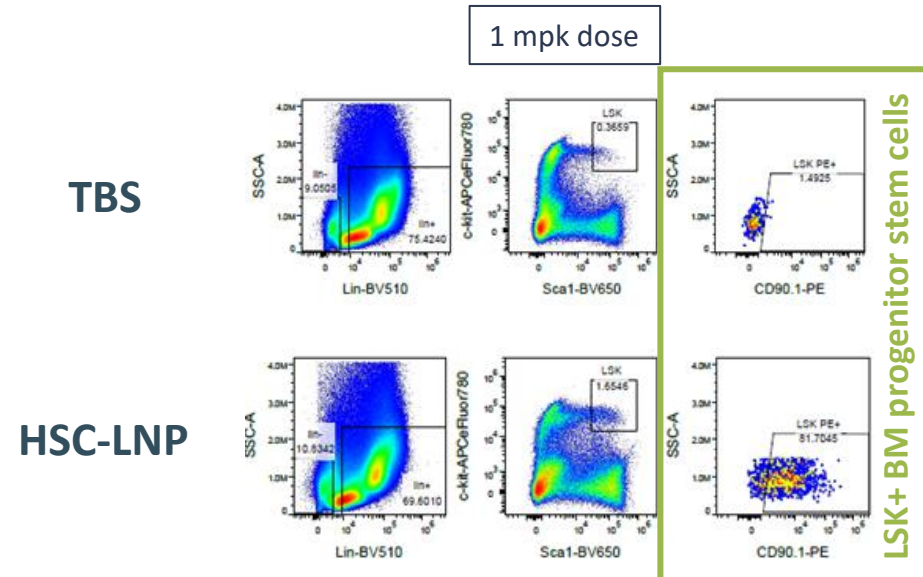
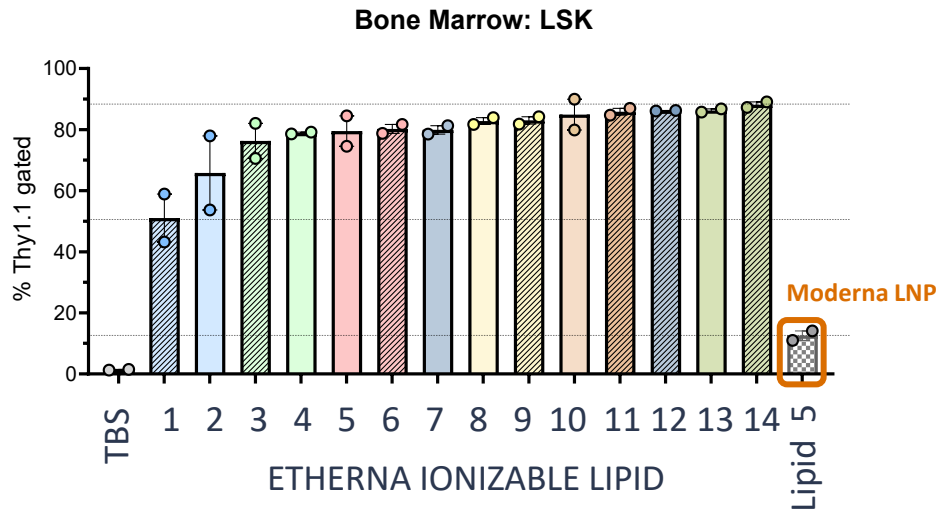
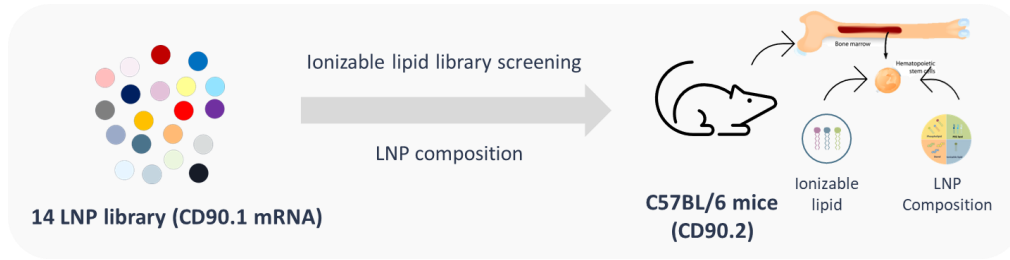
HSC-cLNP: liver de-targeting and HSC delivery

Extrahepatic delivery to HSCs



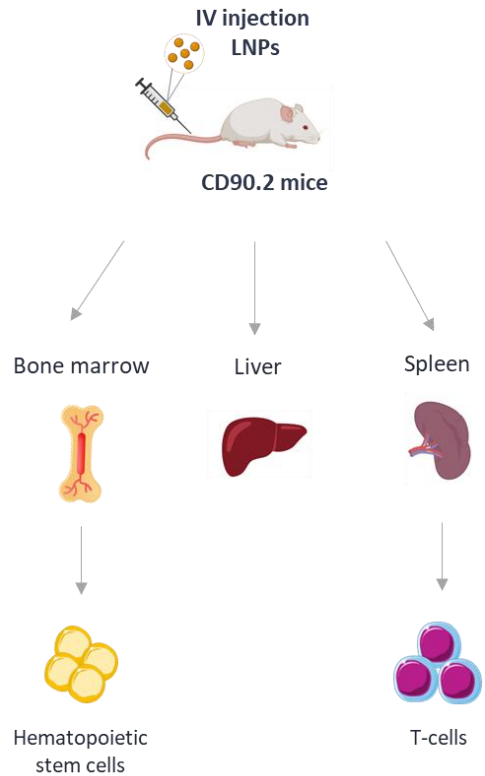
- etherna has established proprietary LNP formulation conditions that promote liver de-targeting and increased delivery to spleen and BM
- Involves lowering the chol/DSPC ratio
- These conditions work across a wide variety of ionizable lipid chemistries

Screening of ILs and LNP compositions for HSC delivery in BM

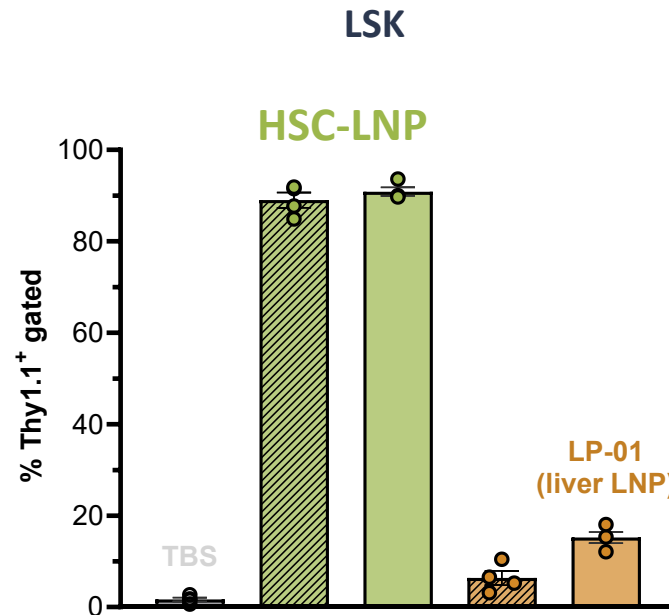


etherna's lead LNPs result in ~80-90% of bone marrow HSCs expressing the payload of interest

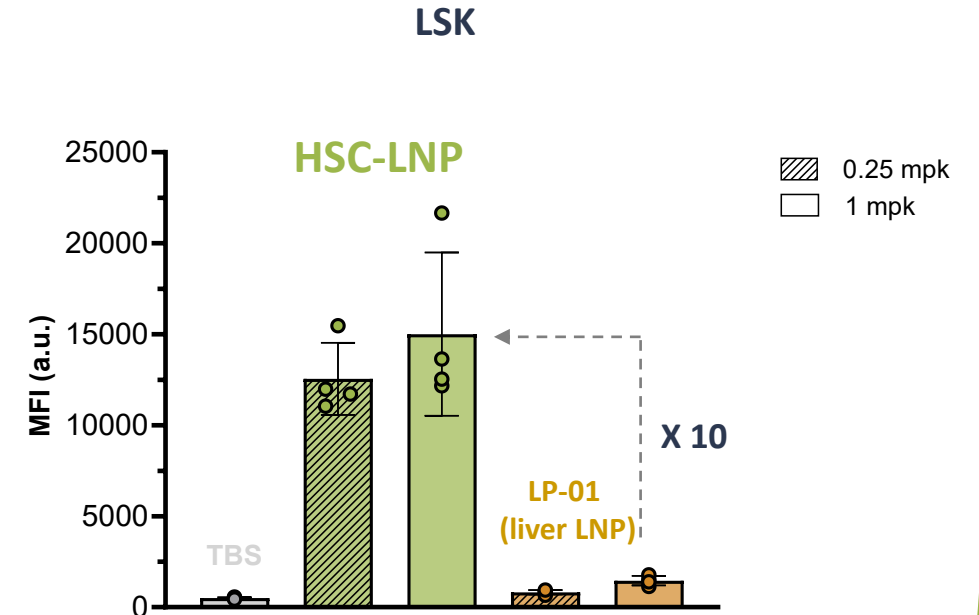
HSC-LNP shows targeted delivery to HSCs in bone marrow & strong drop in liver expression



> 80% delivery in HSCs

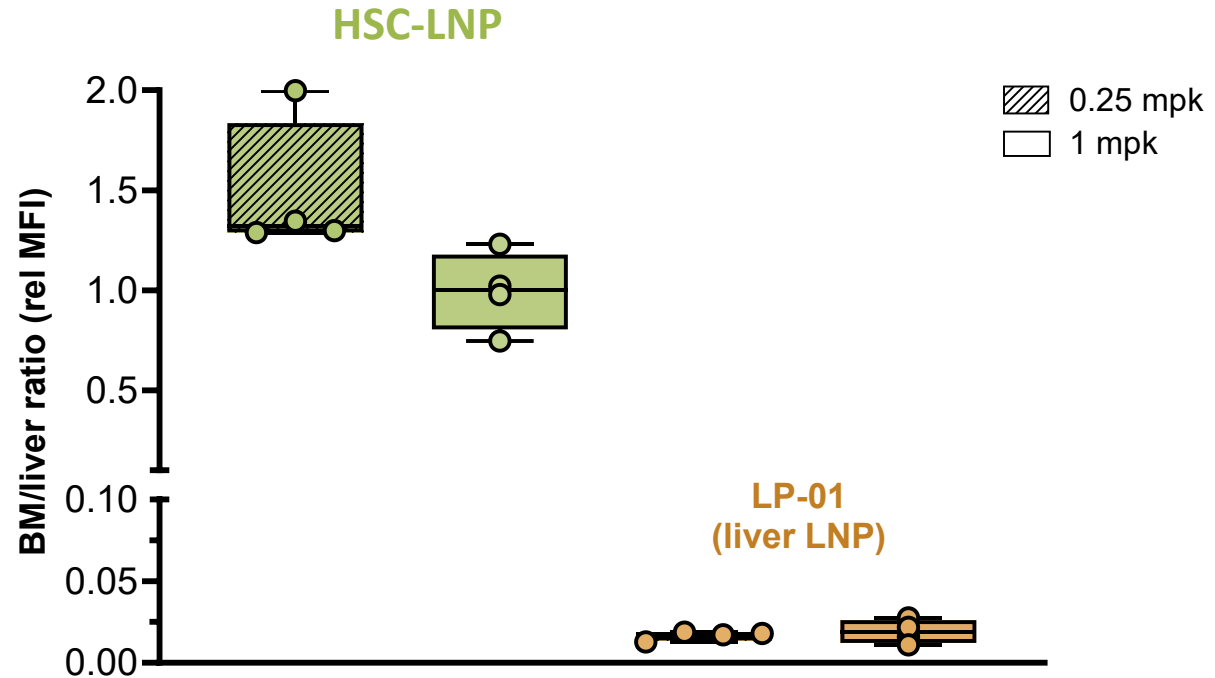
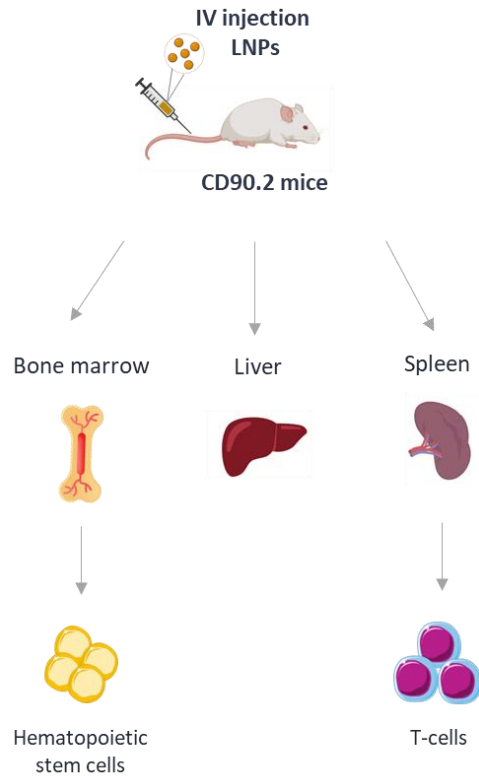


10-fold higher expression in HSC



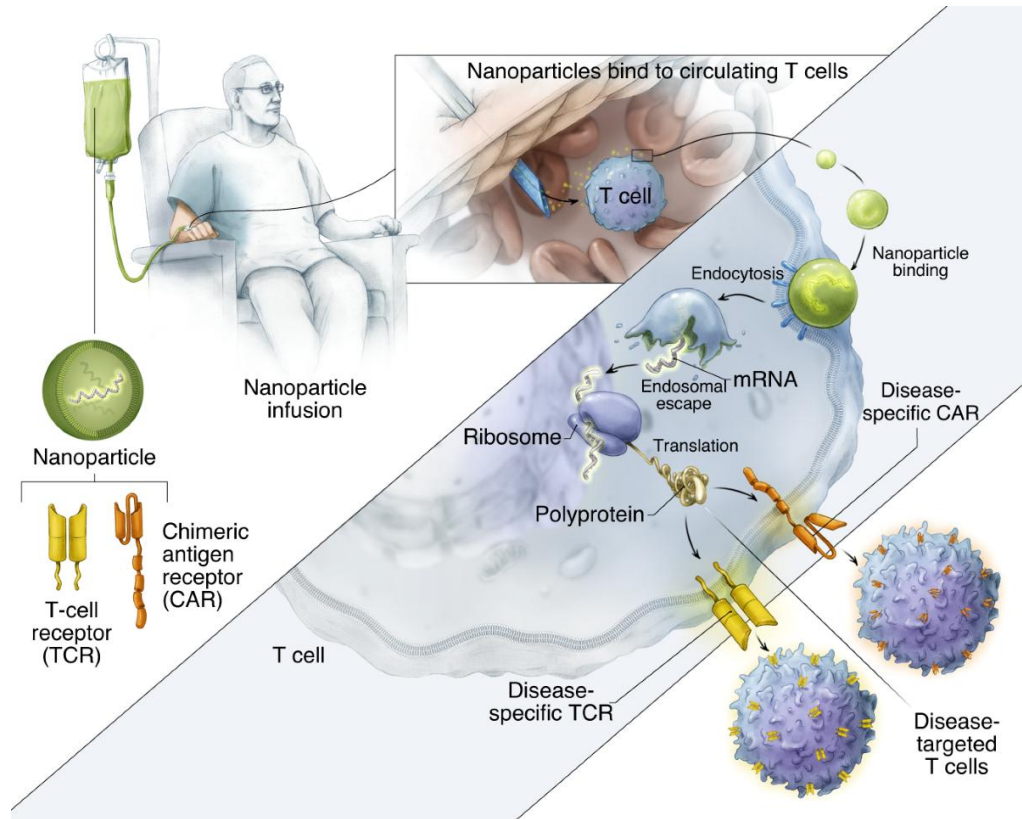
10 times higher % of HSCs/LSK transfected and 10-fold higher expression intensity in HSCs compared to Intellia's LP-01

HSC-LNP shows targeted delivery to HSCs in bone marrow & strong drop in liver expression – HSC-LNP nearly 100-fold better than liver LNP



etherna HSC-LNP combines high expression in HSCs with strong drop in liver expression compared to Intellia's LP-01

LNPs for *in situ* CAR-T cell generation - rationale



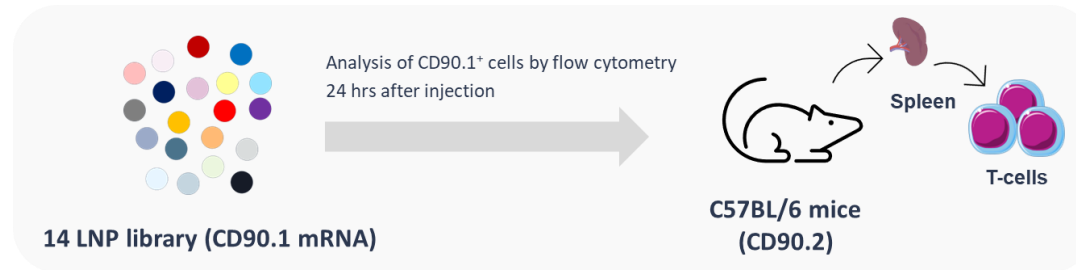
In situ CAR-T cell generation: benefits

- Reduced risk of cytokine release syndrome
- No need for preconditioning lymphodepleting therapies
- Off the shelf: faster & cheaper
- Improved activity

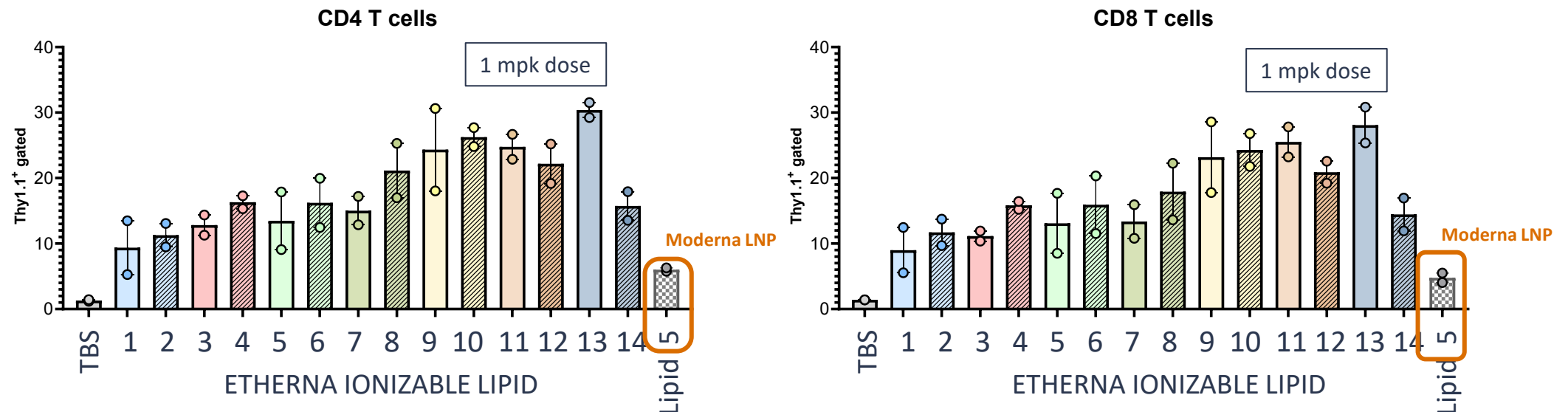
***In situ* CAR-T cell generation – the next frontier**

LNPs for *in vivo* T-cell delivery

Passive targeting

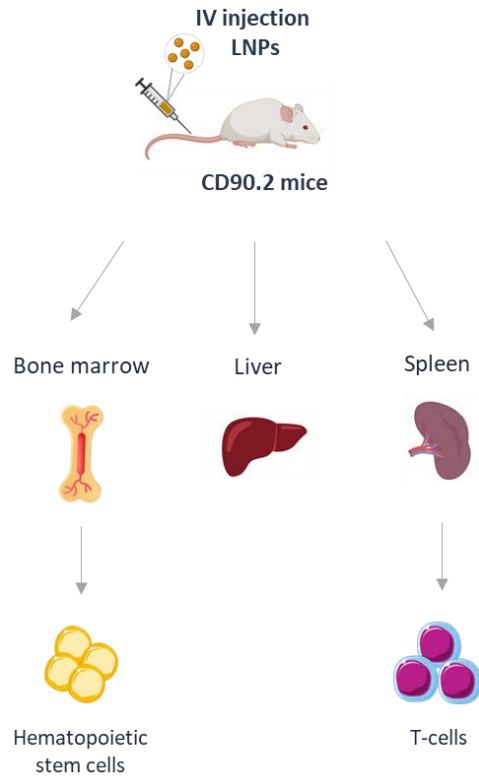


- etherna optimized composition parameters for extrahepatic delivery
- etherna enhanced ionizable lipid chemistries

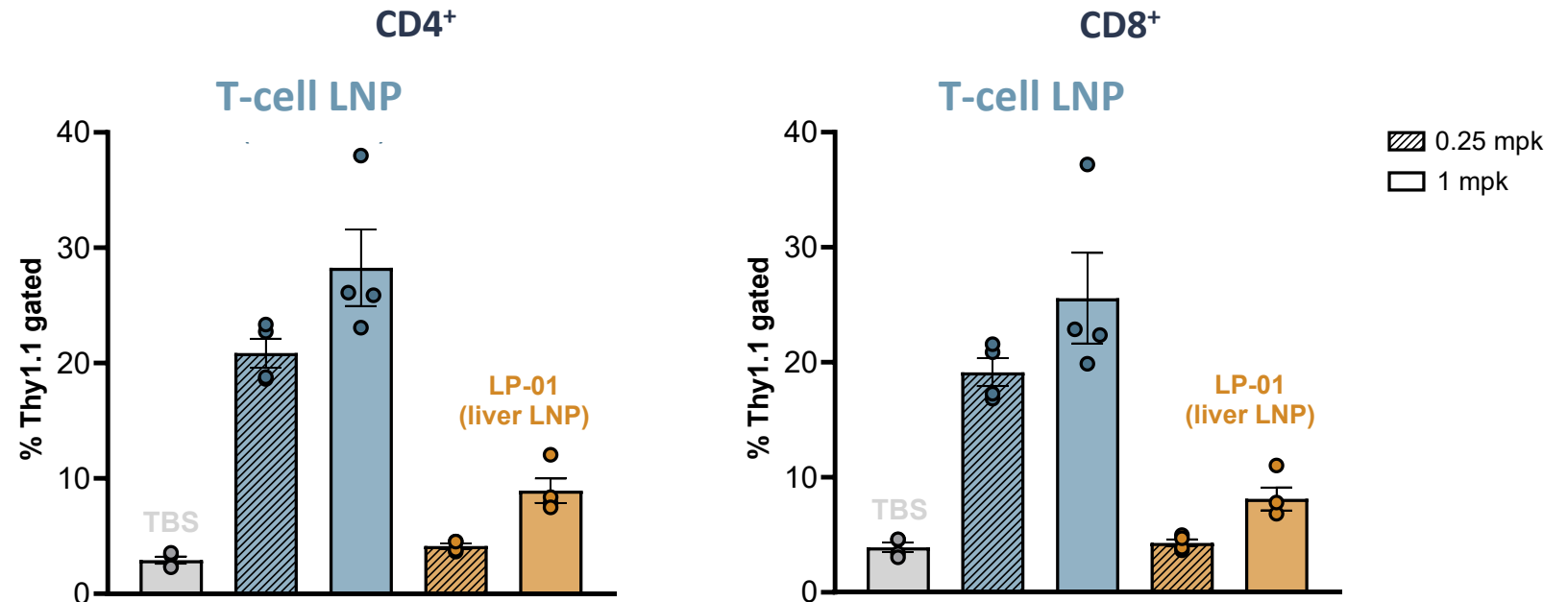


**etherna optimized LNPs enable high *in vivo* transfection of splenic T cells
with superior activity to benchmarks**

Extrahepatic T-cell LNP enables strong delivery to T cells in spleen



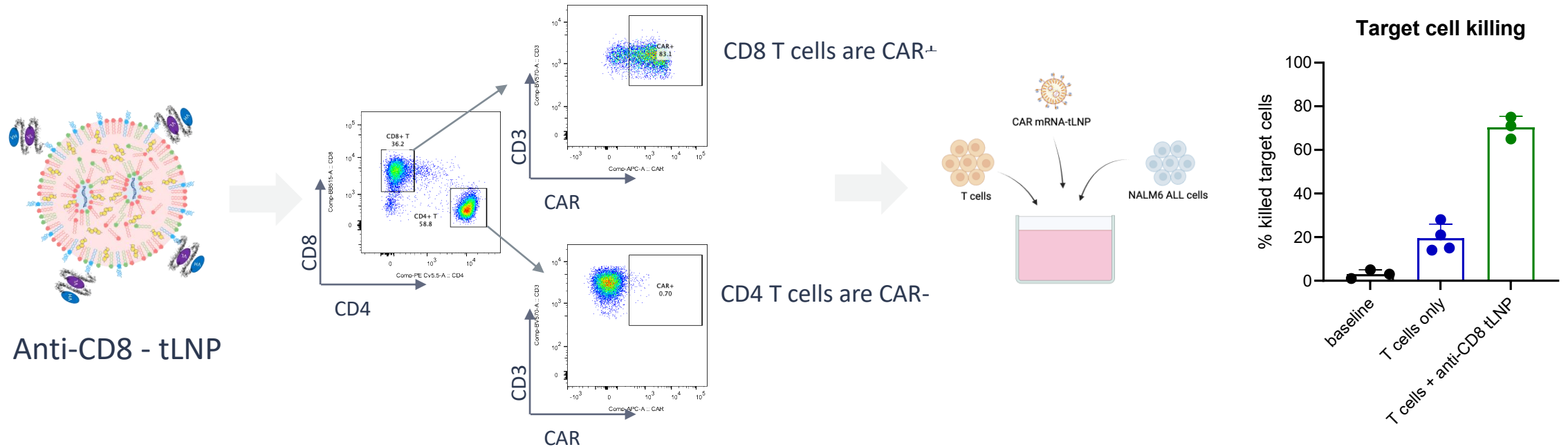
30% delivery to CD4⁺ and CD8⁺ T cells in spleen



etherna's lead T-cell LNP transfects 4-5 times more CD4 and CD8 T cells compared to Intellia's LP-01

Ligand-targeted LNPs for T cell delivery

Active targeting using anti-CD8 decorated LNP

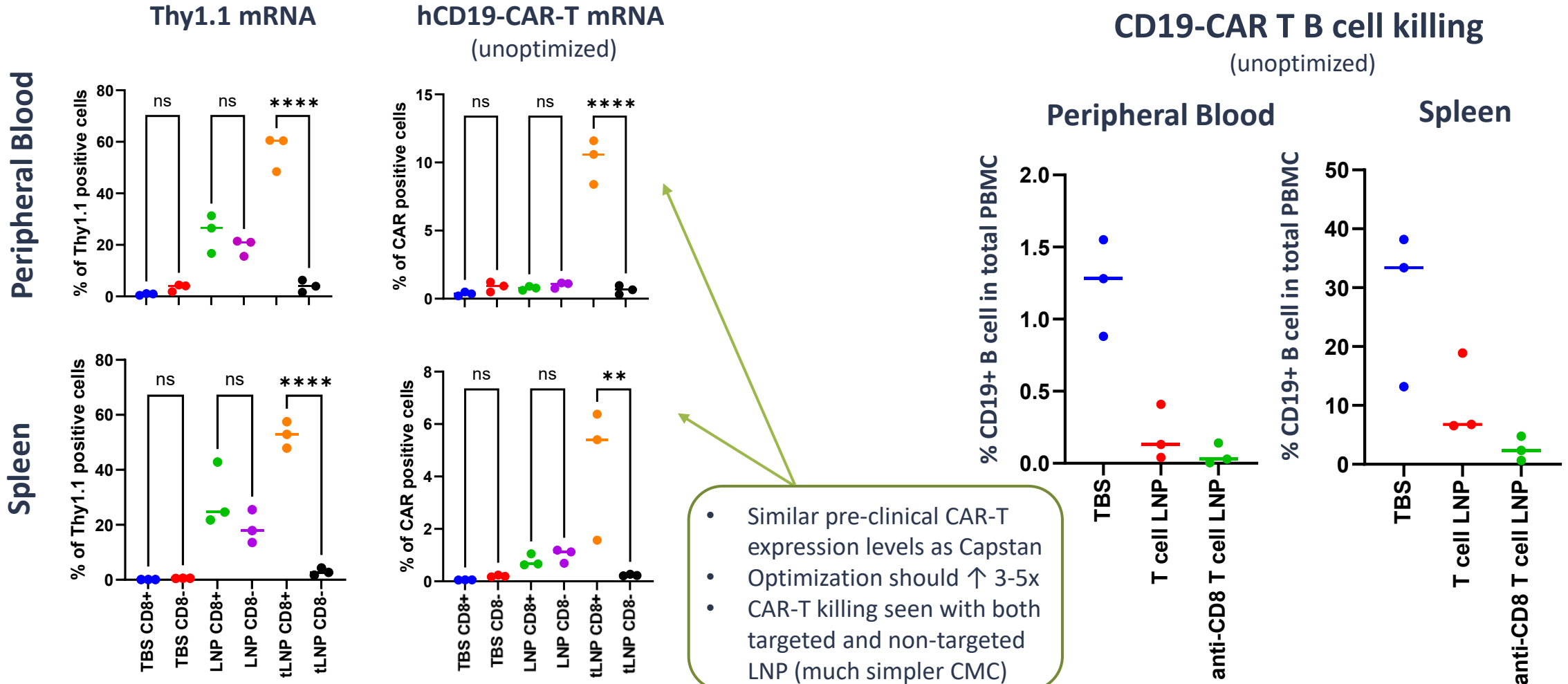


Actively Targeted LNPs:








- Improved conjugation chemistries to enable site specific conjugation of scFv and nanobodies to ensure all ligands are properly oriented at LNP surface
- *In vitro* POC studies with CD8-targeted LNP show strong CAR expression only in primary human CD8 T cells and not CD4
- Generated CAR-T cells are functional at killing target cells
- ***In vivo* studies with actively targeted LNP on-going**

Potent CAR-T activity of etherna T cell-directed LNP in humanized mice

Both passive and active CD8 Ab-based targeting of LNP show robust CAR-T mediated killing *in vivo*



etherna's LNPs: best-in-class profile across multiple therapeutic areas

Therapeutic area	Data highlights
 Prophylactic vaccination	<ul style="list-style-type: none">• Rodent & NHP: Superior antibody titers compared to LNPs used in SpikeVax™ and Comirnaty™• NHP: well-tolerated; no changes in blood chemistry, hematology, no liver damage
 Liver diseases	<ul style="list-style-type: none">• Increased activity (expression & gene editing efficiency) in rodents and NHP compared to Intellia's LP-01• Excellent tolerability in rodents & NHP
 HSC targeting	<ul style="list-style-type: none">• Strong expression of payload of interest in bone marrow HSCs (>90%)• 90% reduction in liver expression
 T cell targeting	<ul style="list-style-type: none">• In vivo: Robust T cell delivery and CAR-T activity with passive and actively targeted LNP in rodent & humanized mice• Ex vivo: high % of T-cell editing (95% TRAC KO) with superior T cell viability to electroporation protocols
 Myeloid cell reprogramming	<ul style="list-style-type: none">• Efficiently enables mRNA expression in macrophages and monocytes and is highly suited for <i>in situ</i> reprogramming of myeloid cells
 Intra-tumoral delivery	<ul style="list-style-type: none">• Lead LNP for IT delivery produces 4x higher IT expression with 20x lower liver expression compared to MC3 LNP• High anti-tumor efficacy in rodent models upon delivery of etherna's immune-stimulatory Triplet mRNA
 Tolerizing / auto-immune disease	<ul style="list-style-type: none">• Tolerizing mRNA vaccine restores immune homeostasis in rodent models of autoimmune disease outperforming the approved medicine fingolimod

ethernä's mission is to be a **technology leader** in the development of **nucleic acid-based medicines** which enables our **partners** to create new therapeutics and improve patients' lives

ethernä

Partner



mRNA



Custom LNPs



Process tech



Cell biology &
pharmacology



mRNA/LNP clinical &
regulatory expertise



Disease biology

Target selection
Indication selection
Disease models
Biomarkers



Clinical expertise

Treatment protocol
Patient selection

Transformational medicines for patients

ethernä



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