



Stanford | Office of Technology Licensing

Nucleic Acid Delivery Platform

June 2025

The RNA/DNA Delivery Problem

Rising Demand for RNA/DNA Therapies

- Vaccines: infectious disease, cancer, animals
- Gene editing with CRISPR
- Protein replacement therapies
- Immunotherapy
- Cell therapies and cell reprogramming
- Rejuvenation

Problems

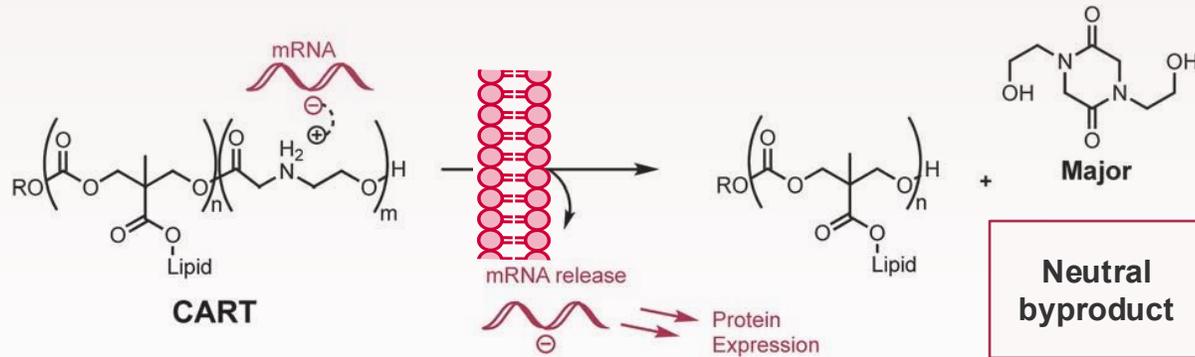
- **For RNA, Delivery is the Bottleneck**
 - Fragile → easily degraded by RNases
 - Large, negatively charged → can't enter cells on their own
- **LNPs Limitations**
 - Inflammation & toxicity at high doses
 - Liver-restricted targeting
 - Complex, hard-to-scale manufacturing
 - Poor repeat dosing profile

What's Needed

- **High transfection efficiency**
 - Efficient intracellular delivery across diverse cell types
- **Tunable organ/cell targeting**
 - Deliver beyond liver — e.g., lung, spleen, eye
- **Biodegradable & non-toxic**
 - Safe degradation, minimal immune response
- **Safe for repeat dosing**
 - No polyethylene glycol, no cumulative toxicity, low immunogenicity
- **Room-temperature stability**
 - Reduces dependence on cold chain for global access
- **Scalable & modular manufacturing**
 - Flexible chemistry adaptable to new therapeutic needs

CARTs: Transient Polymers for Potent Drug Delivery

Charge Altering Releasing Transporters



Mechanism of Action

- Charged polymer → binds negatively charged RNA/DNA
- Self-immolative rearrangement → polymer neutralizes and disassembles
- Releases RNA/DNA into cytosol for translation

Novel Features

- Transient charge → avoids long-term toxicity
- Breaks down into small, neutral byproducts
- Efficient intracellular delivery
- Includes both polymeric and non-polymeric delivery systems
- Adaptable to diverse applications and regulatory needs

CARTS

- Neutral, non-toxic
- Stable on formulation, degradable in administration
- Efficient cytosolic release
- Single-component, easily scalable synthesis

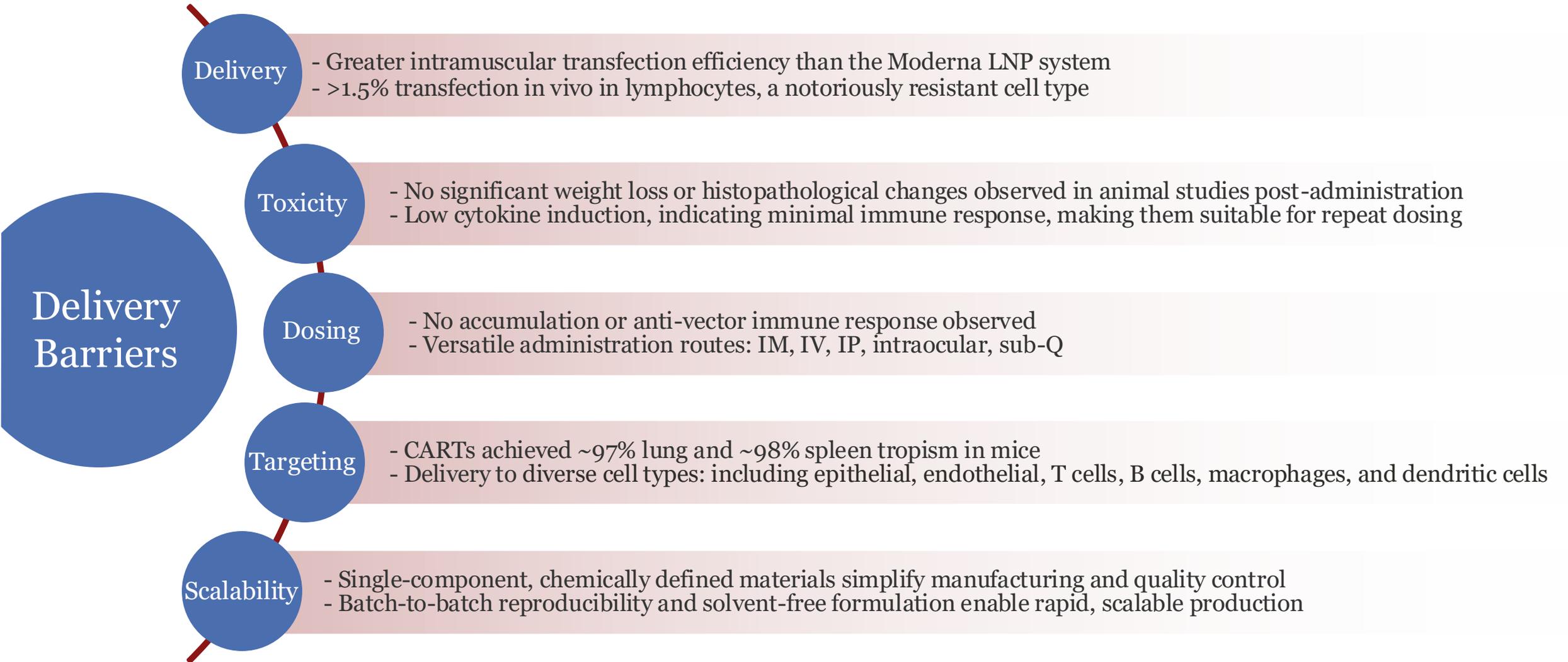
LNPs

- Persistent cationic charge
- Accumulating lipid byproducts
- Endosomal entrapment

Viral Vectors

- Immunogenic capsid proteins
- Risk of insertional mutagenesis
- Scalability challenges

Overcoming RNA/DNA Delivery Barriers with CARTs



Tunable CART Architectures for Targeted Applications

ONA-CARTs

Lymphoid and spleen targeting; immune modulation, curative cancer vaccines, and SARS-CoV2 vaccine

G-CART

High-efficiency delivery in vitro; efficient endosome escape, research applications

K-CART

Lung-selective delivery; respiratory therapy, inhaled vaccines

GSER-CARTs

Tunable lung, spleen, and macrophage targeting; inflammatory disease, cancer, lung disorders

bAC-CARTs

Spleen-selective, targeting lymphocytes and APCs in spleen, efficient T-cell engineering

Fingolimod-CARTs

Drug-modified CARTs for receptor-targeted specific in-vivo delivery (lung/spleen); therapeutic versatility

Branched Lipid CARTs

Improved endosomal escape; versatile platform design

DIGIT-CARTs

Single-component, degradable lipids, batch-consistent alternatives to oligomers; suitable for reg. advancement

O-CART

Efficient lung delivery; potential for differential biodistribution

BAAI-CARTs

Enhanced delivery upon IV admin, tunable spleen, lung and liver selectivity

STAT-CARTs

High transfection in vitro and in vivo; scalable formulation

Targeted Tissue Delivery Enabled by Distinct CART Chemistries

Current Limitation:

- LNPs predominantly accumulate in the liver, limiting targeted delivery

Lysine-Derived CARTs (K-CARTs)*

- >90% selective protein expression in the lungs post-IV injection, without the need for targeting ligands
- Demonstrate no significant toxicity

Glycine-Derived CARTs (G-CARTs)

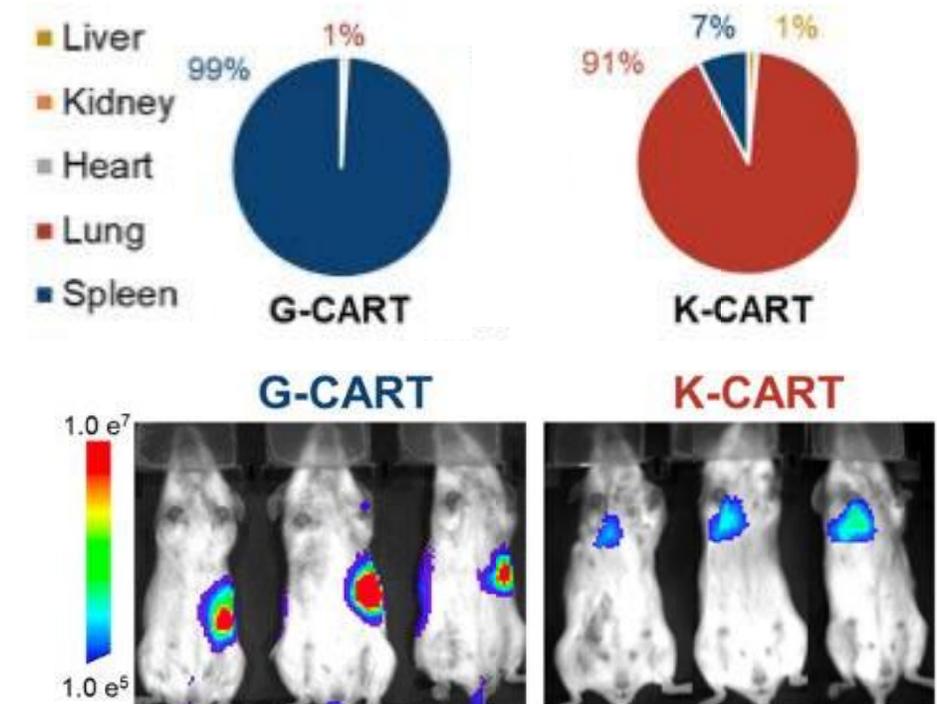
- > 99 % protein expression in spleen post-IV injection

Beta-Amino Carbonate–Backbone CARTs (bAC-CARTs)+

- Up to ~97 % spleen tropism and transfection of ~8 % primary splenic T cells without any targeting ligands

Targeted delivery with or without targeting ligands

- Organ tropism is encoded in CART chemistry, not dependent on external ligands
- Changing lipid composition or polymer architecture redirects delivery to desired organs or cell types



- G-CARTs and K-CARTs are chemically tuned CART variants enabling organ-specific mRNA delivery
- K-CARTs → >90% luciferase expression in lungs
- G-CARTs → preferential delivery to spleen
- Bioluminescence measured 8 hours post IV injection

CARTs Enable Efficient Ex Vivo CAR-NK and CAR-T Cell Engineering

High Transfection Efficiency

- CARTs achieved up to 70% transfection efficiency in primary human T cells*
- Outperformed traditional methods like electroporation and Lipofectamine in delivering mRNA encoding for chimeric antigen receptors (CAR) constructs

Functional CAR-NK and CAR-T Cells

- T cells transfected with CARTs expressing anti-CD19 CARs demonstrated:
 - Increased expression of activation markers
 - Enhanced cytotoxicity, achieving up to 80% killing of CD19+ target cells in co-culture assays

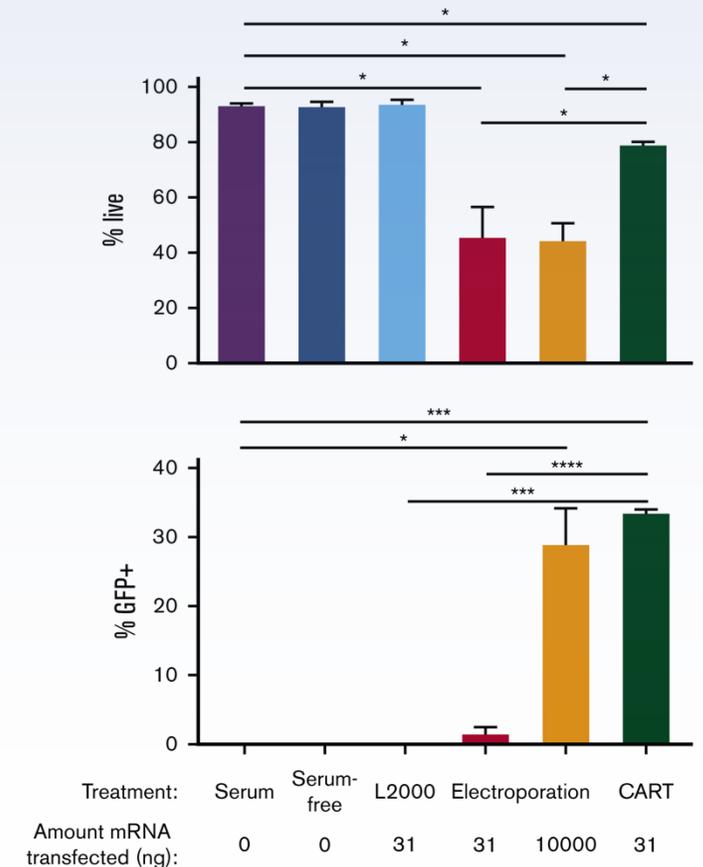
Preserved NK and T Cell Phenotype

- No significant changes in exhaustion markers (e.g., PD-1) post-transfection
- Maintained proliferation capacity and viability comparable to controls

Advantages Over Current Methods

- Simplified protocol without the need for electroporation equipment
- Reduced cytotoxicity, leading to higher yields of functional CAR-NK and CAR-T cells

CARTs outperformed electroporation for mRNA delivery into primary NK cells, preserving NK phenotypes and achieving significantly higher transfection efficiency at low doses⁺



IP Estate

- Stanford holds a diverse and expanding patent portfolio covering multiple CART chemistries and applications
- 16 issued patents across 12 jurisdictions
- Issued patents in major jurisdictions including the U.S., China, Japan, Australia, and Europe
- Leading families like O-CARTs, CART x CAR-T, and Original Recipe CARTs are covered in 8+ countries
- Emerging variants like DIGIT-CARTs, bAC-CARTs, and STAT-CARTs are in earlier stages, offering freedom to operate and license flexibility

The CART technology is available for licensing and sponsored research partnerships to tailor CARTs for specific applications

CART Publications

- ❖ Charge-altering releasable transporters (CARTs) for the delivery and release of mRNA in living animals
- ❖ Functional DNA Delivery Enabled by Lipid-Modified Charge-Altering Releasable Transporters (CARTs)
- ❖ mRNA vaccination with charge-altering releasable transporters elicits human T cell responses and cures established tumors in mice
- ❖ Charge-altering releasable transporters enhance mRNA delivery in vitro and exhibit in vivo tropism
- ❖ Enhanced mRNA delivery into lymphocytes enabled by lipid-varied libraries of charge-altering releasable transporters
- ❖ Charge-altering releasable transporters enable phenotypic manipulation of natural killer cells for cancer immunotherapy
- ❖ Lysine-Derived Charge-Altering Releasable Transporters: Targeted Delivery of mRNA and siRNA to the Lungs
- ❖ Isoprenoid CARTs: In Vitro and In Vivo mRNA Delivery by Charge-Altering Releasable Transporters Functionalized with Archaea-inspired Branched Lipids
- ❖ Organ- and Cell-Selective Delivery of mRNA In Vivo Using Guanidinylated Serinol Charge-Altering Releasable Transporters
- ❖ Local Delivery of *Ox40l*, *Cd80*, and *Cd86* mRNA Kindles Global Anticancer Immunity
- ❖ An mRNA SARS-CoV-2 Vaccine Employing Charge-Altering Releasable Transporters with a TLR-9 Agonist Induces Neutralizing Antibodies and T Cell Memory