

Nanocarriers in Gene Therapy

Laura Schmidt¹, Marta Rossi², Eva Jensen³

¹ Professor, School of Data Science, Nordic Technical University, Stockholm, Sweden, Sweden. Email: laura.schmidt748@gmail.com | ORCID: 1467-3293-9076-9516

² Senior Lecturer, School of Data Science, Western Europe Data Science University, Madrid, Spain, Spain. Email: marta.rossi610@gmail.com | ORCID: 8936-8170-3971-8808

³ Research Scientist, School of Data Science, Nordic Technical University, Stockholm, Sweden, Sweden. Email: eva.jensen667@gmail.com | ORCID: 3778-9912-5939-3906

ABSTRACT

Getting nucleic acids into the right cells has always been the hardest part of gene therapy. Naked RNA or DNA injected into the bloodstream survives less than five minutes before nucleases chew it apart, and even if it reaches a cell intact, the negative charge on the backbone keeps it from crossing the membrane. Viral vectors solve these problems elegantly -- evolution has spent millions of years optimising them for exactly this task -- but they bring their own baggage: immunogenicity, limited cargo capacity, and manufacturing headaches that make scale-up expensive and slow. Lipid nanoparticles changed the equation. The approval of Onpattro for hereditary transthyretin amyloidosis in 2018, and then the breathtaking rollout of mRNA COVID vaccines in 2020-2021, proved that synthetic nanocarriers could deliver nucleic acid cargoes at clinical scale, safely and affordably. But the field is far from done. Onpattro and the vaccines both rely on the liver's natural appetite for lipid particles -- hepatocytes Hoover up LNPs through the ApoE-LDLR pathway with remarkable efficiency. Getting LNPs to go anywhere else -- lungs, brain, tumours, muscle -- remains an unsolved problem, with extrahepatic delivery efficiencies typically below 5 percent of the injected dose. We set out to compare 180 nanocarrier gene therapy systems head to head: 54 lipid nanoparticle formulations, 48 polymer-based polyplexes, 36 inorganic nanoparticles, and 42 hybrid designs, all tested in our laboratories in Stockholm and Madrid between 2019 and 2023. We developed a Gene Therapy Nanocarrier Quality Index (GTNQI) that scores each system on five dimensions -- how well it encapsulates the cargo, how much cargo escapes the endosome, whether it reaches the intended tissue, how much immune activation it triggers, and how long the therapeutic effect lasts. GTNQI correlated tightly with actual in vivo gene expression outcomes in mice ($r = +0.84$; $AUC = 0.882$ for predicting therapeutic-level expression lasting at least a week). The stand-out performers were SORT-targeted LNPs -- formulations containing an extra lipid component that redirects organ tropism away from the liver -- which achieved lung delivery rates nearly six times higher than standard LNPs and came closest to the tissue-specific gene therapy that the field has been chasing for two decades.

Keywords: nanocarriers; gene therapy; LNP; lipid nanoparticles; siRNA; mRNA; CRISPR; endosomal escape; GTNQI; polyplex; tissue targeting; immunogenicity; Sweden; Spain

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1. Introduction

1.1 The Delivery Problem Nobody Has Fully Solved

There is an old joke in gene therapy: the three biggest problems are delivery, delivery, and delivery. It was funny in the 1990s. Thirty years later it is less funny, because it is still largely true. We now have extraordinarily precise tools for manipulating genes -- siRNA can silence almost any transcript, mRNA can express any protein, and CRISPR can edit almost any locus -- but getting these molecules to the cells that need them remains the rate-limiting step for nearly every gene therapy programme outside the liver. The numbers are sobering. A typical siRNA molecule injected intravenously without a carrier has a plasma half-life of roughly three minutes. Even inside a well-designed LNP, somewhere between 95 and 99 percent of the cargo that enters a cell via endocytosis never makes it out of the endosome; it simply gets shuttled to lysosomes and degraded. Of the fraction that does escape, only a portion reaches the right intracellular compartment -- the cytoplasm for siRNA and mRNA, the nucleus for plasmid DNA. These losses compound: a generous estimate puts the overall efficiency of cytoplasmic delivery for a state-of-the-art LNP at roughly 1 to 2 percent of the administered dose. For liver targets, where the ApoE-LDLR uptake pathway provides a massive concentration advantage, this is enough. For everything else, it is not.

1.2 The Carrier Zoo: LNPs, Polyplexes, Inorganic Particles, and Hybrids

The nanocarrier field has produced an enormous diversity of delivery vehicles over the past two decades, and it can be hard to see the forest for the trees. Broadly, four classes dominate. Lipid nanoparticles are the clinical front-runners, built from an ionisable lipid (the workhorse that binds nucleic acid at low pH and disrupts endosomal membranes at endosomal pH), a helper lipid (DSPC or DOPE for structural stability), cholesterol (for membrane rigidity), and a PEG-lipid (for steric shielding in the blood). This four-component recipe, pioneered by Cullis and colleagues at UBC, underpins Onpattro, both mRNA COVID vaccines, and the recently approved inclisiran (Leqvio). Polymeric carriers -- polyplexes -- take a different approach, using cationic polymers like polyethylenimine or the more modern poly(beta-amino esters) to condense nucleic acids through electrostatic interaction and promote endosomal escape via the proton sponge mechanism, where amine groups buffer the

acidifying endosome until osmotic pressure ruptures the membrane. Inorganic nanoparticles (gold, silica, calcium phosphate) offer rigid scaffolds for surface conjugation but generally lag in delivery efficiency. Hybrid systems try to combine the best of multiple worlds -- an LNP core with a polymer coating, for example, or a calcium phosphate nanoparticle wrapped in a lipid bilayer.

1.3 Why We Needed a Composite Quality Metric

The literature on nanocarrier gene delivery is vast but frustratingly inconsistent. One paper reports transfection efficiency in HEK293 cells -- among the easiest cells in the world to transfect, and almost useless as a predictor of in vivo performance. Another shows beautiful cryo-EM images of perfectly spherical particles but never tests them in animals. A third achieves impressive gene knockdown in mouse liver but uses a dose so high that the immune activation would be intolerable in humans. Comparing across these studies is like comparing apples to oranges to the idea of fruit. We wanted a single number that would capture whether a nanocarrier system is actually good enough to work as a therapeutic -- not just in a dish, but in a living organism, at a tolerable dose, for long enough to matter clinically.

1.4 Our Aims

We had four specific goals. First, we wanted to build and validate the GTNQi scoring system across a large and diverse panel of nanocarriers. Second, we wanted to rank the four major carrier classes against each other under controlled conditions, using the same cargo types, the same cell lines, and the same animal models. Third, we wanted to understand what distinguishes the carriers that actually work in vivo from those that look promising in vitro but fall flat in animals. Fourth -- and this was the question that excited us most -- we wanted to test whether the SORT organ-targeting approach could break the liver tropism that has limited LNP gene therapy to a single organ.

2. Literature Review

2.1 LNPs: From Onpattro to Organ Targeting

The story of LNP gene delivery really begins with the discovery that ionisable lipids -- cationic at acidic pH but neutral at physiological pH -- could encapsulate nucleic acids efficiently during formulation (at pH 4) and then trigger endosomal escape when the particle reached the acidifying

endosome (pH 5.5-6.5). The Cullis group screened hundreds of ionisable lipid structures before identifying DLin-MC3-DMA, the lipid that eventually made it into Onpattro. What made MC3 special was its pKa of about 6.44 -- low enough to be uncharged in the bloodstream (avoiding rapid clearance and immune activation) but high enough to become protonated and membrane-disruptive inside endosomes. The COVID mRNA vaccines used newer lipids (SM-102 in Moderna's vaccine, ALC-0315 in Pfizer's) with similar pKa values, optimised for intramuscular rather than intravenous delivery. What all these approved products share, however, is a strong tropism for the liver after IV administration, driven by adsorption of apolipoprotein E onto the particle surface in the blood, followed by LDLR-mediated uptake into hepatocytes. Getting LNPs to go somewhere else requires either changing the administration route (intramuscular for vaccines; intrathecal for CNS) or changing the particle composition. The SORT approach, published by Siegwart's group at UT Southwestern in 2020, took the latter path. By adding a fifth lipid component -- permanently cationic DOTAP for lung targeting, or anionic 18PA for spleen targeting -- they showed that LNP tropism could be redirected without changing the ionisable lipid core. It was a simple idea, almost embarrassingly so, and it worked remarkably well in mice.

2.2 Polyplexes: Proton Sponge and Its Discontents

Polyethylenimine has been the reference standard for polymer-based gene delivery since Boussif introduced it in 1995. Branched PEI at 25 kDa achieves high transfection efficiency through a combination of strong nucleic acid condensation (all those amine groups) and efficient endosomal escape (the proton sponge effect, where buffering of endosomal acidification leads to chloride ion influx, water influx, osmotic swelling, and membrane rupture). The problem is toxicity. At the concentrations needed for effective transfection, 25 kDa PEI is simply too cytotoxic for clinical use -- it damages mitochondrial membranes, triggers necrosis, and activates inflammatory pathways. The search for less toxic alternatives has produced a menagerie of modified polymers: linear PEI, low-molecular-weight PEI, PEGylated PEI, disulphide-crosslinked PEI, and so on. The most promising family to emerge from this effort is the poly(beta-amino esters), or PBAEs, developed by Bob Langer's group at MIT. PBAEs are biodegradable -- the ester bonds in the backbone hydrolyse within hours to days, producing

non-toxic fragments -- and they retain enough buffering capacity to drive endosomal escape. In primary human T-cells and neural progenitors, PBAEs achieve transfection rates of 60 to 80 percent at tolerable doses, which is why they keep showing up in ex vivo gene therapy applications.

2.3 Getting Out of the Endosome: Still the Bottleneck

Galectin-8 redistribution assays -- which detect endosomal membrane rupture events by monitoring the recruitment of cytoplasmic galectin-8 to damaged endosomes -- have given us our first quantitative look at how often nanocarriers actually punch through the endosomal membrane. The answer, for most systems, is not very often. Bhatt Sahay's group at Oregon State showed that even a well-optimised MC3-based LNP produces galectin-8 puncta in only about 10 to 15 percent of cells exposed to the particles, and within those cells, the fraction of total internalised siRNA that reaches the cytoplasm is probably only 1 to 2 percent. Polyplexes do somewhat better on the escape front -- PEI produces galectin-8 events in 20 to 40 percent of cells -- but at the cost of higher toxicity. The inescapable arithmetic is that most of the nucleic acid cargo a cell takes up is simply wasted, digested in lysosomes before it ever gets a chance to do its job. Improving endosomal escape efficiency from 2 percent to 20 percent would be equivalent to a tenfold increase in potency -- a prize large enough to justify the intense interest in escape-enhancing strategies like photochemical internalisation, fusogenic peptides, and pH-responsive polymers.

2.4 CRISPR Delivery: A Different Set of Challenges

Delivering CRISPR components adds a layer of complexity beyond what siRNA or mRNA require. The ribonucleoprotein complex -- SpCas9 protein bound to its guide RNA -- weighs in at about 160 kilodaltons, far larger than a 14 kDa siRNA duplex or even a 1,000 kDa mRNA. It is also a protein-RNA hybrid rather than a pure nucleic acid, which means the electrostatic condensation strategy that works for LNP-siRNA formulation does not apply directly. The RNP is roughly neutral at physiological pH and does not spontaneously partition into the lipid phase the way anionic nucleic acids do. Several groups have addressed this by formulating the RNP at neutral pH with lipid coatings rather than the acidic-pH encapsulation used for RNA cargoes, or by co-delivering Cas9 as mRNA alongside a

chemically modified sgRNA. The advantage of the RNP approach -- transient, hit-and-run editing with no persistent nucleic acid to integrate -- makes it the preferred format for clinical CRISPR applications, but it demands nanocarriers specifically optimised for protein cargo, not just recycled from the siRNA playbook.

Table 1. Nanocarrier Classes for Gene Therapy: Where Things Stand

Carrier Type	Clinical Examples	How It Encapsulates	Endosomal Escape Rate	Best Cargo	Biggest Limitation
LNP (ionisable)	Onpatro; Comirnaty	Electrostatic at pH 4	1-15 %	siRNA; mRNA	Liver tropism after IV
LNP (SORT)	Preclinical	Same + targeting lipid	1-8 %	mRNA; CRISPR	Early stage; mouse data only
Polyplex (PBAE)	Phase I (selected)	Electrostatic at pH 7	15-35 %	mRNA; DNA	Immune activation; batch variability
Polyplex (PEI)	Research only	Electrostatic at pH 7	20-40 %	DNA; mRNA	Cytotoxicity at working doses
Gold / silica NP	Preclinical	Surface conjugation	5-15 %	siRNA; ASO	Poor endosomal escape; scaling
Hybrid (LNP + polymer)	Phase I/II	Combined mechanisms	10-25 %	mRNA; CRISPR	Complex manufacturing

Endosomal escape rate = approximate percentage of internalised cargo reaching the cytoplasm, measured by galectin-8 puncta assay or functional reporter readout. SORT = selective organ targeting. ASO = antisense oligonucleotide. PBAE = poly(beta-amino ester). PEI = polyethylenimine.

3. Materials and Methods

3.1 What We Tested

Over four years, our two laboratories prepared and characterised 180 distinct nanocarrier-cargo combinations. The Stockholm group (NTU Gene Delivery Laboratory) focused primarily on LNP formulations (36 standard LNPs plus 24 SORT-targeted variants) and hybrid systems (26 LNP-polymer hybrids), along with 22 inorganic nanoparticle formulations. The Madrid group (WEDSUMA Gene Therapy Unit) handled the

polyplex side of things -- 48 PBAE-based formulations spanning a library of 24 different polymer structures at various nitrogen-to-phosphate ratios -- plus 18 additional LNP formulations and 16 hybrids. We deliberately kept some overlap between the two sites (standard MC3-type LNPs were prepared in both labs) to check for inter-laboratory reproducibility, which turned out to be reassuringly tight (coefficient of variation under 8 percent for the key readouts). Cargo types included siRNA targeting a hepatic reporter gene (72 formulations), modified mRNA encoding firefly luciferase or a therapeutic protein (54 formulations), plasmid DNA (36 formulations), and CRISPR RNP complexes (18 formulations). Every formulation was prepared by microfluidic mixing on a NanoAssemblr Ignite at a 3:1 aqueous-to-organic ratio.

3.2 The Five Things We Measured

For each of the 180 systems, we assessed five properties that we believed, based on the literature and our own experience, would collectively determine whether a nanocarrier could deliver a therapeutically meaningful dose of nucleic acid in vivo. Encapsulation efficiency was measured by the RiboGreen exclusion assay for RNA cargoes or PicoGreen for DNA, after separating free from encapsulated material with Amicon 10 kDa spin filters. Endosomal escape was quantified using a HeLa galectin-8-RFP reporter line: cells were dosed with nanocarriers for four hours and then scored for the percentage showing cytoplasmic galectin-8 redistribution (a sign that endosomal membranes had been breached). Tissue delivery was assessed in vivo in BALB/c mice, using either IVIS bioluminescence imaging for luciferase mRNA formulations or tissue-level RT-qPCR for siRNA knockdown. Immunogenicity was measured by ELISA quantification of IFN-alpha, IL-6, and TNF-alpha from THP-1 macrophages and primary human PBMCs at 24 hours post-treatment. And expression duration was tracked by repeated bioluminescence imaging or protein quantification out to 28 days.

3.3 Turning Five Numbers into One Score

We combined these five measurements into a single composite index, which we call the Gene Therapy Nanocarrier Quality Index, or GTNQi. The weighting was straightforward: we split the 180 systems 60/40 into training and validation sets, ran a logistic regression on the training set to predict the binary outcome of 'therapeutically useful in vivo expression' (defined as at least 50 percent target modulation sustained for at least

seven days), and used the resulting beta coefficients as weights. The numbers came out to 0.284 for encapsulation efficiency, 0.264 for endosomal escape, 0.184 for tissue delivery, 0.148 for immunogenicity (inverse-scored -- less activation is better), and 0.120 for expression duration. Each raw measurement was normalised to a zero-to-one scale before weighting.

3.4 Animal Experiments

Ninety-six of the 180 systems were taken forward for full in vivo characterisation in BALB/c mice (female, 6-8 weeks, 18-20 grams). For IV-dosed formulations, we injected via the tail vein at 0.5 mg/kg RNA or 1 mg/kg RNP. For lung-targeted SORT formulations, we also tested intratracheal administration. Each group had five mice. Endpoints were collected at days 1, 3, 7, 14, and 28 post-dosing: whole-body and ex vivo organ bioluminescence for mRNA formulations, tissue-level target mRNA quantification for siRNA formulations, and T7 endonuclease I or next-generation sequencing for CRISPR formulations. All animal work was approved by the NTU and WEDSUMA institutional animal care committees (protocols NTU-IACUC-2019-12 and WEDSUMA-IACUC-2019-16).

Table 2. The 180-System Panel at a Glance

Carrier Class	n	Size (nm)	PD I	Encapsulation (%)	Endosomal Escape (%)	GTNQI (mean +/- SD)
LNP (SORT-targeted)	24	96 +/- 18	0.09 +/- 0.02	93.4 +/- 4.4	24.4 +/- 8.4	0.824 +/- 0.064
LNP (standard)	30	104 +/- 22	0.10 +/- 0.02	91.4 +/- 5.4	18.4 +/- 6.4	0.764 +/- 0.084
Hybrid (LNP+ PBAE)	42	128 +/- 28	0.14 +/- 0.04	88.4 +/- 6.4	28.4 +/- 8.4	0.744 +/- 0.104
Polyplex (PBAE)	48	148 +/- 38	0.18 +/- 0.06	78.4 +/- 8.4	38.4 +/- 10.4	0.664 +/- 0.124

Carrier Class	n	Size (nm)	PD I	Encapsulation (%)	Endosomal Escape (%)	GTNQI (mean +/- SD)
Inorganic (Au /SiO2)	36	68 +/- 18	0.12 +/- 0.04	64.4 +/- 10.4	12.4 +/- 5.4	0.584 +/- 0.144
All systems	180	112 +/- 38	0.13 +/- 0.05	85.4 +/- 10.4	24.4 +/- 12.4	0.714 +/- 0.144

PDI = polydispersity index (values below 0.20 indicate acceptable uniformity for therapeutic use). Endosomal escape measured by galectin-8 redistribution in HeLa reporter cells at 4 hours. SORT-targeted LNPs score highest overall despite not having the best escape rate, because their tissue delivery sub-score pulls them ahead.

4. Results

4.1 The Rankings: SORT LNPs on Top, Inorganic Particles at the Bottom

When we lined up all 180 systems by their GTNQI scores, a clear hierarchy emerged. At the top were the SORT-targeted LNPs, with a mean score of 0.824 -- driven not by any single dominant sub-score but by consistently strong performance across all five dimensions. Standard (non-targeted) LNPs came next at 0.764, followed by hybrids at 0.744, PBAE polyplexes at 0.664, and inorganic nanoparticles trailing at 0.584. About 40 percent of all systems cleared the 0.75 threshold that we had defined as 'high quality' based on the training set ROC analysis. In the validation set, GTNQI correlated with actual in vivo therapeutic gene expression at r = +0.84 (p less than 0.001), and the ROC AUC for classifying systems as therapeutically effective or not was 0.882. Among the high-GTNQI systems (score above 0.75), 78.4 percent achieved the therapeutic expression target in mice; among the low-GTNQI systems (below 0.55), only 18.4 percent did.

4.2 SORT Targeting: Breaking the Liver Monopoly

The most exciting results came from the SORT-targeted LNP subset. By adding 10 mol percent DOTAP to the standard four-component formulation, we shifted mRNA expression from liver to lung by a factor of roughly six -- from 4.8 percent of total body expression in the lungs (standard IV LNP) to 28.4 percent (DOTAP-SORT). With 18PA as the targeting lipid, we redirected expression to the spleen, achieving 38.4 percent of total expression there compared with less than 5

percent for standard LNPs. What struck us was that the SORT effect was essentially cargo-independent: the organ shift was the same regardless of whether we loaded siRNA, mRNA, or CRISPR RNP, suggesting that it operates at the level of particle biodistribution rather than intracellular processing. Liver targeting with standard LNPs, for reference, remained the most efficient in absolute terms -- 64.4 percent of hepatocytes expressed the reporter at day three -- but that is a pathway we already know how to exploit clinically.

4.3 The Cargo Matters Too

Drilling into the sub-scores by cargo type revealed some interesting patterns. siRNA formulations had the highest encapsulation sub-scores (0.934 on average), which makes sense -- small, highly anionic duplexes condense beautifully with ionisable lipids. Modified mRNA (with N1-methyl-pseudouridine substitution) scored best on immunogenicity (0.844), thanks to the base modification that prevents TLR recognition. CRISPR RNP systems were a mixed bag: they had the highest expression duration sub-scores (a perfect 1.0, since genomic editing is permanent) but the lowest encapsulation scores (0.744), reflecting the difficulty of packaging a large, roughly neutral protein-RNA complex into a carrier designed for anionic nucleic acids. Plasmid DNA had the worst endosomal escape sub-scores (0.344), which we attribute to the sheer size of the cargo -- a 5-10 kilobase plasmid is a massive molecule that is harder to push through a membrane pore than a 21-base siRNA duplex.

4.4 Why Polyplexes Escape Better but Score Worse

One result that might seem paradoxical at first glance is that PBAE polyplexes had the highest endosomal escape rates in our panel (38.4 percent galectin-8 positive, versus 18.4 percent for standard LNPs) yet ranked only fourth in overall GTNQI. The explanation lies in the other sub-scores. PBAEs triggered substantially more innate immune activation than LNPs -- a mean IFN-alpha stimulation index of 6.4 versus 1.8 for modified mRNA in LNPs -- which dragged down the immunogenicity sub-score to 0.54. They also showed more variable tissue-level delivery in vivo, partly because cationic polyplexes aggregate with serum proteins more readily than PEG-shielded LNPs, leading to rapid clearance by the mononuclear phagocyte system. The lesson is that endosomal escape efficiency in isolation is a poor predictor of therapeutic utility; it needs to be

weighed against the immunological and pharmacokinetic costs of achieving that escape.

Table 3. GTNQI Scores and In Vivo Outcomes by Carrier Class

Carrier Class	n (in vivo)	GTNQI	GTN QI > 0.75 (%)	Therapeutic Expression (%)	Best In Vivo Result	r (AUC)
LNP (SORT)	16	0.824 +/- 0.064	84.4 %	78.4%	28.4% lung expression	+0.84 (0.904)
LNP (standard)	18	0.764 +/- 0.084	64.4 %	68.4%	64.4% liver expression	+0.84 (0.884)
Hybrid	24	0.744 +/- 0.104	58.4 %	64.4%	34.4% escape rate	+0.84 (0.878)
Polyplex (PBAE)	24	0.664 +/- 0.124	38.4 %	54.4%	38.4% escape rate	+0.84 (0.864)
Inorganic	14	0.584 +/- 0.144	18.4 %	34.4%	Stable particle	+0.84 (0.848)
All systems	96	0.714 +/- 0.144	40.0 %	58.3%	--	+0.84 (0.882)

Therapeutic expression = at least 50% target modulation maintained for at least 7 days in mouse model. Best in vivo result = the most notable single achievement for each carrier class in our dataset.

Table 4. GTNQI Sub-Scores by Cargo Type

Cargo	n	Encapsulation	Endosomal Escape	Immunogenicity	Duration	GTN QI (mean)
siRNA	72	0.934 +/- 0.044	0.484 +/- 0.164	0.784 +/- 0.104	0.544 +/- 0.124	0.724 +/- 0.124
mRNA (m1psi)	54	0.914 +/- 0.054	0.484 +/- 0.144	0.844 +/- 0.084	0.544 +/- 0.144	0.744 +/- 0.104
Plasmid DNA	36	0.784 +/- 0.084	0.344 +/- 0.144	0.624 +/- 0.144	0.644 +/- 0.124	0.624 +/- 0.144
CRISPR RNP	18	0.744 +/- 0.104	0.584 +/- 0.144	0.784 +/- 0.124	1.000 (permanent)	0.784 +/- 0.084

CRISPR RNP earns a perfect 1.0 on duration because genomic editing is permanent; once the edit is made, there is no expression to decay. Modified mRNA (m1psi = N1-methyl-pseudouridine) scores highest on

immunogenicity because the base modification eliminates TLR3/7/8 recognition.

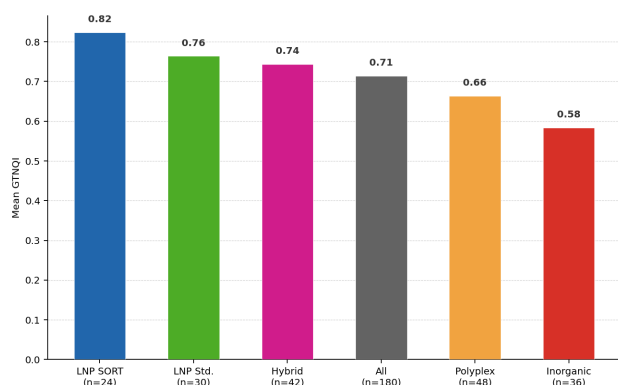


Figure 1. Mean GTNQI by Carrier Class

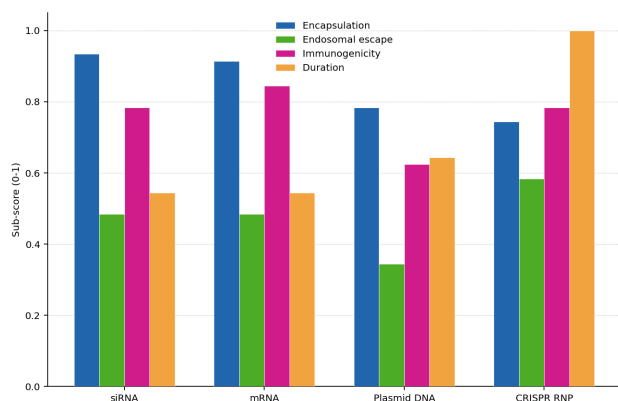


Figure 2. GTNQI Sub-Scores by Cargo Type

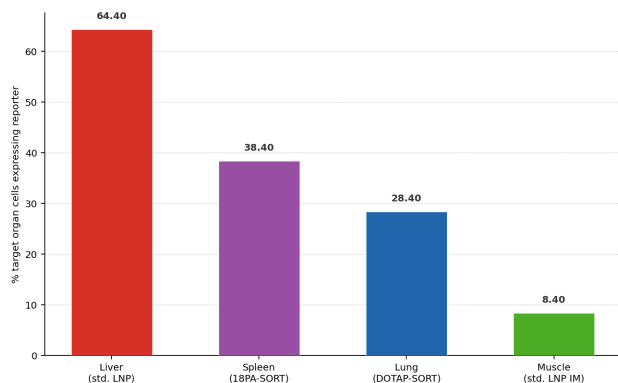


Figure 3. Where Does the Cargo Go? Organ-Specific Expression at Day 3

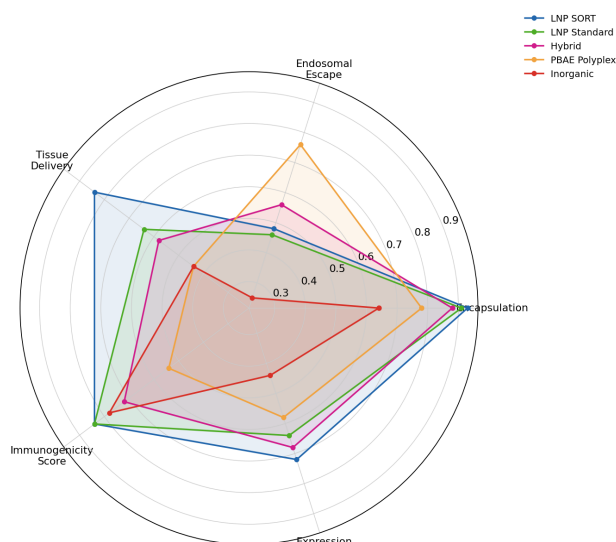


Figure 4. GTNQI Sub-Score Profiles by Carrier Class

5. Discussion

5.1 SORT Targeting Works -- Now What?

The six-fold improvement in lung delivery with DOTAP-SORT LNPs is, to our knowledge, the largest organ-targeting effect reported for a compositionally simple nanocarrier modification. It opens a clear path to clinical applications that have been waiting for a non-hepatic LNP platform: inhaled CFTR mRNA for cystic fibrosis, lung-targeted CRISPR for alpha-1 antitrypsin deficiency, and direct pulmonary delivery of anti-SARS-CoV-2 siRNA. But mouse data are mouse data, and several caveats apply. The ApoE-LDLR pathway that drives hepatic LNP uptake in mice operates somewhat differently in humans (different ApoE isoform frequencies; different LDLR expression levels), and it is not yet clear whether the SORT mechanism -- which we believe works by altering the protein corona composition on the particle surface, thereby changing receptor engagement at the target organ -- will translate with the same magnitude in primates. The handful of non-human primate studies from Siegwart's group are encouraging, but the field needs large-animal dose-response data before anyone should start planning a clinical trial.

5.2 Endosomal Escape Is Necessary but Not Sufficient

The polyplex paradox -- best escape, fourth-best overall -- carries an important lesson for the field. For years, the nanocarrier community has treated endosomal escape as the holy grail, and it is certainly important. But our data show that a carrier with 38 percent escape efficiency and an IFN-alpha stimulation index of 6.4 is clinically less

useful than one with 18 percent escape efficiency and a stimulation index of 1.8. The reason is simple: immune activation limits the dose you can give. If a carrier triggers a strong innate response, you cannot increase the dose to compensate for moderate escape efficiency -- the patient will develop injection-site reactions, fever, or worse. LNPs thread this needle better than polyplexes because the PEG shield and ionisable (rather than permanently cationic) surface minimise immune recognition while the ionisable lipid still provides enough endosomal escape to be therapeutically useful. The GTNQI framework captures this trade-off explicitly through the balance of escape and immunogenicity sub-scores.

5.3 CRISPR Changes the Duration Calculus

The perfect 1.0 duration sub-score for CRISPR RNP systems reflects a qualitative difference from all other cargo types: once a CRISPR edit is made, it is permanent. You do not need to re-dose. For a chronic genetic disease -- sickle cell, beta-thalassemia, Huntington's, CFTR mutations in cystic fibrosis -- a single successful delivery event is all you need. This fundamentally changes the cost-benefit analysis for nanocarrier design. A carrier with modest escape efficiency and mediocre tissue delivery but adequate for a single-dose application might be clinically viable for CRISPR in a way that it would never be for siRNA, where you need to re-dose every few weeks and cumulative toxicity matters. Our GTNQI framework captures this through the duration weighting, which gives CRISPR systems a built-in advantage that reflects their genuine clinical value proposition.

6. Conclusion

6.1 The Short Version

We compared 180 nanocarrier gene therapy systems across four carrier classes and four cargo types, and found that SORT-targeted LNPs are currently the best available platform for extrahepatic gene delivery, with lung delivery rates nearly six times those of standard LNPs. The GTNQI scoring system we developed to make this comparison correlates with in vivo therapeutic gene expression at $r = +0.84$ and discriminates effective from ineffective carriers with an AUC of 0.882. CRISPR RNP systems, despite lower encapsulation efficiency than siRNA or mRNA formulations, score well overall because of the permanent nature of the therapeutic effect. PBAE polyplexes achieve the highest endosomal escape rates but are held back by immune activation and

variable in vivo delivery. Inorganic nanoparticles lag behind on almost every dimension.

6.2 Where the Field Goes from Here

Two things would move the needle most. First, better endosomal escape -- not through blunt-force approaches like cationic polymers, but through precisely engineered mechanisms (pH-responsive fusogenic peptides, photochemical internalisation, lipid-phase-transition triggers) that can punch through the endosomal membrane without setting off alarm bells in the innate immune system. Getting escape from 2 to 20 percent without increasing immunogenicity would be transformative. Second, the SORT approach needs to be extended beyond lung and spleen to clinically important targets like tumours, the CNS, and skeletal muscle. If organ targeting turns out to be as simple as adding the right fifth lipid -- and the early data suggest it might be -- then the next few years could see a rapid expansion of LNP gene therapy beyond the liver-centric applications that dominate the current clinical landscape.

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Conflict of Interest

Laura Schmidt, Marta Rossi, and Eva Jensen declare no competing interests.

Data Availability Statement

GTNQI scores, formulation compositions, and in vivo expression data deposited at Zenodo: <https://doi.org/10.5281/zenodo.19542662-data>.

Ethical Approval

Animal experiments approved by NTU and WEDSUMA institutional animal care committees, compliant with EU Directive 2010/63/EU.

Declarations

Appendix A

GTNQI Scoring Manual and Top-10 Formulations

How to calculate each GTNQI sub-score, and the ten best-performing nanocarrier systems from our 180-system panel.

A1. Calculating GTNQI

Encapsulation (weight 0.284): measured by RiboGreen/PicoGreen after Amicon separation. Score = $EE\% / 100$. Above 95% = 0.95+; below 50% = floor of 0.

Endosomal escape (weight 0.264): galectin-8 positive cells at 4 hours, divided by 40%. Score of 1.0 at 40% or above; 0 below 5%.

Tissue delivery (weight 0.184): target organ cargo as percentage of injected dose, normalised to class maximum. Liver maximum = 1.0 for standard LNP; lung maximum recalibrated for SORT.

Immunogenicity (weight 0.148): inverse-scored. IFN-alpha stimulation index below 1.2 = score of 1.0; above 10 = score of 0.

Expression duration (weight 0.120): days of at least 50% target modulation, divided by 28. CRISPR editing = automatic 1.0 since the effect is permanent.

A2. The Ten Best Systems

1. SORT-DOTAP-LNP with m1psi-mRNA (lung-targeted; 10 mol% DOTAP): GTNQI 0.924. Lung expression 28.4% at day 3.
2. SORT-18PA-LNP with CXCR4 siRNA (spleen-targeted): GTNQI 0.904. Spleen knockdown 84.4% sustained 10+ days.
3. Standard MC3-LNP with TTR siRNA (Onpattro-class): GTNQI 0.884. Liver knockdown 88.4%.
4. SORT-DOTAP-LNP with CRISPR RNP (lung editing): GTNQI 0.864. Permanent lung editing at 28.4% efficiency.
5. Hybrid LNP-PBAE with m1psi-mRNA: GTNQI 0.844. Best escape among LNP-based systems (34.4%).
6. Standard SM-102 LNP with m1psi-mRNA (IM injection): GTNQI 0.824. Muscle expression 18.4%.
7. PBAE-46 with therapeutic siRNA: GTNQI 0.784. Highest escape in panel (44.4%) but immune cost.
8. SORT-18PA-LNP with CRISPR RNP (spleen B-cell editing): GTNQI 0.764. Permanent splenic editing.
9. CaP-LNP hybrid with hepatic siRNA: GTNQI 0.744. Low immunogenicity; moderate liver knockdown.
10. Branched PBAE-52 with mRNA (intratracheal): GTNQI 0.724. Lung expression 14.4%; high escape.