

OVERCOMING THE DELIVERY BOTTLENECK:

Can Existing Drug Delivery Technologies Solve CRISPR-Cas9's Biggest Limitation in Treating Inherited Disease?

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INTRODUCTION

- Inherited diseases result from DNA mutations passed from parent to offspring.
- Four categories: single-gene defects, chromosomal defects, mitochondrial disorders, multifactorial disorders.
- Traditional treatments manage symptoms (bandage approach); gene editing offers a potential permanent correction.
- CRISPR-Cas9 is the leading gene editing technology and can correct genome errors or turn genes on/off (Redman et al., 2016).
- Natural CRISPR = bacterial immune system (adaptation, expression, interference) (Hossain, 2021; Karimi et al., 2026; Redman et al., 2016).

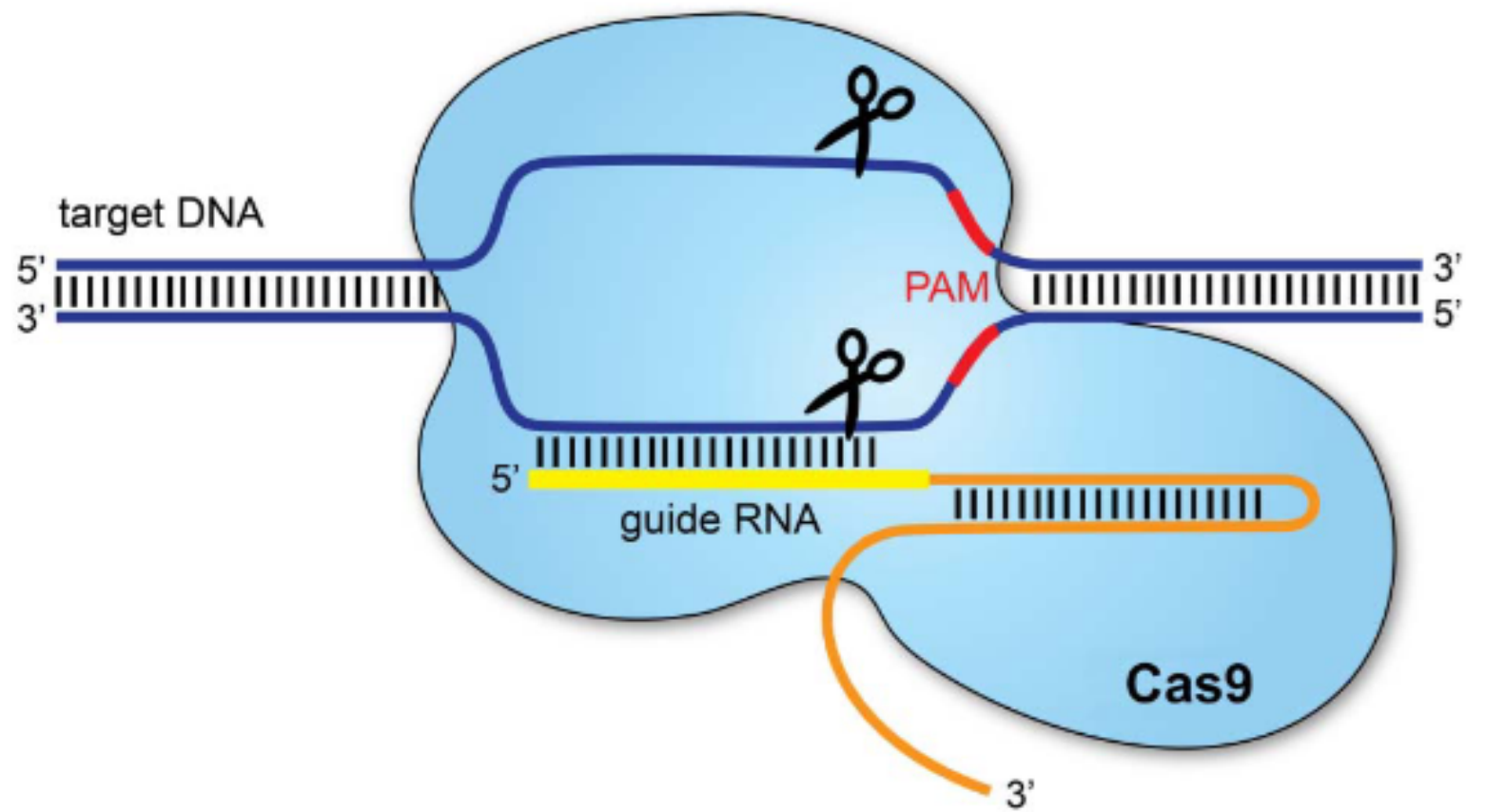


FIGURE 1. THE CRISPR/CAS9 SYSTEM. ADAPTED FROM REDMAN ET AL. (2016), ARCHIVES OF DISEASE IN CHILDHOOD, EDUCATION AND PRACTICE EDITION, 101(4), 213–215. LICENSED UNDER CC BY 4.0.

Modern applications bypass adaptation by introducing synthetic guide RNA (sgRNA) with Cas9 enzyme. Cas9 is guided to its DNA target by a sgRNA, which recognizes a sequence adjacent to a protospacer adjacent motif (PAM). Once bound, Cas9 cuts both DNA strands, creating a double-strand break. The cell's own repair machinery then fixes the break either disrupting the gene (NHEJ) or precisely correcting it using a donor template (HDR).

OBJECTIVE

The editing mechanism is well studied; delivery solutions are often discussed only briefly in the literature (based on review of Hossain, 2021; Karimi et al., 2026; Redman et al., 2016).

Objective: Connect CRISPR-Cas9's specific delivery/safety limitations to recent, non-CRISPR-specific drug delivery advances (mainly LNP engineering) to evaluate how much of the 'delivery problem' is already being solved (Chen et al., 2025; Gillmore et al., 2021; Song et al., 2024).

METHODOLOGY

- Narrative literature review.
- Databases: PubMed, PMC, Google Scholar.
- Inclusion:** peer-reviewed, 2015–2026 (preference 2020–2026), English, CRISPR/inherited disease/delivery focus.
- Exclusion:** conference abstracts, non-peer-reviewed sources, opinion pieces, unrelated studies, inaccessible full text.

REFERENCES

THE CORE PROBLEM: DELIVERY

- Main clinical barrier = efficient cellular delivery, not the editing mechanism itself (Karimi et al., 2026).
- Cas9 cDNA ~ 4.2 kb size; with regulatory elements, it approaches/exceeds the ~4.7 kb AAV packaging limit (Redman et al., 2016).
- Leaves little room for donor DNA or regulatory sequences; viral delivery (AAV) is size-constrained (Redman et al., 2016).
- Three delivery modalities: physical, electrical and carrier-based (lipid nanoparticles, engineered viral vectors) (Karimi et al., 2026).

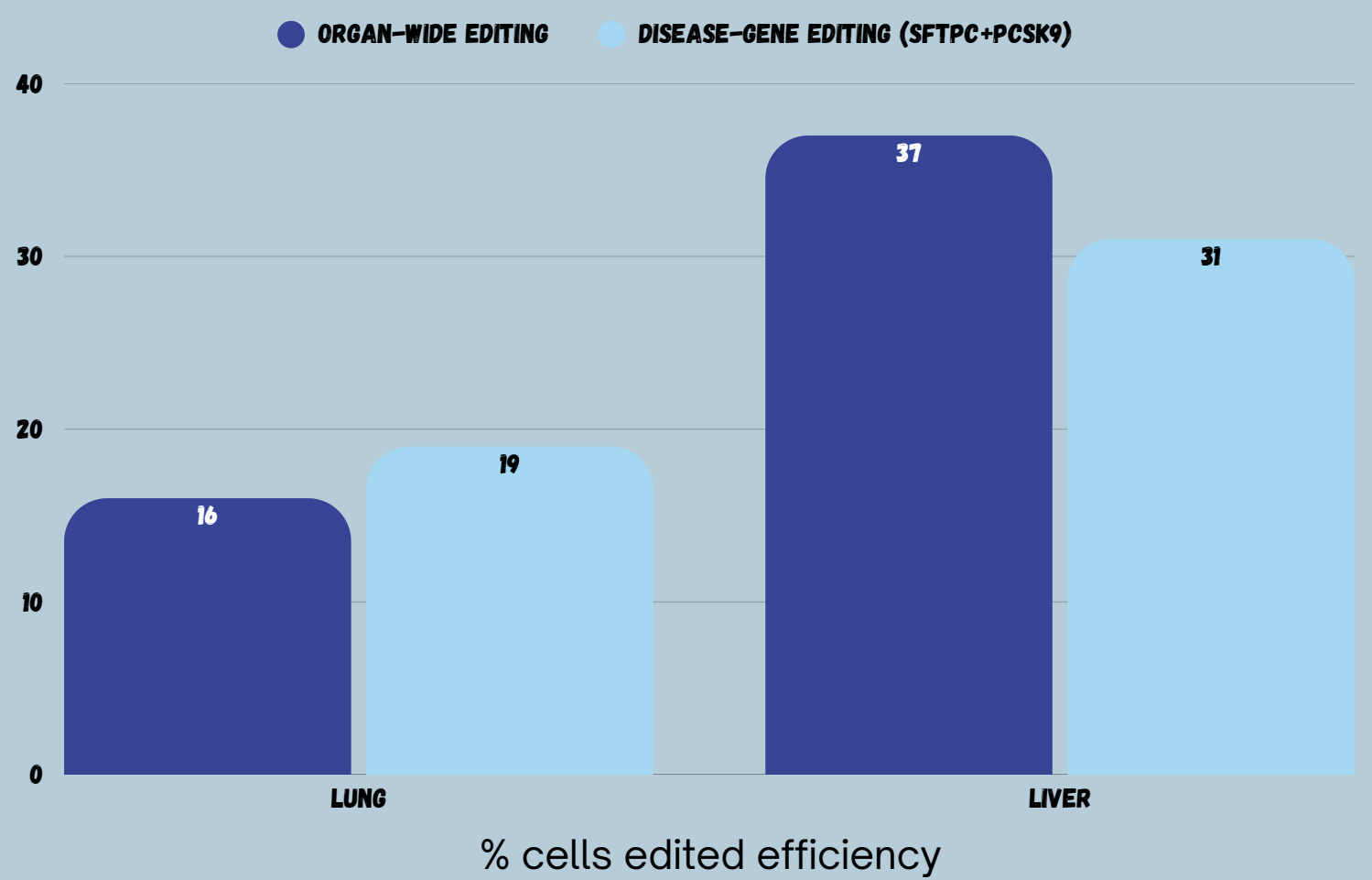
ETHICAL & SAFETY CONCERNS

- Germline editing:** irreversible, heritable changes raise concerns about genetic determinism, inequality, discrimination (Karimi et al., 2026; Redman et al., 2016).
- Off-target cleavage:** unintended edits can cause gene disruption, chromosomal rearrangements, cancer-linked mutations (Karimi et al., 2026; Redman et al., 2016).
- Delivery format directly affects off-target risk: mRNA keeps producing Cas9 longer (higher risk); RNP degrades quickly (lower risk) (Chen et al., 2025).

KEY FINDINGS

A. Engineered RNP-LNP Delivery (Chen et al., 2025)

- Problem solved: Cas9 denatures during LNP assembly; RNPs don't encapsulate well; prior work mostly liver-only
- Solution: heat-stable GeoCas9, improved via directed evolution; iGeoCas9, 100x more efficient editing.
- Delivered as RNP (not mRNA); breaks down fast; lower off-target risk
- No detectable immune response; minimal off-target editing



In Vivo Results in Mice

Single IV Injection Achieved: organ-wide editing shown in graph.

Therapeutic Target Validation: the formulations also edited real disease genes, confirming they work on actual therapeutic targets, not just reporter genes.

B. Human Clinical Validation (Gillmore et al., 2021)

- Phase 1 trial, hereditary transthyretin (ATTR) amyloidosis
- LNP carrying Cas9 mRNA + guide RNA targeting TTR gene (contrast to RNP approach above)
- ApoE protein coating naturally targets LNP to the liver; no viral vector needed (Gillmore et al., 2021; Song et al., 2024).
- Result: Dose-dependent; only mild adverse events
- First published human evidence that LNP-delivered CRISPR is safe and effective

Patients	single IV dose each	TTR Reduction (Day 28)
3	0.1 mg/kg	52%
3	0.3 mg/kg	87%

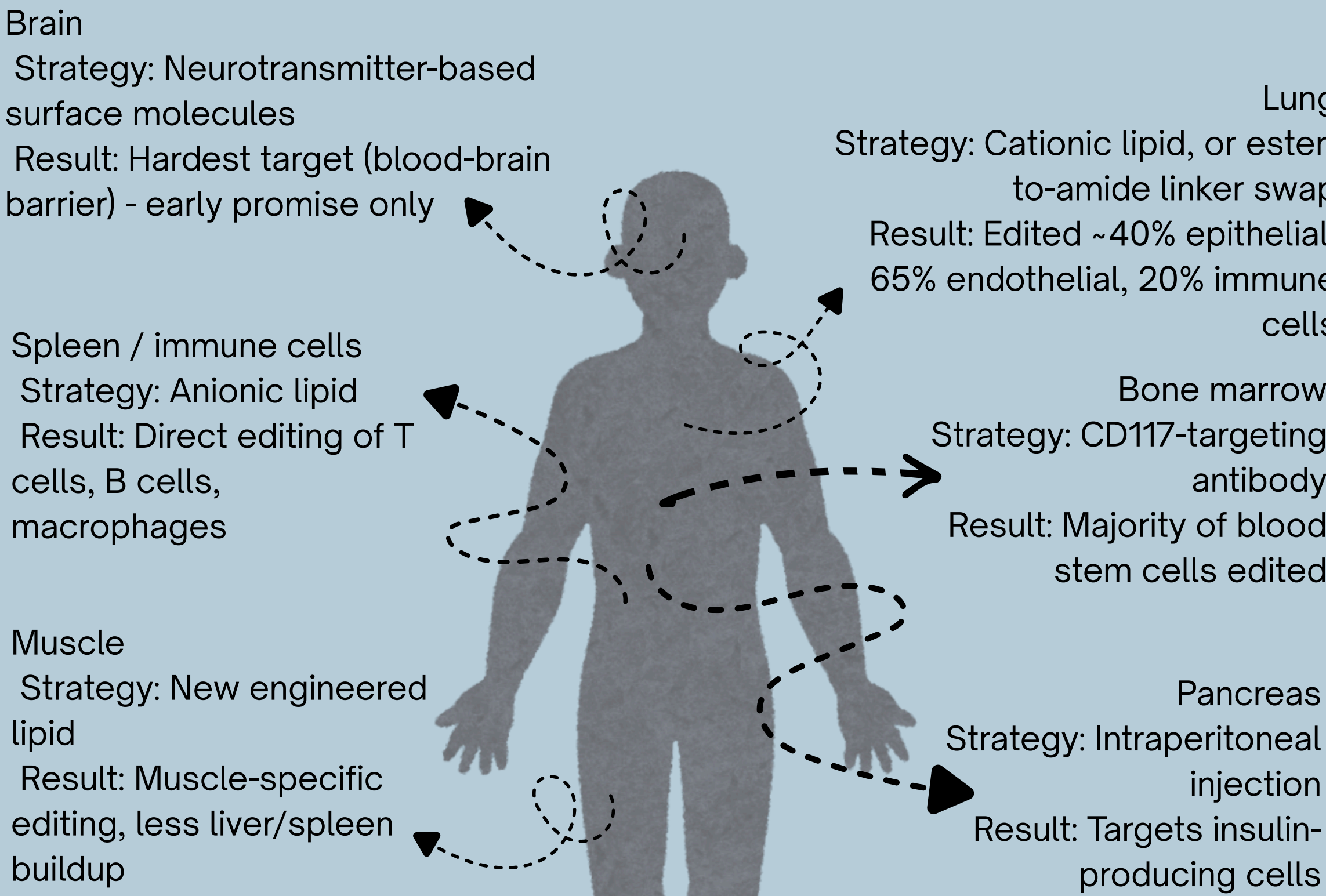
C. Extending Delivery Beyond the Liver (Song et al., 2024)

- Problem: LNPs naturally accumulate in the liver regardless of design
- Why: liver macrophages clear particles; leaky liver blood vessels trap them; slow blood flow increases liver exposure; ApoE coating pulls LNPs into liver cells
- Key concept: biomolecular corona, blood proteins that coat the LNP determine which organ it targets, not just the LNP's own chemistry

Charge-Dependent Targeting:

Surface Charge	Protein Attracted	Target Organ
Slightly acidic	Specific protein	Spleen
Moderate	ApoE	Liver
More basic	Vitronectin	Lung

Organ-Specific LNP Formulations



CONCLUSION

CRISPR-Cas9's limitation isn't editing precision; it's delivery (Karimi et al., 2026).Existing (non-CRISPR-specific) drug delivery science, especially LNP engineering, is actively closing this gap:

- More stable Cas9 variants survive LNP packaging (Chen et al., 2025).
- Human trials confirm LNP-CRISPR is safe and effective (Gillmore et al., 2021).
- Tunable LNP surface chemistry extends editing beyond the liver to lung, spleen, bone marrow, pancreas, and muscle (Song et al., 2024).

Germline-editing ethics remain unresolved and separate from the delivery question (Karimi et al., 2026; Redman et al., 2016).

Takeaway: delivery technology, not the CRISPR mechanism itself, is now the main lever determining how soon CRISPR-Cas9 becomes a practical therapy for inherited disease.

CRISPR-Cas9 delivered by advanced LNP systems has the potential to permanently correct inherited diseases, fundamentally transforming medicine from symptom management to genetic cure.