vaccines

Review

From mRNA Vaccines to *In Vivo* CAR-T Therapy: RNA-LNP Design and the Pharmacology of CAR-Positive Cells

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Abstract

mRNA vaccines established scalable manufacturing and lipid nanoparticle (LNP) delivery for sequence-defined, non-integrating RNA medicines. In vivo CAR-T therapy repurposes this platform to generate CAR-positive lymphocytes after intravenous dosing, but its active product is a time-varying cell population rather than the injected RNA mass. This review critically examines the pharmacological sequence from biodistribution and cell-specific uptake to cytosolic RNA release, surface CAR expression, effector function, and disease response. The evidence comprises primary human T cells, mice, humanized mice, non-human primates, and 11 patients described in one correspondence and two meeting abstracts (as of July 2026). Matched studies show that receptor internalization can outweigh surface abundance, ligand density follows a non-monotonic relationship with delivery, and lipid chemistry and ligand presentation independently alter productive T-cell transfection. Preclinical models demonstrate transient CAR expression and target-cell depletion, whereas current human reports establish feasibility but not durable efficacy, a dose–response relationship, or the incidence of uncommon toxicities. We therefore distinguish administered dose, cell-resolved CAR-positive-cell exposure, and pharmacodynamic response, and propose their joint measurement as the basis for comparing platforms and selecting repeat-dose regimens.

Keywords: mRNA; lipid nanoparticle; *in-vivo* CAR-T; T-cell targeting; cellular exposure

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1. Introduction

mRNA vaccines established scalable *in-vitro* transcription, nucleoside modification, purification, and LNP formulation for cytosolic protein expression [1–4]. *In-vivo* CAR-T uses this manufacturing platform for a different pharmacological objective: intravenous delivery, productive RNA release, and transient CAR expression in selected lymphocytes rather than intramuscular antigen expression [1,2,4–13].

A chimeric antigen receptor (CAR) couples recognition of an intact surface antigen to T-cell activation. The single-chain variable fragment (scFv) defines antigen, affinity, and epitope, whereas the hinge, transmembrane, costimulatory, and CD3ζ modules govern receptor geometry, stability, and signaling [12–17]. Recognition is independent of major histocompatibility complex (MHC) presentation, but CAR-T cells remain vulnerable to antigen loss, lineage switching, poor tissue access, and local immunosuppression [14–16].

Multiple molecular properties of CAR RNA jointly determine translation and innate sensing [1,3,18,19]; these properties include cap structure, untranslated regions, coding sequence, poly(A) tail, nucleoside chemistry, RNA integrity, and residual double-stranded RNA. Because a soluble reporter can be expressed without correct receptor assembly, productive delivery requires cell-resolved measurement of intact RNA, surface CAR density and duration, phenotype, and antigen-specific function [10–13].

Ex-vivo CAR-T provides the clinical benchmark and safety context. The U.S. Food and Drug Administration (FDA) lists seven autologous CD19- or B-cell maturation antigen (BCMA)-directed products, including obecabtagene autoleucel [20,21]. These products established efficacy in B-cell leukemias, lymphomas, and multiple myeloma, while defining cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), cytopenias, infections, prolonged B-cell or plasma-cell depletion, and delayed adverse events [20–23]. In 2024, the FDA required a class-wide boxed warning for secondary T-cell malignancies and lifelong monitoring after approved BCMA- or CD19-directed autologous products [24]. Transient RNA avoids genomic integration, but this distinction does not eliminate risks from off-target expression, prolonged immune-cell depletion, or repeated dosing.

Ex-vivo manufacture delivers a released cell lot with measured identity, number, phenotype, and potency. *In-vivo* delivery instead creates the cellular product after dosing through a serial chain: lymphocyte exposure, receptor binding and internalization, endosomal escape, cytosolic RNA release, translation, receptor assembly, and antigen-dependent function [1–3,5–13,25]. Failure at any step can produce clinical nonresponse but requires a different corrective strategy.

The central translational variable is CAR-positive-cell exposure: the number, phenotype, receptor density, anatomical distribution, and persistence of CAR-positive cells generated by a defined dose [10–13,26–31]. Neither RNA mass nor organ-level signal reports this exposure.

This review uses that exposure framework to compare *ex-vivo* controls with post-dose cellular pharmacology; assess linear mRNA, circular RNA, targeting ligands, and LNP chemistry; and examine evidence from primary cells to early human reports. Alternative RNA formats and effector cells are included only when they address a defined limitation. Distinct from prior descriptive reviews summarizing technical platforms, this review emphasizes a pharmacology-oriented conceptual framework of CAR-positive-cell exposure, stratified evidence grading, and practical translational decision-making criteria.

We classify evidence by the claim it can support: cell-resolved CAR measurement after systemic dosing constitutes direct *in-vivo* evidence; *ex-vivo* CAR-mRNA and reporter studies are enabling evidence; trial registries show development activity; and patient reports provide early clinical evidence. We do not treat organ exposure, cellular engineering, pharmacodynamic activity, and clinical benefit as interchangeable outcomes.

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Throughout the manuscript, the terms '*in-vivo* CAR-T', '*in-vivo* CAR-T therapy', and 'systemic RNA-LNP CAR-T' all refer to systemic intravenous administration of lipid nanoparticle (LNP) formulated RNA to generate chimeric antigen receptor (CAR)-expressing lymphocytes within the patient, as distinct from *ex-vivo* manufactured CAR-T cell products, while '*in-vivo* CAR-T cells' broadly describes any cell population successfully expressing CAR transgene, and '*in-vivo* CAR-T generation' specifically denotes the biological process by which host cells are transfected for induction of CAR expression after administration of RNA-LNP formulations.

Narrative Review Methodology

This narrative review searched PubMed and Embase using the terms '*in-vivo* CAR-T', 'mRNA-LNP', and 'lipid nanoparticle CAR-T'. Clinical trial records were retrieved from ClinicalTrials.gov; the last search was performed on 15 July 2026. Peer-reviewed articles, meeting abstracts, preprints and registry entries reporting LNP-mRNA-mediated *in-vivo* CAR-T were included. Studies without LNP-mediated CAR mRNA delivery were excluded.

Patient-level records from HN2301 (n = 5), GT801 (n = 3) and STR-P004 (n = 3) were cross-checked by trial ID, eligibility criteria and treatment regimen; no overlapping subjects were identified. Registry entries were assigned to platforms based on NCT number, CAR target, RNA format, and delivery carrier.

2. From *Ex-Vivo* Manufacturing to In-Vivo Cellular Pharmacology

2.1. Controls Built into Ex-Vivo Manufacturing

Autologous manufacture comprises leukapheresis, T-cell enrichment, activation, genetic modification, expansion, formulation, testing, transport, and reinfusion. Before infusion, the manufacturer measures identity, purity, viability, sterility, potency, and stability [20,21,23,26]. These controls define a countable, phenotyped, and functionally tested cell population.

Long vein-to-vein times delay treatment in rapidly progressive disease, and prior therapy and disease state alter starting material. Manufacturing failure, specialized infrastructure, transport, and cost restrict access [10,11,14,26,27]. Allogeneic products decouple manufacture from the patient but remain *ex-vivo* products and introduce graft rejection, limited persistence, and genome-editing challenges [10,15,26]. *In-vivo* development must map each ex-vivo release criterion to either retained drug-product release testing, preclinical characterization, or post-dose in-patient measurement (Table 1). *Ex-vivo* CAR-T products benefit from well-established product characterization, predefined potency testing, precise infused cell-dose control, *ex-vivo* expansion protocols, known *in-vivo* persistence profiles, and mature standardized clinical safety monitoring frameworks, all of which remain major developmental challenges for in-vivo-generated CAR-positive cell products.

Table 1. *Ex-Vivo* Manufacturing Controls and Corresponding In-Vivo Cellular Pharmacology Measurements.

Control Domain	*Ex-Vivo* Control	*In-Vivo* Change	Post-Dosing Measurement
Starting material and cell identity	Leukapheresis material is enriched and characterized before engineering	The accessible T-cell pool varies with disease, prior therapy, receptor abundance, and tissue distribution	Baseline absolute CD4/CD8 counts, subset phenotype, targeting-receptor abundance, and disease-donor uptake
Genetic modification	Vector exposure and transduction are controlled during manufacture	RNA delivery occurs after dosing and competes with uptake by non-target cells	Intact RNA and surface CAR in intended T cells and predefined off-target immune and hepatic cell types

Cell number and composition	Expansion yields a countable lot with a measured subset composition	CAR-positive cell number, CD4/CD8 balance, and activation state emerge in the patient	Serial absolute CAR-positive cell counts, subset frequencies, phenotype, and protocol-defined tissue sampling where accessible
Potency	The released cell lot is tested for antigen-specific activity before infusion	Potency is inferred from sampled cells and downstream pharmacodynamics	Surface CAR density, antigen-specific activation and killing, target-cell depletion, and disease response
Dose	A defined number of viable CAR-positive cells is infused	Milligrams of RNA are administered, but the active cellular exposure changes over time	Injected RNA dose reported separately from peak and time-integrated CAR-positive cell exposure
Release and repeatability	Identity, purity, viability, sterility, potency, and stability are assessed before treatment	Vial release remains necessary but cannot predict cell-specific exposure or later-dose response	RNA-LNP quality attributes linked to cellular pharmacology, repeat-dose recovery, and anti-carrier immunity

Some release attributes remain performed on the RNA-LNP drug substance/drug product and are not replaced by post-patient-dosing measurements. Abbreviations: CAR, chimeric antigen receptor; CD, cluster of differentiation; LNP, lipid nanoparticle; RNA, ribonucleic acid.

2.2. *In-Vivo* Generation of the Cellular Product

In-vivo CAR-T shifts cell generation from the manufacturing facility to the patient. The administered RNA-LNP generates CAR-positive cells through distribution, binding, internalization, endosomal escape, translation, and receptor assembly. Injected RNA dose and cellular exposure are distinct [10–13,32].

An *in-vivo* product must deliver intact RNA to the cytosol of intended cells and sustain sufficient surface CAR for a defined interval. Spleen-biased expression and antibody-mediated uptake describe distribution and uptake; surface CAR and downstream pharmacodynamics test productive cellular engineering and function [33–38]. Whole-organ reporter signal does not quantify the number or function of CAR-positive T cells [8–13]. Of note, reporter signal is not equivalent to functional CAR expression and cannot confirm productive CAR assembly and antigen-specific effector function. Figure 1 maps each stage to its failure-localizing measurement. Key stage-specific analytical readouts (product quality attributes, biodistribution, cell-resolved delivery metrics, CAR-positive-cell kinetics, and PK/PD safety endpoints) are described in the main text rather than within the schematic diagram.

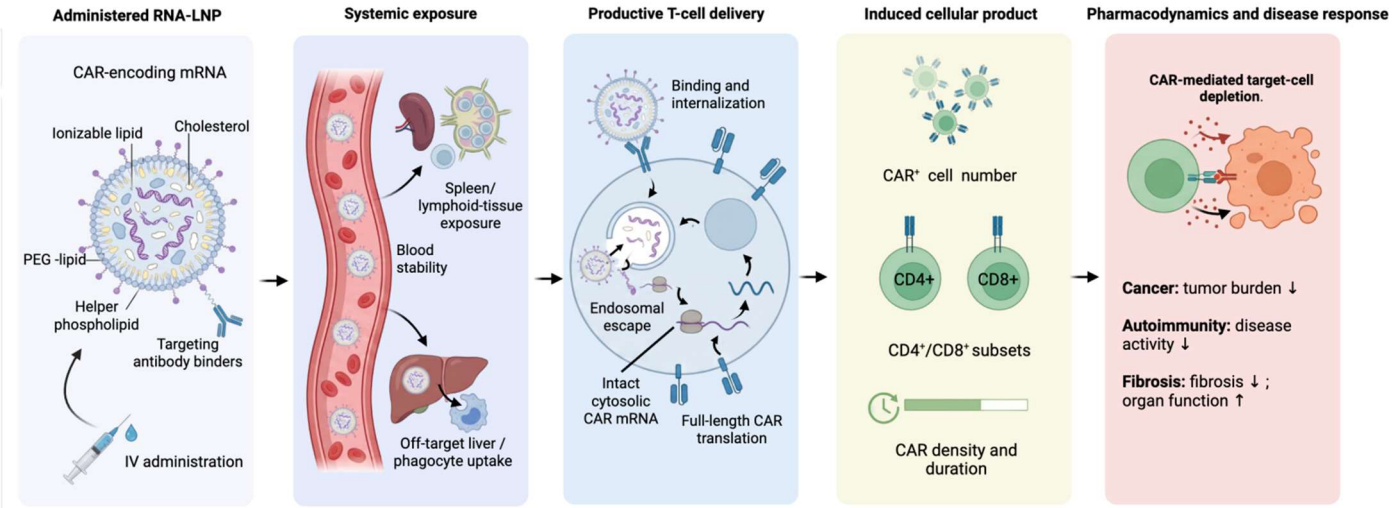


Figure 1. Conceptual framework linking systemic RNA-LNP dosing to functional *in-vivo* CAR-T generation**.** After intravenous administration, biodistribution and productive T-cell delivery (internalization, endosomal escape, RNA release, and translation) determine the number, phenotype, CAR density, and persistence of CAR-positive CD4+ and CD8+ T cells. These cellular exposures

drive antigen-positive target-cell depletion, disease response, and toxicity. Organ exposure or particle uptake alone does not establish productive T-cell delivery or antigen-specific function. Abbreviations: CAR, chimeric antigen receptor; CD, cluster of differentiation; LNP, lipid nanoparticle; mRNA, messenger RNA; PK/PD, pharmacokinetics/pharmacodynamics.

2.3. Transient CAR Expression as a Design Variable

RNA produces transient, non-integrating CAR expression. In leukemia models, repeated infusion of mRNA-electroporated CAR-T cells matched the antitumor activity of stable modification; the surface receptor disappeared as RNA cleared [39]. LNPs avoid electroporation-associated membrane damage and specialized equipment [25,40]. Expression duration should be matched to disease control and redosing feasibility.

LNPs introduce innate immune activation, complement activation, altered tissue distribution, and repeat-dose tolerance risks [5–9]. CD3- or CD28-directed ligands also deliver signaling inputs; anti-ligand immunity and productive off-target uptake require measurement. Route of administration alone does not establish superiority: generated CAR-positive cells must be selective, functional, and reproducible.

3. Evidence Base for *In-Vivo* RNA-LNP CAR-T Development

3.1. Foundational Evidence from Primary Human T Cells

Primary-cell studies establish that LNP delivery can generate functional CAR expression without establishing systemic targeting (Table 2). Billingsley et al. selected C14-4 from 24 ionizable lipids; it produced CAR expression comparable to electroporation with lower cytotoxicity and supported Nalm-6 cell killing [25]. Picht et al. found greater and more sustained surface CAR after electroporation, but LNP-treated cells showed fewer stress-, senescence-, and exhaustion-associated signatures and stronger antigen-driven activation, chemotaxis, and cytotoxicity [41]. Thus, expression magnitude and cell state can diverge, and both require measurement.

Table 2. Primary studies of *in-vivo* RNA-LNP CAR-T development, categorized by evidentiary layer and scope of interpretation.

Study	Evidence Tier	System and Comparator	Cell-Targeted Delivery	Functional Outcome	Limitations
Billingsley et al. [25] (2020)	Enabling, *ex-vivo*	24-lipid screen; C14-4 versus lipofectamine and electroporation in primary human T cells	CAR expression comparable to electroporation with lower cytotoxicity	Antigen-specific Nalm-6 killing	No systemic exposure or *in-vivo* targeting; denominator/time-point/dose/schedule:not reported
Picht et al. [41] (2026)	Enabling, *ex-vivo*	LNP delivery versus electroporation of CAR mRNA in primary human T cells	Electroporation gave higher efficiency and longer surface expression; LNP delivery reduced stress-, senescence-, and exhaustion-associated signatures	Greater antigen-driven activation, chemotaxis, and cytotoxicity after LNP delivery	No systemic targeting; comparison of engineering methods; denominator/time-point/dose/schedule:not reported

Kahwaji et al. [42] (2026)	Enabling, *ex-vivo*	Unmodified uridine versus modified CAR mRNA in activated primary human T cells	Unmodified CAR mRNA produced higher surface expression without increased measured innate activation in T cells	Greater cytotoxic potency in the reported assay with unmodified CAR mRNA	Activated *ex-vivo* cells; no resting-cell or systemic comparison; denominator/time-point/dose/schedule:not reported
Rurik et al. [43] (2022)	Direct *in-vivo* CAR	CD5-targeted modified mRNA-LNP; mouse cardiac injury	FAP-CAR was transient and absent from splenic T cells at one week	Reduced activated fibroblasts and fibrosis; improved cardiac function	Disease-specific mouse model; no malignant target; dose/schedule: not reported; time-point: 7-day post-dose; denominator: splenic T-cell population
Liu et al. [44] (2026)	Direct *in-vivo* CAR	CD3-targeted LNP co-delivering FAP-CAR and truncated human epidermal growth factor receptor mRNAs; mouse myocardial fibrosis	CAR-positive T-cell generation with a cetuximab-responsive deletion switch	Reduced fibrosis and improved cardiac function	Mouse proof of concept; CD3 signaling, redosing, and human tolerability unresolved; denominator/time-point/dose/schedule:not reported
Alvarez-Benedicto et al. [33] (2023)	Direct *in-vivo* CAR	10% 18:1 PA SORT LNP; lymphoreplete mouse lymphoma	CAR expression in splenic CD3, CD4, and CD8 T cells	Reduced liver burden; survival gain only in the less aggressive model	Organ tropism does not establish lineage selectivity; denominator: splenic CD3+ lymphocytes; time-point/dose/schedule: not reported
Billingsley et al. [34] (2024)	Direct *in-vivo* CAR	Extrahepatic antibody-LNP; antibody and dose comparisons in mice	Target- and dose-dependent CAR expression in T cells	Up to 90% circulating B-cell depletion at 36 h	Observation ended at 36 h; redosing not tested; denominator: circulating T-cells; time-point: 36 h post-dose; dose/schedule: not reported
Zhang et al. [45] (2025)	Direct *in-vivo* CAR	Anti-CD5 LNP co-delivering CD19 CAR and PSMA-tag mRNAs; IL-7 preconditioning and repeated dosing	PET confirmed generation and tumor infiltration of CAR-positive cells	75% tumor-free survival in the reported lymphoma model	Multicomponent regimen prevents attribution to one element; denominator/time-point/dose/schedule:not reported

Bajbouj et al. [46] (2026)	Direct *in-vivo* CAR	Anti-CD5 FAP-CAR mRNA-LNP versus 1 × 10^7 *ex-vivo* retroviral CAR-T cells; mouse pancreatic cancer	>45% splenic, >69% circulating, and >35% tumor-infiltrating T cells were CAR-positive after one dose	Tumor inhibition comparable to or greater than the *ex-vivo* arm within the study	One stromal target and mouse model; no general product equivalence; denominator: splenic/circulating/tumor-infiltrating T-cell subsets; time-point/dose/schedule: not reported
Hunter et al. [35] (2025)	Direct *in-vivo* CAR	CD8-targeted linear mRNA-LNP; human cells, humanized mice, and non-human primates	CD8-biased CAR-positive T-cell generation after repeated dosing	Tumor control; B-cell depletion with predominantly naive recovery	Preclinical; human dose and long-term safety unknown; denominator/time-point/dose/schedule:not reported
Lemgart et al. [47] (2026)	Direct *in-vivo* CAR	CD8-targeted VHH-LNP carrying CD22 CAR mRNA	Transient CAR expression in human CD8 T cells after repeated dosing	Inhibited Nalm-6 tumor growth	CD8 is not T-cell-exclusive; no non-human-primate or human outcome data; denominator/time-point/dose/schedule:not reported
Zhang et al. [48] (2025)	Direct *in-vivo* CAR	Ligand-free PL40 LNP; linear and circular RNA; mouse fibrosis and arthritis	Approximately 20% of the relevant population was CAR positive in the reported experiment	Reduced liver fibrosis and arthritis; human uPAR CAR activity in NCG mice	Not T-cell-exclusive; lipid and RNA-format effects partly entangled; denominator: relevant T-cell population; time-point/dose/schedule: not reported
Choy et al. [49] (2026)	Direct *in-vivo* CAR	pMHC-II LNP carrying CD19 CAR mRNA; T-cell-receptor-transgenic mice	Selective expression in cognate antigen-specific CD4 T cells	Peripheral B-cell depletion	Peptide- and MHC-restricted transgenic model; denominator/time-point/dose/schedule:not reported
Hashiba et al. [50] (2026)	Direct *in-vivo* CAR	CD8-targeted VHH-LNP with quantified functional ligand density	Bell-shaped delivery curve; optimum near 0.1 VHH per 100 nm^2; excess density degraded receptor	Dose-dependent B-cell depletion at 10–30 μg/kg	One receptor, VHH, and LNP core; human dose unknown; denominator: CD8+ T-cells; time-point: not reported; schedule: single injection; dose: 10–30 μg/kg

Ji et al. [51] (2026)	Direct *in-vivo* CAR	Citronellol-tailored H3T4 lipid; CD3-targeted LNP; murine CD19 solid-tumor model	Approximately 85% of the relevant population was CAR positive in the reported experiment	83% tumor clearance in the reported model	Percentages are comparator- and model-specific; denominator: relevant T-cell population; time-point/dose/schedule: not reported
Patel et al. [52] (2026)	Direct *in-vivo* CAR	Anti-CD3/anti-CD28/IL-7 LNP; primary human T cells and mice	IL-7 co-presentation increased uptake and expression and biased functional expression toward splenic T cells	CAR-dependent target-cell depletion	Targeting and signaling effects cannot be separated without matched surface controls; denominator/time-point/dose/schedule:not reported
Yin et al. [53] (2026)	Direct *in-vivo* CAR	Immune-cell-tropic LNP carrying HER2 CAR mRNA; three murine solid-tumor models	CAR expression in T cells, macrophages, dendritic cells, and natural killer cells	Tumor inhibition, longer survival, and tumor-microenvironment remodeling	Efficacy cannot be attributed to T cells alone; denominator/time-point/dose/schedule:not reported
Zeng et al. [36] (2026)	Direct *in-vivo* CAR	CD2/CD4/CD5/CD7/CD8-targeted LNPs; human cells and humanized mice	CD7 yielded 33.3% versus 11.5% CAR-positive CD4 cells and 50.6% versus 20.2% CD8 cells relative to CD2	Functional anti-CD20 CAR-T generation	One optimized ligand, LNP, and model system; denominator: CD4+/CD8+ T-cell subsets; time-point/dose/schedule: not reported
Guo et al. [37] (2026)	Direct *in-vivo* CAR	300-lipid screen; 113-AA-C8C14 versus benchmark SORT in mice	57-fold higher splenic expression and 2.7-fold more CAR-T cells	2.3-fold greater tumor-growth inhibition	Fold changes are comparator-, tissue-, and model-specific; denominator/time-point/dose/schedule:not reported
Chen et al. [38] (2025)	Adjacent delivery mechanism	Nanobody capture of antibody Fc versus non-targeted and conventional functionalization	>1000-fold and >8-fold higher reporter expression, respectively	Selective *in-vivo* T-cell reporter expression	Reporter payload; CAR efficacy not tested; denominator/time-point/dose/schedule:not reported

CAR-positive percentage values are reported as provided in original publications. Denominator cell population, post-dosing measurement time-point, RNA-LNP dose and administration schedule are specified where available from source references; parameters not disclosed in original studies are marked as 'not reported'. Percentages are comparator-, tissue-, and model-specific and cannot be directly cross-study compared. Evidence tier indicates what each experiment can establish; it is not a quality score. 'Direct *in-vivo* CAR' denotes systemic dosing followed by CAR measurement in identified cell types; 'Enabling, *ex-vivo*' denotes primary-cell transfection or reporter studies performed without systemic targeting; and 'Adjacent delivery mechanism' denotes reporter-only evidence supporting a delivery approach. Abbreviations: CAR, chimeric antigen receptor; CD, cluster

of differentiation; FAP, fibroblast activation protein; Fc, fragment crystallizable; HER2, human epidermal growth factor receptor 2; IL-7, interleukin-7; LNP, lipid nanoparticle; mRNA, messenger RNA; NCG, NOD/ShiLtJGpt-Prkdcem26Cd52Il2rgem26Cd22/Gpt immunodeficient mouse strain; PA, phosphatidic acid; PET, positron emission tomography; pMHC-II, peptide-major histocompatibility complex class II; PSMA, prostate-specific membrane antigen; RNA, ribonucleic acid; SORT, selective organ targeting; uPAR, urokinase-type plasminogen activator receptor; VHH, variable heavy-chain domain of a heavy-chain-only antibody.

Kahwaji et al. compared unmodified uridine-containing CAR mRNA with N1-methylpseudouridine- and 5-methoxyuridine-containing transcripts in activated primary human T cells. Unmodified mRNA produced higher surface CAR and cytotoxicity without increasing the measured innate-response endpoints in T cells, whereas other primary cell types mounted innate responses [42]. The study did not isolate the mechanism or establish an *in-vivo* advantage; it supports cargo screening in the intended cell population and final formulation.

3.2. A Therapeutic CAR Pulse in Cardiac Fibrosis

Two mouse studies show that a finite CAR pulse can modify cardiac fibrosis. CD5-targeted LNPs carrying FAP-CAR mRNA generated transient CAR-positive T cells, reduced activated fibroblasts and fibrosis, and improved cardiac function; splenic CAR expression was undetectable after one week [43]. A CD3-targeted formulation co-delivering FAP-CAR and truncated human epidermal growth factor receptor safety-switch mRNAs also reduced fibrosis and improved function, and cetuximab depleted CAR-positive cells in the switch experiment [44]. Notably, uniform co-expression cannot be assumed and requires validation across all CAR-positive cells in co-delivery settings; the fraction of CAR-positive cells carrying functional truncated EGFR, together with sustained bioactivity of this elimination element across the full therapeutic time window, requires further quantitative characterization. These studies establish proof of concept for transient antifibrotic activity, not the human tolerability of CD3 ligation, redosing, or off-target expression.

3.3. Ligand-Free Tropism and Active Targeting

Ligand-free formulations show that lipid composition can bias extrahepatic immune-cell delivery, but neither reported system achieved strict T-cell restriction. A spleen SORT LNP containing 10% 18:1 phosphatidic acid delivered CD19 CAR mRNA to splenic CD3+, CD4+, and CD8+ cells and improved survival only in the less aggressive lymphoma model [33]. PL40 delivered circular uPAR-CAR RNA and generated approximately 20% CAR-positive T cells under the reported experimental setting in mouse fibrosis and arthritis models, yet only approximately half of hemagglutinin-tag-positive cells were CD3-positive in one analysis [48]. Because lipid composition, RNA topology, and exposure were not jointly controlled, the efficacy cannot be assigned to circularization.

Antibody-targeted LNPs increased cell-resolved exposure but also revealed strong regimen dependence. CD3-targeted particles depleted up to 90% of circulating B cells at 36 h, while CAR expression, cytokine release, and depletion varied with ligand and mRNA dose; later persistence and redosing were not measured [34]. Anti-CD5 LNPs co-delivering CD19 CAR and PSMA imaging-tag mRNAs produced PET-detectable tumor infiltration and 75% tumor-free survival with IL-7 preconditioning and repeated dosing [45]. Co-encapsulation of two distinct mRNA species within a single LNP formulation does not guarantee uniform co-delivery and co-expression within every individual cell; protein translation and turnover kinetics for the CAR and the PSMA reporter may also diverge. Accordingly, PET signal from the PSMA tag serves only as a surrogate readout,

and direct empirical validation is required to establish its quantitative correlation with surface CAR abundance. The multicomponent regimen prevents attribution of efficacy to a single design variable.

In a pancreatic cancer model, one anti-CD5 FAP-CAR mRNA-LNP dose produced CAR expression in >45%, >69%, and >35% of splenic, circulating, and tumor-infiltrating T cells, respectively [46]. Tumor inhibition matched or exceeded 1 × 10^7 *ex-vivo* retroviral CAR-T cells within that experiment. This comparison supports activity in the reported model but does not establish cross-product equivalence.

It should be noted that preclinical efficacy outcomes require careful distinction between models bearing naturally occurring endogenous antigens and tumor systems engineered to artificially express target antigens. In particular, antitumor effects observed in CD19-transduced solid-tumor models cannot be extrapolated to predict therapeutic performance against authentic solid-tumor antigens, as target-antigen expression, tumor microenvironment and immune-suppressive features differ substantially.

3.4. Humanized Mice and Non-Human Primates

CD8-targeted platforms provide the strongest cross-species evidence for repeated *in-vivo* generation. Hunter et al. generated CD20 CAR-positive T cells in humanized mice and cynomolgus monkeys; repeated dosing-controlled B-cell malignancy in mice and depleted B cells in monkeys, followed by predominantly naive B-cell recovery [35]. Lemgart et al. generated transient CD22 CAR-positive CD8 T cells and inhibited Nalm-6 tumor growth in a humanized mouse model [47]. These data do not define a human dose, durability, or long-term redosing safety, and CD8 expression on natural killer and natural killer T-cell subsets requires explicit off-target analysis.

3.5. Mechanistic Comparisons of Targeting and Productive Delivery

Aligned comparisons identify receptor trafficking and ligand density as determinants of productive delivery. Zeng et al. found that CD7 internalized rapidly whereas abundant CD2 internalized poorly; CD7-targeted LNPs generated 33.3% versus 11.5% CAR-positive CD4 T cells and 50.6% versus 20.2% CAR-positive CD8 T cells relative to CD2 in humanized mice [36]. Hashiba et al. reported a bell-shaped relationship for CD8-targeted VHH-LNPs, with an optimum near 0.1 VHH molecules per 100 nm^2 and receptor degradation at higher density [50]. Surface abundance and maximal ligand loading are therefore poor surrogates for productive delivery.

Material chemistry and ligand presentation also alter delivery independently of nominal tropism. From a 300-lipid screen, Guo et al. selected 113-AA-C8C14, which yielded 57-fold more splenic mRNA expression, 2.7-fold more CAR-T cells, and 2.3-fold greater tumor inhibition under the defined experimental condition than benchmark SORT in the reported pancreatic model [37]. An Fc-binding nanobody architecture increased reporter expression by more than 1000-fold over non-targeted LNPs and more than eightfold over conventional conjugation [38]. The first comparison is model- and comparator-specific; the second establishes a delivery mechanism, not CAR efficacy.

Strategies that narrow or broaden the cellular product introduce different causal ambiguities. Cognate pMHC-II ligands restricted CD19 CAR expression to antigen-specific CD4 T cells and depleted peripheral B cells in TCR-transgenic mice, but peptide specificity, MHC restriction, and the transgenic repertoire constrain generalization [49]. H3T4, IL-7 co-presentation, and a multilineage immune-cell-tropic LNP produced strong CAR expression or antitumor activity in their respective mouse systems [51–53]. Those results remain within-platform: cytokine co-presentation combines targeting with signaling, and multilineage CAR expression prevents attribution of efficacy to T cells alone.

4. Co-Design of the CAR, RNA Cargo, Targeting Strategy, and LNP

4.1. Receptor Internalization and Ligand Presentation

Productive targeting is a serial process comprising receptor binding and internalization, endosomal escape, cytosolic RNA release, translation, receptor assembly, and antigen-dependent signaling. The comparative studies show that receptor abundance alone is insufficient: internalization kinetics, functional ligand density, antibody orientation, and co-presented signals each alter delivery [36,38,50,52]. Candidate screens should therefore progress from binding to internalization, cytosolic RNA, surface CAR, cell phenotype, signaling, and antigen-specific function under matched conditions. When evaluating targeting-ligand performance, well-matched experimental comparisons are required where LNP core lipid chemistry, RNA cargo, and CAR construct remain constant; otherwise observed differences in transfection efficiency may originate from lipid formulation rather than the targeting strategy itself.

4.2. Target Choice and Cellular Product Characterization

Target choice specifies the cells that can become CAR-positive (Table 3). CD3 provides broad T-cell coverage but engages TCR signaling [34,44,51,52]; CD5 and CD7 provide broad T-cell-biased access, with functional evidence for CD5 and rapid internalization for CD7 [36,43,45,46]; CD8 enriches cytotoxic T cells but also reaches natural killer and natural killer T-cell subsets [35,47,50]. Off-target CAR expression within these CD8-expressing non-T-cell populations may modify overall functional potency and introduce unforeseen safety hazards, requiring dedicated off-target analysis. pMHC-II can restrict delivery to cognate CD4 T cells, although current evidence is peptide-, HLA-, and transgenic-model specific [49]. No receptor is intrinsically superior across ligands and LNP cores.

Selection should be based on the required cell subset and validated with the final ligand, LNP, RNA, and CAR. A cell-resolved profile includes absolute CAR-positive-cell number, subset composition, receptor density, activation state, persistence, and predefined off-target populations [10–13,32]. Target-cell transfection also requires measurement when B cells express the targeted antigen. A deliberately multilineage product, such as panCAR, has efficacy and toxicity that cannot be attributed to T cells alone [53].

Off-target CAR expression has cell-type-dependent consequences. CAR expression in NK/NKT cells may include CAR-mediated cytotoxicity; expression in myeloid cells or hepatocytes does not produce T-cell effector function but carries risk of non-specific protein overexpression. In B-cell malignancies, CAR-mRNA delivery to malignant B cells creates a theoretical risk of antigen masking, which has not been experimentally demonstrated for current LNP formulations. Some T-cell malignancies express LNP targeting receptors (CD3, CD5, CD7, CD8). Multi-subset flow cytometry and functional controls are recommended to separate CAR activity from intended T cells and off-target cells.

Table 3. Receptor-targeted and ligand-free strategies for T-cell-directed RNA-LNP delivery: comparison of intended product, evidence, advantages, risks and manufacturing considerations.

Strategy	Intended Cellular Product	Productive-Delivery Evidence	Principal Advantage	Biological Risk	Evidence Boundary and Manufacturing Implication
CD3	Broad mature T-cell population	Productive CAR expression reported with antibody-targeted LNPs [34,44,51,52]	Broad coverage and access to activation ligands	T-cell-receptor-complex signaling and cytokine release	Clone, orientation, density, and activation require product-specific control

CD5	Broad T-cell-biased population	Direct functional evidence in cardiac fibrosis, imaging, and pancreatic cancer [43,45,46]	Evidence in cardiac fibrosis, lymphoma imaging, and pancreatic cancer models	Cell-state-dependent expression and signaling	Evidence remains formulation- and model-specific
CD7	Broad T-cell-biased population	Rapid internalization correlated with CAR delivery in an aligned comparison [36]	Mechanistic support for productive uptake	Also expressed on natural killer cells	Independent LNP cores, clones, donors, and disease models are needed
CD8	Cytotoxic T-cell-enriched population	Direct evidence across human cells, humanized mice, and non-human primates [35,47,50]	Cross-species evidence and repeated dosing	Excludes CD4 helper cells; CD8-positive NK and natural killer T cells are off-target populations	Requires subset-resolved potency and off-target assays
pMHC-II	Cognate antigen-specific CD4 T cells	Selective CAR-mRNA delivery and B-cell depletion in T-cell-receptor-transgenic mice [49]	High subset precision	Peptide and HLA restriction; T-cell-receptor signaling	Human translation requires allele coverage and antigen-specific safety testing
Ligand-free SORT	Organ-enriched mixed immune population	Splenic exposure and CAR expression reported in mice [33]	Simpler surface chemistry	Myeloid and stromal off-target expression	Organ tropism must be resolved into cell-specific translation
Ligand-free PL40	T-cell-favored mixed-cell population	Direct linear- and circular-RNA CAR evidence in mice [48]	Avoids a targeting ligand	Off-target expression and basis of selectivity remain unresolved	Lipid morphology, RNA format, and disease model require matched controls

Abbreviations: CAR, chimeric antigen receptor; CD, cluster of differentiation; HLA, human leukocyte antigen; LNP, lipid nanoparticle; NK, natural killer; pMHC-II, peptide-major histocompatibility complex class II; SORT, selective organ targeting.

4.3. Re-Evaluating CAR Architecture for Transient Expression

The CAR cassette contributes to both exposure and function. Binding-domain affinity and epitope geometry, hinge and transmembrane regions, and costimulatory and CD3ζ domains govern surface stability, signaling, metabolism, and cell state [15,17]. Pooled screens in stably engineered cells cannot be transferred directly to transient RNA because receptor abundance changes over hours to days.

Comparisons should therefore match surface CAR exposure rather than RNA mass; otherwise, differences in translation, trafficking, or receptor stability can be misclassified as signaling effects. A transient-CAR comparison requires time-resolved receptor density, antigen-triggered signaling, serial killing, and T-cell phenotype [10,11,17].

4.4. Lipid Chemistry and Cytosolic RNA Delivery

Ionizable lipids govern encapsulation, stability, biodistribution, membrane disruption, and endosomal escape, whereas helper and PEG lipids modify particle structure, circulation, fusion, and redosing [2,5–9]. In an aligned comparison with similar lymphoid tropism, the CD3-targeted H3T4 formulation generated approximately 85% versus 40% CAR-positive cells in the reported murine CD19 solid tumor at the defined sampling time-

point relative to its comparator [51]. The result shows that tissue tropism and productive T-cell transfection are separable formulation properties; it does not rank unrelated platforms.

Ionizable headgroup and tail architecture affect endosomal escape, T-cell transfection, and innate signaling; DOPE favors membrane fusion and endosomal disruption, whereas DSPC increases particle stability [2,5,6,9,37,51,54]. Cholesterol and its analogs tune rigidity, integrity, release, and tropism [2,9]. PEG-lipid acyl-chain length governs shedding. Faster loss favors uptake, whereas slower loss prolongs circulation [2,5,8]. Anti-PEG antibodies, complement activation, and innate sensing alter later-dose exposure [5–7]. Targeting comparisons must therefore control the complete formulation.

Fluorescent lipid and total cellular RNA include particles or cargo retained in endosomes or committed to degradation [2,8,9,36,38]. Productive delivery therefore requires concordant evidence of intact intracellular RNA, translated protein, calibrated surface CAR, and antigen-specific function in defined primary-cell subsets [2,18,19,36,38].

4.5. Determinants of Surface CAR Exposure

Linear mRNA has the most mature sequence-design, manufacturing, purification, and LNP-formulation workflow [1–3,18,19]. Cap structure, untranslated regions, coding sequence, poly(A) length, integrity, residual double-stranded RNA, and nucleoside chemistry jointly determine translation and innate sensing [1,3,5–7,18,19]. Vaccine-derived modifications therefore require testing with the intended CAR, cell population, and formulation. In activated primary human T cells, the unmodified transcript tested by Kahwaji et al. produced higher surface CAR and cytotoxicity than the modified comparators without increasing measured innate-response endpoints [42].

That experiment did not distinguish effects on ribosome kinetics, innate sensing, RNA stability, or surface-receptor turnover and did not establish an *in-vivo* advantage [42]. Its inference is therefore limited to formulation-specific screening rather than a general preference for unmodified CAR mRNA.

RNA length and structure affect encapsulation, particle organization, release, and innate recognition, whereas lipid composition can change biodistribution and inflammation for the same transcript [5–9,18,19,54–59]. Ligand functionalization further changes particle properties and, for CD3/CD28 ligands, adds receptor-signaling inputs [34,38,50,52]. These coupled variables require evaluation as a final RNA-LNP-ligand product with a primary T-cell potency assay.

5. From Administered Dose to Clinical Cell Exposure

Dose denotes the administered amount of RNA-LNP, usually reported as RNA mass. RNA integrity and particle attributes describe the administered product and can alter cellular exposure. Cellular exposure denotes a time-varying CAR-positive cell population defined by absolute number, subset composition, surface density, distribution, and persistence. The pharmacodynamic window links that exposure to target-cell depletion, cytokines, toxicity, recovery, and disease response. Separating these levels identifies where a formulation succeeds or fails [10–13,32].

5.1. Quantifying CAR-Positive Cell Exposure

Percentage-only readouts can be misleading in lymphopenic patients; early trials should report absolute CAR-positive CD4+ and CD8+ counts. The minimum exposure profile comprises peak count, area under the cell-count curve, calibrated surface density, subset composition, persistence, and sampling compartment. Given intrinsic biological differences across species, disease status, and tissue compartments, full quantitative normalization across independent published studies remains challenging; well-controlled

within-study comparisons therefore are preferred over direct cross-study numerical comparison. Serial flow cytometry should include receptor calibration and activation/exhaustion phenotyping, with protocol-defined tissue sampling where accessible. A co-delivered PSMA reporter enabled PET tracking in mice [45] but adds a second cargo that requires separate product validation. Exposure can then be related to target depletion, cytokines, toxicity, recovery, and response [10–13,32].

CAR-positive cell exposure is a multidimensional parameter, comprising absolute CAR-positive count, surface CAR density, CD4+/CD8+ subset, antigen-specific effector function, tissue distribution and duration. Four separate biological events should be distinguished: lymphocyte survival/proliferation, intracellular mRNA persistence, surface CAR protein retention, and antigen-dependent functional activity. Loss of surface CAR does not equal cell death. T-cell proliferation dilutes intracellular mRNA and CAR receptors in daughter cells. Peripheral blood CAR-positive measurements do not necessarily reflect CAR-T exposure in target lesions. This framework will be referred to in Section 5.4 for redosing evaluations.

5.2. A Minimum Translational Evidence Set

A translational package should resolve intact RNA, surface CAR, and function by cell type and time, including off-target expression and redosing (Table 4). Primary human studies require healthy and disease-affected donors because prior therapy and disease alter receptor abundance, internalization, activation, and fitness. Large-animal studies should address dose scaling, subset coverage, cytokines, tissue distribution, recovery, and anti-ligand or anti-PEG responses. Chemistry, manufacturing, and controls should include RNA identity and integrity, cap and poly(A) attributes, residual double-stranded RNA, encapsulation and free RNA, particle size, sterility, endotoxin, stability, and cell-based potency [11,18,19,23].

Short-amplicon RT-qPCR/RT-ddPCR only detects partial RNA sequences and cannot confirm full-length transcript integrity. Full-length RNA sequencing or long-range RT-PCR is required for integrity validation. Total cellular RNA cannot distinguish endosome-trapped RNA from translatable RNA, which requires subcellular fractionation. Genomic DNA CAR copy counting is not applicable to the non-integrating mRNA platform. Surface CAR detected by flow cytometry requires negative controls and calibration beads for quantitative receptor density. Absolute CD4+ and CD8+ counts using counting beads are preferred over percentages, especially in lymphopenic patients. Antigen-specific killing and cytokine release assays measure effector function. Mechanistic assays (subcellular fractionation, long-read sequencing) are separated from clinically feasible assays (calibrated flow cytometry, absolute counting, short-amplicon RT-ddPCR, target depletion and cytokine markers).

Table 4. Proposed minimum pre-clinical and translational evidence package for *in-vivo* RNA-LNP CAR-T development: required measurements linked to disease, safety, repeated-dosing, and CMC-potency assessment.

Domain	Required Endpoint	Minimum Assay Package	Decision Criterion
Cell specificity	CAR-positive CD4/CD8 cells; off-target immune and liver cells	Flow cytometry or imaging with functional CAR readout	Advance only with cell-resolved selectivity
Productive delivery	Uptake, endosomal escape, translation, surface CAR	Orthogonal RNA, protein, and functional assays	Reporter uptake alone is insufficient
Disease response	Target depletion, tumor burden, fibrosis, recovery	Time-resolved CAR exposure and disease endpoint	Match duration to indication

Safety and redosing	Cytokines, complement, liver, neurotoxicity, recovery	Repeat-dose and delayed-observation studies	Non-integrating RNA does not ensure safe redosing
Manufacturing and potency	RNA identity/integrity, LNP attributes, cell potency	Release panel linked to primary-T-cell function	Vial quality does not fully define induced cell product

The package defines the minimum evidence needed for a development decision; it is not a regulatory checklist. Abbreviations: CAR, chimeric antigen receptor; CD, cluster of differentiation; LNP, lipid nanoparticle; RNA, ribonucleic acid.

5.3. Indication-Specific Pharmacology

Indication changes the exposure-response relationship. Autoimmune studies should connect CAR-positive-cell kinetics with B-cell depletion, autoantibodies, immunoglobulins, infection, clinical response, and B-cell reconstitution [35,60]. B-cell malignancies require exposure sufficient to control an expanding tumor burden [14,16], whereas fibrosis requires durable remodeling after a finite CAR pulse [43,44]. Solid tumors add antigen heterogeneity, restricted vascular access, stromal exclusion, and suppressive metabolism that prolonged RNA expression alone cannot correct [16,46,53].

5.4. Repeat-Dose Considerations

Non-integrating RNA does not guarantee reproducible redosing. Hunter et al. generated CAR-positive T cells after repeated dosing in cynomolgus monkeys and documented predominant naive-phenotype B-cell recovery; Lemgart et al. applied repeat-dose regimens in humanized mice [35,47]. However, neither study reported dose-resolved RNA pharmacokinetics, quantitative cellular exposure metrics, anti-carrier immunity readouts, or long-term multi-lineage immune reconstitution upon cycles of repeated dosing. Anti-PEG or anti-ligand antibodies, complement, innate sensing, receptor availability, and cumulative target depletion alter later-dose exposure [5–7,10–13]. Repeat-dose studies must test exposure reproducibility and distinguish carrier immunity from altered target biology or exhausted targetable pools. Escalation requires predefined recovery criteria and stopping rules.

Clinical translation also requires scalable manufacturing, lot consistency, and long-term safety. Scale-up must preserve RNA integrity, particle size, encapsulation, and primary T-cell potency while controlling residual double-stranded RNA across batches [11,18,19]. Non-integrating RNA avoids insertional mutagenesis but not prolonged B-cell or plasma-cell depletion, infection, delayed reconstitution, off-target expression, or cumulative effects. Lineage-resolved biodistribution and extended follow-up are required [20–24].

Important safety risks beyond classical *ex-vivo* CAR-T toxicities include off-target cellular transfection, hepatic RNA-LNP exposure, innate immune activation, complement-mediated effects, developing anti-LNP or anti-targeting-ligand antibodies upon repeated administration, and undesired CAR expression in non-target immune cell populations. These risks should be systematically assessed in preclinical repeat-dose studies.

5.5. Early Clinical Evidence Landscape

Registered clinical programs include linear mRNA-LNPs, LNPs with incomplete public disclosure, a polymer-lipid dual-CAR construct, and circular RNA-LNPs [61–87]. Table 5 summarizes public cargo, carrier, target, indication, trial status, and patient-level evidence as of July 2026.

Table 5. Registered clinical studies of lipid and polymer-lipid RNA platforms designed to generate CAR-expressing cells *in-vivo*.

Program and Trial(s)	Public RNA and Delivery Description	CAR Target	Indication(s)	Current Status and Evidence Level	Interpretive Limit
CPTX2309/NCT06917742 [64]	Linear mRNA; CD8-targeted LNP	CD19	RA, SLE, and healthy participants	Recruiting, Phase 1	Named program; no posted peer-reviewed clinical results
STR-P004/NCT07003178, NCT07143617, NCT07245251 [65,66,77,87]	Reported LNP-mRNA platform; registry disclosure varies	CD19 or undisclosed	B-cell NHL, B-ALL, and autoimmune disease	Recruiting/not yet recruiting; three-patient meeting abstract	Transient B-cell depletion and mixed clinical markers; no full peer-reviewed report
JCXH-213/NCT06618313, NCT07622329 [67,68]	mRNA-LNP *in-vivo* CAR platform	B-cell target not fully disclosed	B-cell NHL and immune thrombocytopenia	Recruiting/not yet recruiting	Registry-level evidence; no peer-reviewed clinical outcomes
GT801/NCT07205315, NCT07614191 [63,78,79]	Antibody-displayed LNP; linear CD19 CAR mRNA	CD19	B-cell malignancies, AIHA, and refractory autoimmune disease	Recruiting; three-patient meeting abstract	Three-patient abstract; durability and uncommon toxicity unresolved
HN2301/NCT06801119, NCT06965309, NCT07333677, NCT07556120 [70,80–82,86]	CD8-antibody LNP with CD19 CAR mRNA where disclosed *	CD19	SLE/SSc/RA, myasthenia gravis, and Graves disease	Recruiting/not yet recruiting; one terminated record; five-patient peer-reviewed correspondence	Five-patient correspondence with 3-month follow-up; durability and tissue B-cell depletion unresolved
HN2302/NCT07413835, NCT07523282 [84,85]	*In-vivo* CAR-T product; RNA format, target, and carrier not disclosed in registry records	Not disclosed	Myasthenia gravis, SLE, and systemic sclerosis	Recruiting/not yet recruiting	Program identity is public, but target and carrier details are insufficient for comparison
Arnovie101/NCT07629596 [69]	mRNA-LNP; targeting ligand not disclosed in the registry	B-cell target not stated	SLE and autoimmune hemolytic anemia	Recruiting, Early Phase 1	Five-participant study; no posted results
AFN50/NCT07448298, NCT07408336 [71,72]	T-cell-targeted LNP carrying CAR RNA or mRNA	Not disclosed	Refractory autoimmune diseases	Recruiting, Early Phase 1	RNA and CAR details differ between records
InViVoCAR1920/NCT07321301 [83]	Polymer-lipid particle carrying dual CAR mRNA	CD19/CD20	R/R B-cell lymphoma or leukemia	Recruiting, Phase 2	Registry record; delivery ligand and clinical results not public
Undisclosed linear programs/NCT07349849, NCT07362758, NCT07362602, NCT07388212 [73–76]	LNP-mRNA; product identity or targeting ligand undisclosed	CD19, CD20, or CD19/BCMA	B-cell malignancies and autoimmune diseases	Recruiting/not yet recruiting	Registry records establish modality only

Circular-RNA comparators/NCT07413341, NCT07322718 [61,62]	T-cell-targeted or immunotropic LNP; circular RNA	CD19	Relapsed or refractory autoimmune diseases	Recruiting, early phase	Different RNA format; registration does not establish superiority to linear mRNA

Trial status is extracted from ClinicalTrials.gov registry records accessed in July 2026 and does not constitute proof of clinical efficacy. Note that multiple independent NCT identifiers may be grouped within a single table row; the reported "current status and evidence level" column provides a collective summary for all registry entries listed in that row and may not exactly reflect the individual status of each separate trial record. Readers can retrieve real-time, trial-specific status information for each study by searching the corresponding unique NCT number on ClinicalTrials.gov. Patient-level evidence comprises a five-patient peer-reviewed correspondence for HN2301 and two three-patient meeting abstracts for GT801 and STR-P004; none permits a comparative efficacy estimate. No trial in this table explicitly identifies an saRNA cargo. * Inferred from partial trial-registry disclosures, not explicitly stated in official registry entries. Abbreviations: AIHA, autoimmune hemolytic anemia; B-ALL, B-cell acute lymphoblastic leukemia; BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; CD, cluster of differentiation; LNP, lipid nanoparticle; mRNA, messenger RNA; NHL, Non-Hodgkin lymphoma; RA, rheumatoid arthritis; R/R, relapsed/refractory; RNA, ribonucleic acid; saRNA, self-amplifying RNA; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

The registered programs concentrate in autoimmune disease and CD19/CD20-positive B-cell malignancies [61–87]. Autoimmune programs seek finite B-cell depletion followed by immune reconstitution, whereas oncology programs must control an expanding malignant burden. Public records identify CD8-targeted LNPs for CPTX2309 and HN2301, an antibody-displayed linear-mRNA LNP for GT801, and a polymer-lipid dual-CD19/CD20 construct for InViVoCAR1920; many other records omit the ligand, RNA format, or CAR construct. The registries also lack common measurements of absolute CAR-positive subsets, receptor density, off-target expression, and dose-resolved cellular exposure, preventing mechanistic comparison.

Peer-reviewed human evidence is limited to a five-patient correspondence on HN2301 in refractory systemic lupus erythematosus. CD8-targeted LNPs delivered CD19 CAR mRNA without lymphodepletion; transient CAR-positive CD8 T cells preceded peripheral B-cell depletion and lower disease activity at three months, with no major toxic effects reported [86]. These observations establish delivery and pharmacodynamic activity, but not durable remission, tissue B-cell clearance, or the incidence of uncommon toxicities.

A 2026 American Society of Clinical Oncology abstract reported GT801 in three adults with B-cell non-Hodgkin lymphoma [63]. One patient received three weekly 0.5 mg doses and two received four weekly 1.5 mg doses without lymphodepletion. CAR-positive CD8 T cells appeared within 4 h and peaked on day 1; reported reprogramming reached 93%, although the denominator was unclear. Peripheral B cells were depleted within 4 h for at least 7 days, and all patients responded at week 4. Grade 1–2 adverse events included infusion-related symptoms; no grade ≥ 3 hematologic toxicities, cytokine release syndrome, immune-effector-cell-associated neurotoxicity syndrome, or organ failure were documented.

A second 2026 abstract described STR-P004 in three patients with autoimmune disease receiving up to eight infusions [87]. CAR-positive cells peaked at 12 h; peripheral B-cell depletion occurred within 72–156 h and lasted 3–12 days. Grade 1 CRS followed the first two infusions, with no reported dose-limiting toxicity or ICANS. Clinical and serological responses were inconsistent, including a rebound in anti-double-stranded DNA

antibodies and nonsustained platelet gains. The report demonstrates transient pharmacodynamic activity, not durable remission.

In aggregate, publicly available patient-level data encompass 11 individuals across three independent reports. The HN2301 cohort includes five patients with refractory systemic lupus erythematosus who each received a single infusion without prior lymphodepletion; circulating CAR-positive CD8+ T-cells were quantified via peripheral-blood flow cytometry. These subjects demonstrated transient B-cell depletion and reduced disease activity, though the available follow-up does not establish the durability of remission; longer observation periods will be required to rigorously assess sustained clinical benefit across these early clinical cohorts [86]. The GT801 cohort enrolled three patients with relapsed/refractory B-cell non-Hodgkin lymphoma, receiving either three weekly 0.5 mg infusions or four weekly 1.5 mg infusions with no lymphodepletion. CAR-positive CD8+ T-cell kinetics were assessed by peripheral-blood flow cytometry; clinical responses were observed at week 4, with grade 1–2 infusion-related adverse events and absence of grade ≥ 3 hematologic, cytokine-release, neurotoxic, or organ-related adverse events, with at least four-week follow-up [63]. The third published cohort (STR-P004) comprises three patients with refractory autoimmune disease treated with up to eight infusions in the absence of lymphodepletion. Circulating CAR-positive cells were measured by peripheral-blood flow cytometry. Transient peripheral B-cell depletion persisted for 3–12 days, but serological and clinical responses were inconsistent; only grade-1 cytokine-release syndrome was observed after early infusions, without dose-limiting toxicity or ICANS [87]. Important gaps remain for all cohorts: complete dose details are not fully disclosed for HN2301 and STR-P004, and durability beyond the reported follow-up windows remains undetermined.

These observations stem from one peer-reviewed correspondence and two meeting abstracts [63,86,87]. Among these 11 patients, indications include refractory systemic lupus erythematosus and relapsed/refractory B-cell malignancy; note that repeated-infusion regimens were adopted for GT801 and STR-P004, while HN2301 used a single-infusion schedule. Where reported, CAR-positive T-cells peaked at Day 1 for GT801 and 12 h for STR-P004, whereas corresponding peak-time data are not publicly available for HN2301. All cohorts showed transient peripheral B-cell depletion, together with partial clinical or serological responses. CRS was observed only in the STR_P004 cohort. Reported patient follow-up durations are at least four weeks for GT801 and STR-P004, and three months for the HN2301 cohort. Comprehensive baseline patient characteristics cannot be obtained from current public sources. Collectively, these reports establish transient CAR-positive-cell generation and target depletion in humans, but not comparative efficacy, durability, a dose–response relationship, or the incidence of uncommon toxicities.

5.6. Limits of the Current Evidence

Cross-study numerical comparison is not valid because studies differ in denominators, tissues, sampling times, reporters, CAR constructs, RNA chemistry, dose, species, disease burden, LNP composition, targeting-ligand density, and assay methodology [25,33–38,41–53]. These confounding factors greatly complicated cross-platform inference. Aligned within-study comparisons can identify mechanisms when key variables are controlled [36,50]. Blood CAR-positive-cell percentages, organ bioluminescence, and splenic dose recovery are not commensurate; incomplete formulation and assay disclosure further limit replication. Registry records, correspondence, and abstracts also lack a shared endpoint set [61–87]. Interpretation should therefore keep administered dose, cellular exposure, pharmacodynamic activity, and clinical outcome separate [10–13,26,27].

6. Future Directions for RNA Cargo and Effector-Cell Selection

Linear mRNA is the clinical manufacturing benchmark, but its finite expression can create a redosing requirement. Circular RNA and self-amplifying RNA (saRNA) are designed to extend expression, while adding topology- or replication-specific manufacturing and innate-sensing constraints. Multigenic cargo adds logic or safety functions at the cost of greater product complexity, and CAR-NK introduces a separate cell-targeting problem. These extensions should be compared by administered dose, cell-resolved exposure, expression duration, innate sensing, manufacturability, and direct functional evidence (Table 6) [12,13,18,19,54–59,88,89].

Table 6. Translational Properties of RNA Formats for *In-vivo* CAR-T Cell Engineering.

RNA Format	Expected Duration	Dose Rationale	Payload Capacity	Innate Sensing	Manufacturing Maturity	Experimental Evidence (*Ex-Vivo* Primary Cell/Systemic *In-Vivo* Preclinical)	Unresolved Risk
Linear mRNA	Days-scale; non-replicating	Finite exposure; redosing when longer exposure is needed	Supports single-CAR and compact multigenic payloads; length limits yield and encapsulation	Modified bases, double-stranded RNA impurities, and lipid sensing	Established clinical mRNA-LNP workflow	Direct functional evidence in primary human T-cells; systemic *in-vivo* evidence in mice, humanized mice and non-human primates [25,33–38,41–53]	Finite exposure can require redosing for durable control
Circular RNA	Longer expression than matched linear RNA in the cited PL40 comparison [48]	Longer exposure without a replicase	Circularization and purification become harder as transcripts lengthen [90]	Junctions, impurities, and translation elements	Format-specific circularization and purification; no approved CAR product	*Ex-vivo* human T-cell data; systemic *in-vivo* mouse CAR evidence; two registered clinical programs [48,61,62]	Selectivity, manufacturing control, persistence, and redosing remain undefined
saRNA	Replicase-dependent amplification; systemic duration unknown	Designed to reduce input dose or extend expression	Large replicon cargo	Replicase, double-stranded RNA, and innate sensing	Clinical vaccine experience; no reported *in-vivo* CAR-T product	*Ex-vivo* modified-saRNA human T-cell data; no published systemic saRNA-LNP CAR-T evidence	Requires matched mRNA and off-target controls

No appropriately matched head-to-head *in-vivo* comparisons are available among these RNA formats with fixed LNP chemistry, targeting ligand, CAR construct and dose. Observed differences cannot be solely attributed to RNA topology given co-varying confounding factors. Abbreviations:

CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; LNP, lipid nanoparticle; mRNA, messenger RNA; RNA, ribonucleic acid; saRNA, self-amplifying RNA.

6.1. Circular RNA

Circularization removes free RNA ends and is hypothesized to increase exonuclease resistance, but transcript length, circularization efficiency, nicking, purification, and impurities affect quality and innate sensing; CAR-encoding transcripts often exceed 3 kb, reducing circularization and purification yields and creating a manufacturing bottleneck [90]. In isolated mouse T cells, circular uPAR-CAR RNA produced approximately 99% CAR-positive cells at 24 h versus 80% with linear RNA and approximately tenfold higher receptor fluorescence; approximately 15% remained positive on day 5, whereas linear RNA fell below 5% by day 2.

PL40 circular-RNA CARs reduced disease in mouse fibrosis and arthritis models [48], but the *in-vivo* experiments did not isolate topology using exposure-matched linear and circular products, and cell selectivity was incomplete. Whether prolonged CAR expression conferred by circular RNA can translate into superior *in-vivo* therapeutic benefit remains a hypothesis awaiting rigorous validation. Two registered circular-RNA programs establish clinical development activity, not superiority [61,62]. Relevant assays include junction identity and purity, translation initiation, surface-CAR exposure, tissue selectivity, and repeat-dose behavior.

Topology and carrier effects require a two-dimensional matched design. The design should use the same LNP to deliver linear versus circular RNA encoding the same CAR, and the same circular RNA delivered by different carriers, with matched dose and cellular exposure [48].

6.2. Roles for saRNA

saRNA encodes a replicase that amplifies RNA in the cytosol, with the hypothesized aim of reducing input dose or extending functional CAR expression *in-vivo*. Replication, translation, and interferon signaling depend on nucleotide substitution, replicon design, vehicle, route, and lipid chemistry [54–59,91–93]. Vaccine trials establish manufacturability and human administration [94–96], and non-CAR studies show sustained expression or antitumor activity [88,97,98]. None demonstrate selective intravenous delivery of saRNA-CAR to T cells.

A 2026 preprint introduced modified saRNA into primary human T cells *ex-vivo* and transferred the cells to leukemia-bearing mice [89]. Relative to conventional mRNA, saRNA prolonged CAR expression and supported multigenic logic, but the study did not test systemic saRNA-LNP delivery or *in-vivo* cell generation. No trial in Table 5 explicitly identifies saRNA [61,62,64–85]. A decisive experiment would combine replication, T-cell-targeted systemic delivery, and cell-resolved surface CAR measurement in the same animal.

6.3. Head-to-Head Evaluation of saRNA-CAR

A head-to-head comparison requires the same CAR, targeting ligand, and LNP for saRNA and linear mRNA. Dose-matched analyses test input-dose reduction, whereas exposure-matched analyses compare function and toxicity at comparable early surface CAR levels. These two represent complementary analytical frameworks; experimental constraints often preclude satisfying both matching criteria within a single study [18,19,54–59,88,89]. Measurements should include RNA integrity, encapsulation, replicative intermediates, CAR-positive-cell number and duration, receptor density, cell fitness, cytokines, killing, off-target expression, recovery, and redosing. Longer expression warrants

advancement only if it improves disease control without unacceptable inflammation or off-target exposure.

6.4. Multigenic Cargo

Alternative cargoes encode dual-target OR gates, AND-gated recognition, safety switches, or immunomodulators [12,13,17,89]. Each addition should address a defined failure such as antigen escape, off-tumor recognition, or inadequate persistence. Longer, structured transcripts complicate full-length yield, integrity, encapsulation, purification, and potency testing [18,19,89], so multigenic products require matched comparison with a simpler single-CAR control.

6.5. CAR-NK as a Separate Delivery Challenge

CAR-NK introduces a separate delivery challenge. NK cells provide innate cytotoxicity and lower graft-versus-host risk in the allogeneic setting. Clinical studies of IL-15-expressing cord-blood-derived CD19 CAR-NK have reported that engineered NK cells can remain molecularly detectable for at least 12 months; nevertheless, sustained molecular detectability does not equate to persistent antigen-specific functional activity. These clinical observations demonstrate limited sustained functional activity despite prolonged transgene detectability. T-cell data do not establish LNP selectivity, uptake, trafficking, or RNA sensing in NK cells. RNA-LNP CAR-NK therefore requires independent optimization of targeting, delivery, expansion, persistence, and distribution [99,100].

7. Conclusions

Systemic RNA-LNP delivery can generate CAR-positive cells and pharmacodynamic activity in primary-cell and animal models, including non-human primates [25,33–38,41–53]. Human evidence is limited to 11 patients in one correspondence and two meeting abstracts [63,86,87], which is insufficient to establish durable benefit, a dose–response relationship, platform equivalence, or the incidence of uncommon toxicities. These 11 reported individuals span distinct diseases (refractory SLE, B-cell NHL, mixed autoimmune disorders), divergent dosing schemes (single-dose versus up-to-eight repeated infusions), and variable lymphodepletion status, and therefore cannot be statistically pooled into a single combined patient cohort. Across studies, receptor trafficking, ligand presentation and density, lipid chemistry, RNA design, and CAR architecture govern conversion of injected RNA into functional surface CAR. Organ accumulation or particle uptake cannot substitute for cell-resolved evidence of translation and antigen-specific function.

Development should begin by defining the intended cell population and exposure window, then selecting the CAR, RNA format, ligand, LNP, and dose around that target product. The decisive questions are the cellular exposure required for durable response, whether redosing reproduces that exposure without anti-carrier immunity or diminishing returns, and whether transient expression reduces toxicity without sacrificing disease control. No targeting ligand has shown platform-wide superiority; equivalence to *ex-vivo* CAR-T remains unproven; and no peer-reviewed study has demonstrated systemic T-cell-targeted saRNA-LNP generation of functional CAR-T cells. Advancement should depend on reproducible cell-resolved exposure, pharmacodynamic linkage, recovery criteria, and long-term safety rather than injected RNA mass alone.

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Statement

We have noted that some of the authors are employees of a biotechnology company that is developing *in-vivo* CAR-T therapeutics; the evidence-tiering and measurement framework presented herein is derived from the publicly published literature and does not constitute product-specific claims.

Abbreviations

Abbreviation	Definition
AIHA	Autoimmune hemolytic anemia
ASCO	American Society of Clinical Oncology
B-ALL	B-cell acute lymphoblastic leukemia
BCMA	B-cell maturation antigen
CAR	Chimeric antigen receptor
CAR-NK	Chimeric antigen receptor-engineered natural killer cell
CAR-T	Chimeric antigen receptor T cell
CD	Cluster of differentiation
CMC	Chemistry, manufacturing, and controls
CRS	Cytokine release syndrome
FAP	Fibroblast activation protein
Fc	Fragment crystallizable
FDA	U.S. Food and Drug Administration
HER2	Human epidermal growth factor receptor 2
HLA	Human leukocyte antigen
ICANS	Immune effector cell-associated neurotoxicity syndrome
IL-7	Interleukin-7
LNP	Lipid nanoparticle
MHC	Major histocompatibility complex
mRNA	Messenger RNA
NCG	NOD/ShiLtJGpt-Prkdcem26Cd52Il2rgem26Cd22/Gpt immunodeficient mouse strain
NHL	Non-Hodgkin lymphoma
NK	Natural killer
PA	Phosphatidic acid
PEG	Polyethylene glycol
PET	Positron emission tomography
PK/PD	Pharmacokinetics/pharmacodynamics
pMHC-II	Peptide-major histocompatibility complex class II
PSMA	Prostate-specific membrane antigen
RA	Rheumatoid arthritis

RNA	Ribonucleic acid
R/R	Relapsed/refractory
saRNA	Self-amplifying RNA
scFv	Single-chain variable fragment
SLE	Systemic lupus erythematosus
SORT	Selective organ targeting
SSc	Systemic sclerosis
TCR	T-cell receptor
uPAR	Urokinase-type plasminogen activator receptor
VHH	Variable heavy-chain domain of a heavy-chain-only antibody

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