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Review article

In vivo nano-engineering T cells for CAR-T therapy☆

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ABSTRACT

In vivo nano-engineering of T cells has moved from a delivery question to an integrated framework that combines targeting, exposure, and expression to build CAR T therapies within patients. We distill the design rules of active targeting on viral and nonviral carriers (ligand architecture, avidity, and linker chemistry shaping receptor engagement, uptake) and endosomal escape, and contrast them with practical constraints from variable EPR, off-targeting, and batch consistency. At the organ scale, spleen-tropic designs provide a pragmatic exposure platform shaped by microanatomy, hemodynamics, and protein-corona programming. Unlike traditional liver-directed systems, spleen-targeting nanocarriers as a novel and underexplored therapeutic direction for in vivo T-cell programming, offer the potential for selective delivery to lymphoid-resident T cell precursors, facilitating more precise immunomodulation. Because distribution is not the same as expression, specificity therefore comes from payload logic, including promoters tuned to lineage or activation state, UTR designs with miRNA detargeting, and protein or logic-gated circuits, which confine expression without changing biodistribution. We outline translational priorities in targeting precision, analytics and CMC, and regulatory classification, and advocate coordinated design of active targeting, corona-aware exposure, and expression-centric control for scalable, indication-tailored in vivo CAR T.

1. Introduction

Over the past decade, cell therapy, which primarily utilizes engineered T cells for target cell killing, has made significant advancements for cancer treatment [1]. Through various techniques in biology, genetics, and materials science, T cell functions are enhanced to recognize specific targets or epitopes. Viral (or non-viral) vectors facilitate the genetic engineering of T cells, enabling them to express chimeric antigen receptors (CARs) or transgenic T cell receptors (TCRs) specifically designed for therapeutic applications at targeted disease sites [2–4]. Once reinfused into the patients, these in vitro processed T cells trigger an anti-tumor immune response. In 2017, the first CAR-T therapies were introduced into clinical practice. As of 2025, seven CAR-T cell products have received U.S. FDA approval—tisagenlecleucel (Kymriah), axicabtagene ciloleucel (Yescarta), brexucabtagene ciloleucel (Tecartus), lisocabtagene maraleucel (Breyanzi), idecabtagene vicleucel (Abecma), ciltacabtagene autoleucel (Carvykti) and obecabtagene autoleucel (Aucatzyl)—spanning B-ALL, LBCL/MCL/CLL and multiple myeloma [5]. Notably, in June 2025, FDA removed the REMS (Risk evaluation and mitigation strategies) requirement for approved CAR-T products, signaling the maturation of risk controls, while boxed warnings remain in place.

To minimize the adverse effects associated with adoptive cell therapy and improve patient outcomes, personalized treatment plans tailored to each individual patient are imperative [6]. This personalized approach underscores the necessity for engineered T cells to be derived either from the patient themselves or from healthy donors (Fig. 1a). However, additional complex genetic engineering maneuvers are required to mitigate graft-versus-host reactions. The specific drug formulation and its source are crucial factors in guaranteeing consistent production [7]. Typically, T cells from a patient are collected by leukapheresis, transduced with retroviruses or lentiviruses derived by complex processes,

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and expanded into individualized cellular products [6]. Advanced design and rigorous processing endow engineered T cells with the ability to recognize epitope antigens; however, these processes can also introduce some negative effects. Due to the complexity of this process, the time interval between T cell harvesting and engineered T cell infusion typically takes several weeks, which can lead to missed opportunities for optimal treatment, especially in patients with acute diseases [8]. Moreover, the economic aspect of this process cannot be overlooked. Recent analyses suggest that manufacturing CAR T-cell therapies using current methods remains costly, typically in the high six-figure range per product, while some approved therapies are priced above that level [9]. Therefore, it is of utmost importance to identify the primary cost drivers and the timeliness of T cell engineering, as well as to stay abreast of trends in cell therapy development strategies. (See BOX 1.)

In recent years, breakthroughs in gene delivery technologies have revolutionized our capacity to direct and regulate cellular functions. Techniques in viral vector engineering and alternative nucleic acid delivery methods, including liposomal nanoparticles (NPs) and polymer-based NPs, have significantly broadened the range of achievable genomic alterations [10]. These genomic delivery systems serve as a foundational platform for in vivo T cell engineering, which presents a versatile strategy that shortens the manufacturing process and facilitates swift access to therapy for patients experiencing disease progression [11] (Fig. 1b). Viral or non-viral vectors are administered directly to the patient to program circulating T cells in the body; delivered nucleic acids are translated by ribosomes to produce the CAR/TCR that is displayed on the T-cell surface (non-viral mRNA—transient; viral vectors—more durable). This approach is expected to overcome the prevailing clinical challenges associated with in vitro T cell engineering, such as the lack of lymphocyte depletion chemotherapy and its subsequent symptoms [12]. Moreover, an intact immune system may more effectively promote epitope spreading and elicit a robust anti-tumor immune response [13]. Parallel to ex vivo manufacturing, direct in vivo CAR engineering has rapidly advanced from proof-of-concept in rodents to translational studies. A recent landmark by Hunter et al. reported lipid nanoparticle (LNP)-mediated in vivo generation of functional CAR-T cells in non-human primates (NHP), achieving therapeutic benefits across oncology and autoimmunity models—firmly positioning LNPs as a clinically credible platform for in situ T-cell programming [14]. This trend is comprehensively framed by Li et al., who synthesize mechanistic and translational principles across vector design, immune targeting and clinical readiness [15].

The design of a gene-delivery vector is a central step and the technical basis for implementing in vivo CAR T cells. In this review, we focus on the targeted nanodelivery systems currently used for in vivo CAR T cells. We describe the working principle of the targeted delivery system, the different types of targeted delivery strategies, and discuss their potential application for in vivo CAR T cells. In addition, some progress in front-end clinical research is also discussed here.

2. Overview of targeted nano-engineering systems

The fundamental principles underpinning targeted nanodelivery systems involve three interdependent processes: loading, targeting, and release. The effectiveness of a nanodelivery system depends on the optimal integration of these processes. The initial design phase involves the encapsulation or conjugation of therapeutic agents (e.g., RNA, DNA, proteins, or small-molecule drugs) onto the nanoparticle surface or within its core [40,41]. Depending on the nanoparticle type, loading may occur via physical encapsulation, covalent conjugation, or surface

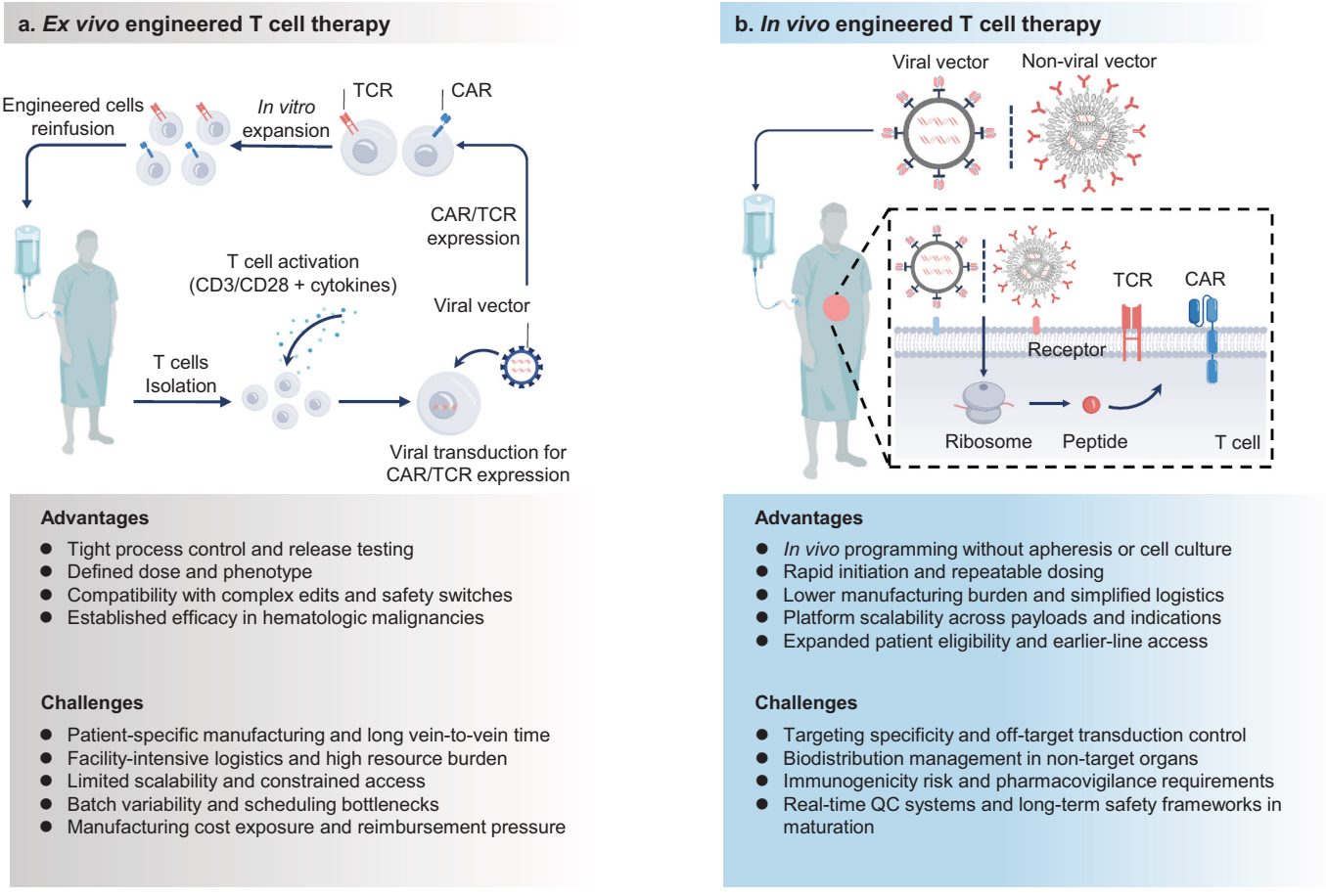

Fig. 1. Overview of (a) ex vivo engineering T cell therapy in comparison with (b) in vivo engineered T-cell therapy.

BOX 1

CAR-T cells from discovery to clinical utility

In 1987, Kuwana and his colleagues first proposed the concept of chimeric antigen receptor (CAR) [16]. When introduced into cytotoxic T cells (CTLs), these chimeric receptors enable T cells to recognize and kill target cells [17]. In the subsequent decade, many pioneering works have significantly contributed to the advancement of CAR in T cells [18–20]. While the first generation of CAR-T showed marginal clinical efficacy due to constrained in vivo proliferative capacity [21–23], the realization emerged that CAR-T therapy faced significant hurdles, including the need for a durable immune response and apoptosis [24,25]. Fortunately, the persistence and tumor control ability of CAR T cells was then improved by adding CD28 or 4-1BB (CD137) costimulatory domain [26,27]. By the early 2010s, the second-generation CAR was gradually being tested in clinical trials and had shown unprecedented clinical application value [28–30].

With the development of gene editing technology, more optimization strategies have been proposed to enhance the durability of CAR T cells [31,32]. The lentivirus-based CAR-T cell technology is considered to be safe in the setting of chronic HIV infection [33]. CD19-targeting CAR T cells showed CD19 specificity and retention in patients' blood and bone marrow samples for several months [29]. In 2017, FDA historically approved the first CAR T cell drug Kymriah® (tisagenlecleucel) for the treatment of acute lymphoblastic leukemia in patients aged 25 or younger [34]. Today, CAR-T cell drugs have entered numerous clinical trials aimed at treating various diseases, including solid tumors and autoimmune diseases. Notably, the re-development of this cell therapy has shifted the focus from oncologic therapy to autoimmune diseases such as systemic lupus erythematosus (SLE), idiopathic inflammatory myositis (IIM), and systemic sclerosis (SSc) [35,36]. This is supported by recent clinical findings of CD19 CAR-T therapy for autoimmune diseases [37].

Allogeneic platforms vs direct in vivo engineering

Allogeneic CAR platforms promise immediate availability, centralized GMP production and lower COGS but require additional edits (e.g., B2M/CIITA KO, HLA-E upregulation) to mitigate host rejection and NK-mediated clearance; they also carry risks of GvHD if TCR is incompletely disabled. In contrast, direct in vivo engineering leverages the patient's own T cells as substrates, potentially bypassing leukapheresis, logistics and batch variability [38]. Allogeneic products often show limited persistence without extensive immune evasion engineering or lymphodepletion; in vivo–engineered cells may inherit autologous persistence traits but place a greater onus on targeting specificity and dose control. For indications that demand rapid deployment across large populations or where patient T cells are deeply compromised, allogeneic strategies are attractive. For precision gene circuits (base/prime editing, regulated CARs) or multi-omics personalization, in vivo engineering offers a uniquely modular path that can iterate per patient without re-manufacture [39].

adsorption. The encapsulation efficiency and payload stability constitute fundamental determinants of nanocarrier performance, critically impacting systemic bioavailability and ultimate therapeutic outcomes. Nanodelivery systems offer distinct advantages for challenging therapeutics, particularly nucleic acids (DNA/RNA) that exhibit pronounced susceptibility to enzymatic degradation in biological systems. Through protective encapsulation within engineered nanoparticle matrices, these labile biomolecules achieve enhanced stability during systemic circulation and targeted delivery to cells of interest [42,43].

Nanodelivery platforms are designed with multifunctional capabilities to achieve targeted recognition, payload protection, controlled release, and efficient cellular uptake [44–46]. Various nanomaterials have been investigated for these purposes, including lipid-based nanoparticles, polymeric nanoparticles, dendrimers, and inorganic nanoparticles, each offering distinct advantages in terms of stability, biocompatibility, and payload capacity [47–49]. Particularly, surface modification of these nanoparticles with ligands, antibodies, or aptamers enables precise targeting of T cells within a physiological environment (Fig. 2). One prominent strategy involves conjugating nanoparticles with T cell-specific ligands that bind to surface receptors, thereby ensuring high specificity and minimizing off-target interactions [50]. Another approach utilizes nanoparticles equipped with pH-sensitive or redox-responsive linkers, which enable controlled release within the specific intracellular environment of T cells [51]. Collectively, these innovations enhance the precision of in vivo T cell engineering, thereby improving therapeutic outcomes while minimizing adverse effects.

The potential of targeted nanodelivery systems extends beyond the delivery of genetic material for T cell modification. These systems also facilitate the direct delivery of drugs, proteins, and CRISPR-Cas9 complexes, thereby enabling in situ reprogramming or enhancing T cell function. As the field progresses, a comprehensive understanding of nanoparticle biodistribution, immune clearance, and long-term safety will be essential for translating these technologies from the laboratory to clinical applications.

3. Implementation strategy of targeted delivery-active targeting

Nanomedicine aims to enhance systemic drug delivery to specific sites, thereby increasing therapeutic efficacy while reducing side effects. Since the concept of targeted cancer therapy emerged, the enhanced permeability and retention (EPR) effect has played a critical role in accumulating macromolecules or NPs in tumors. While initially attributed to tumor-specific vascular abnormalities (e.g., leaky vasculature and impaired lymphatic drainage), the EPR effect is now recognized for its complexity [52–54]. Beyond solid tumors, EPR-like effects have been applied to various diseases such as atherosclerosis, inflammatory bowel disease, and rheumatoid arthritis [55–57]. However, the EPR effect is primarily restricted to inflamed tissues and passive extravasation from the systemic circulation, and the absence of reliable biomarkers for EPR heterogeneity complicates patient stratification [58–60]. This variability substantially hinders the reliable prediction of NPs' biodistribution patterns and accumulation efficiency across different tissues.

To improve organ/tissue targeting beyond the EPR effect, "active" delivery using ligand-conjugated NPs has been widely investigated by leveraging ligand-receptor interactions (Table 1). This approach, which enables the recognition of specific receptors on target cells and enhances therapeutic delivery and retention, has demonstrated considerable success in diverse therapeutic modalities, including antibody-drug conjugates, small peptide-radionuclide conjugates, and GalNAc-siRNA conjugates [61,62]. Ligand-targeted NP delivery also exhibits significant potential for enhancing drug accumulation within specific tissues and overcoming physiological barriers (e.g., the intestinal mucosa and blood-brain barrier) [63,64].

3.1. Envelope protein engineered viral vector for T cell engineering

Among enabling platforms, envelope-engineered viral vectors are pivotal for achieving cell-type–selective gene transfer with high-level, durable expression. Foundational lentiviral and retroviral systems

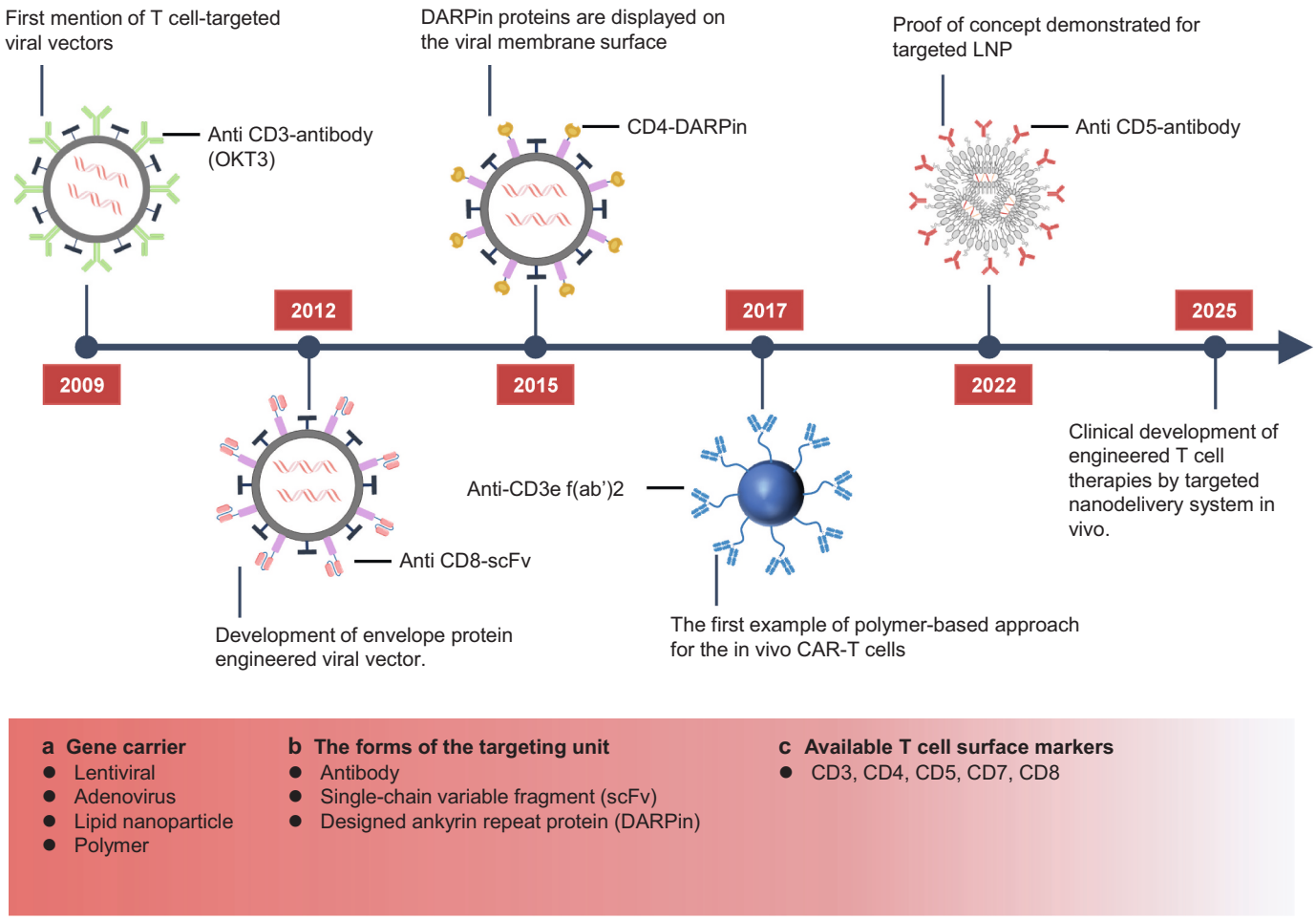

Fig. 2. Timeline of in vivo CAR-T cell production showing key milestones in technological advances.

Table 1
Overview of active targeting platforms for in vivo T cell engineering.

Vectors	Ligand	Payload	Indication	Ref
Lentivirus	Anti-CD8 antibody	DNA (CD19 CAR)	Acute lymphoblastic leukemia	[65]
	CD4 DARPin	DNA (maC46, ErbB2-CAR, FOXP3)	HIV, breast cancer, autoimmune, and inflammatory disease	[66]
	CD3 scFv	DNA (CD19 CAR)	Leukemia	[67]
	CD3 scFv & ligand binding domains of CD80 and CD58	DNA (CD20 CAR)	B-cell malignancies	[68]
AAV	No cell targeting moiety	DNA (CD4 CAR)	Human T-cell leukemia	[69]
LNP	Anti-CD4 antibody	mRNA	HIV	[70]
	Anti-CD5 antibody	mRNA (FAP CAR)	Cardiac injury	[71]
	Anti-CD3 antibody	mRNA (TRR1 CAR & IL-7)	Melanoma	[72]
	Anti-CD8 antibody	mRNA (CD19 CAR)	Leukemia, SLE	[14]
	Anti-CD3e F (ab)2 fragment	DNA (194-1BBz CAR)	Leukemia	[73]
Polymer	Anti-CD8 antibody	mRNA (1928z CAR or HBcore18–27 TCR)	Leukemia, prostate cancer, et al	[74]

established therapeutic delivery to T cells (Fig. 3a), and subsequent envelope retargeting has progressively improved selectivity, potency, and manufacturability. Surface-engineered lentiviral particles that co-display an anti-CD3 binding moiety (OKT3) with a fusogenic protein constitute the first $CD3^+$ T-cell targeted vectors, enabling bona fide in vivo T cell programming and TCR transfer against tumor antigens [75]. This proof-of-concept established the general principle that receptor-guided envelopes can convert broadly tropic vectors into T-cell–selective delivery vehicles in situ. Design experience since then has underscored several practical rules: ligand architecture and density must support high-avidity binding without compromising envelope stability; insertion site and promoter choice tune expression and vector fitness; and payload format (DNA vs mRNA) trades durability against immunogenicity and re-dosability. Remaining challenges include off-target transduction, pre-existing or induced anti-vector immunity, and real-time quality control are increasingly addressable through modular ligand engineering, orthogonal entry pathways, and built-in safety circuitry. Together, these advances frame in vivo T cell engineering as a tractable problem of ligand–receptor biology and vector biomechanics rather than solely one of cargo design.

Building on receptor targeting, in vivo TCR gene transfer elicits functional antitumor responses in relevant models [76]. CD8-targeted lentiviral vectors (CD8-LV) carrying a second-generation anti-CD19 CAR generate CAR-T cells in situ with measurable tumor control in NSG mice [77]. Development of CD4-LV further advanced the field by achieving selective transduction of resting primary $CD4^+$ T cells cell states that generally resist lentiviral entry without mitotic activation [66,78,79]. Despite this capability, the intrinsic restriction factor

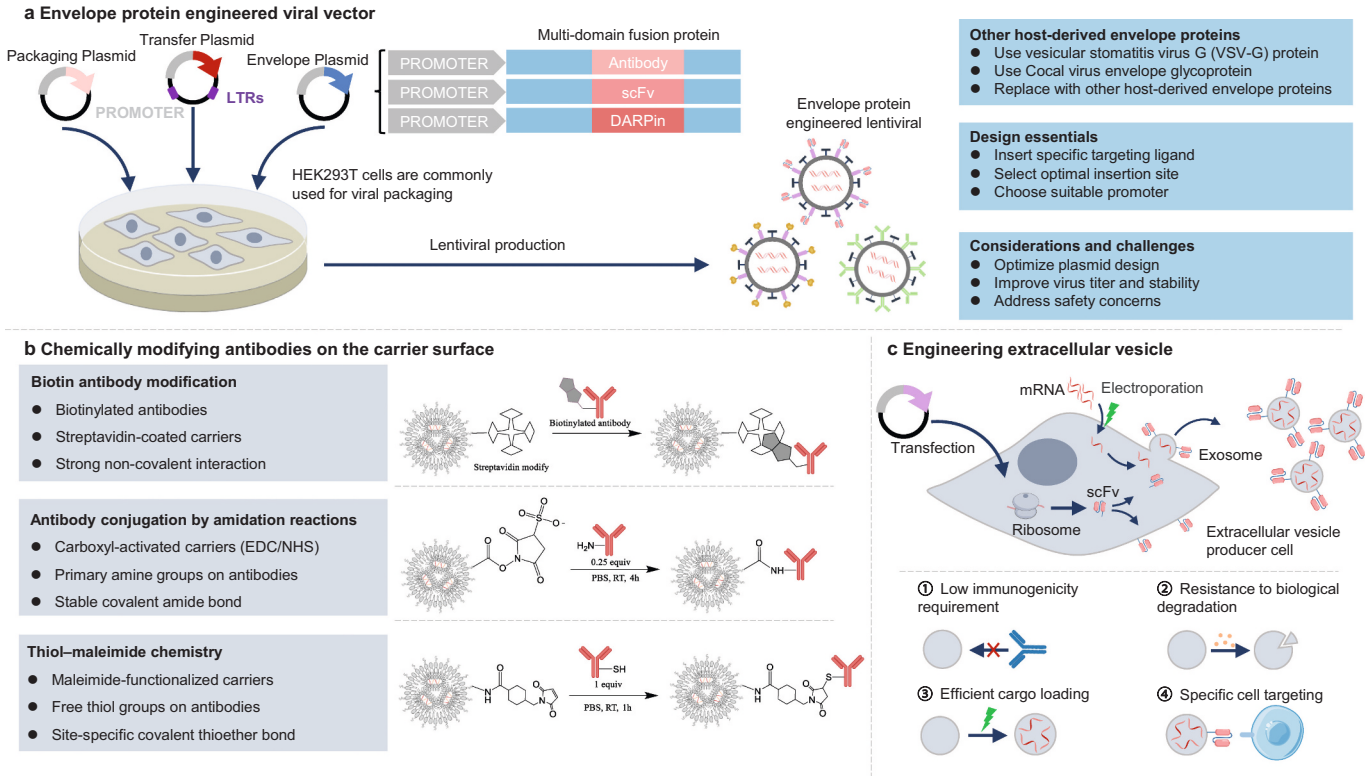

Fig. 3. Platforms for targeted in-vivo T-cell programming. (a) Envelope-engineered viral vectors. (b) Chemical modification strategies for displaying antibodies on the surface of carrier. (c) Engineered extracellular vesicles (EVs).

SAMHD1 continues to limit gene transfer into quiescent T cells, constraining clinical efficiency unless addressed by vector design or dosing strategies [80]. To increase on-target entry, lentiviral envelopes displaying anti-CD3 single-chain fragments have been used to program $CD3^+$ T cells in vivo with anti-CD19 CAR cargo [81]. Anti-CD3 engagement promotes activation and internalization, thereby enhancing transduction, but it also introduces risks of excessive activation and exhaustion. Consequently, entry efficiency must be balanced against T cell fitness particularly under repeated dosing. Pragmatically, co-receptor targeting (CD4 vs. CD8) should be matched to the intended effector repertoire, while CD3-based retargeting may be tempered by weaker affinity binders, transient payloads (e.g., mRNA or integrase defective LVs), or conditional/logic-gated expression to reduce bystander activation. Parallel tactics such as SAMHD1 antagonism, promoter and insertion-site optimization, and microRNA detargeting can further enhance selectivity, durability, and safety.

Adeno-associated virus (AAV) vectors provide a complementary alternative to lentiviral and retroviral platforms. Key attributes include episomal persistence, efficient transduction of non-dividing cells, and continuously evolving capsids with T cell tropism features that can reduce dependence on strong exogenous activation signals [82]. Single-infusion paradigms have generated circulating CAR-T cells with multi-week persistence and in-vivo cytotoxic activity, consistent with a "living drug" profile [69]. These properties make AAV attractive for selected indications and delivery contexts. Constraints remain: limited cargo capacity that restricts the use of complex CAR circuits; pre-existing anti-AAV immunity that complicates re-dosing; and a continuing need for vigilant integration surveillance despite the predominantly episomal fate.

Across viral modalities, class-specific risks shape development strategies. Anti-vector and anti-transgene immune responses may eliminate transduced cells and shorten durability; potent activation during in-situ CAR induction can precipitate cytokine-release syndrome in susceptible settings. Integrating retro/lentiviral vectors carries a non-zero risk of insertional mutagenesis, warranting long-term follow-up and careful vector/payload selection [83]. These considerations motivate precision envelope engineering to minimize bystander transduction and unintended activation, as well as dose-fractionated or step-up regimens, and—where appropriate—non-integrating or transient payloads to widen the therapeutic window.

Taken together, envelope protein engineering has transformed viral vectors from generic carriers into T-cell–addressable medicines capable of in-situ programming. The emerging design space couples receptor-informed entry (e.g., CD3, CD4, CD8, or alternative binders) with safety-by-design principles—controlled activation thresholds, tight off-target control, and explicit management of integration risk—providing a mechanistic bridge to non-viral and hybrid systems that apply the same targeting logic with distinct safety and manufacturability trade-offs.

3.2. Development of targeted non-viral vectors for nano-engineering T cells

Non-viral vectors have matured rapidly as clinically credible gene delivery systems, highlighted by the FDA approvals of mRNA vaccines formulated in LNPs. This clinical familiarity, together with modular chemistry and scalable manufacturing, positions synthetic nanomaterials as an attractive chassis for in vivo T-cell programming. That said, the trajectory for T-cell engineering with non-viral vectors lagged behind viral approaches; the first report of in situ T-cell reprogramming using synthetic nanomaterials appeared only in 2017 [73], and the field remains in an early stage relative to lentiviral/AAV platforms.

Foundational work by Billingsley et al. established that ionizable LNPs can deliver mRNA ex vivo to human T cells, driving transient CAR expression with favorable viability [84]. A follow-up study employed an orthogonal design-of-experiments (DoE) strategy to optimize composition, revealing the central levers of the ionizable lipid fraction/pKa, cholesterol content, helper phospholipid identity, and PEG-lipid mol

%/anchor length for balancing cargo release, endosomal escape, colloidal stability, and cytocompatibility [85]. These compositional rules now underpin in vivo vector tuning as the field moves from "transfection feasibility" to "destination-specific programming". To improve delivery precision, current strategies incorporate surface-engineered nanocarriers functionalized with antibodies or ligands that recognize immune cell populations. These modifications can be achieved through various chemical conjugation techniques — such as biotin–streptavidin interaction, EDC/NHS-mediated amide bond formation, or thiol–maleimide chemistry — as illustrated in Fig. 3b. Such chemically modified carriers enable modular design, enhance immune cell selectivity, and support gene delivery strategies tailored for in vivo T cell reprogramming.

3.2.1. Ligand-functionalized LNP for T cell nano-engineering

LNPs have become a central, clinically validated modality for nucleic-acid delivery in gene therapy and vaccination [86–89]. Beyond their formulation details, two properties primarily motivate their use in immune engineering: efficient cytosolic delivery via ionizable-lipid–mediated endosomal escape and generally favorable tolerability due to metabolizable, membrane-mimetic components [90–95]. The critical question is no longer whether LNPs can deliver mRNA, but how precisely they can be steered to desired immune subsets while avoiding dose-driven off-tissue transfection. Proof-of-concept studies now demonstrate in-body programming of CAR-T cells—for example, anti-CD5–targeted LNPs carrying modified mRNA encoding a FAP-CAR, thereby eliminating the ex vivo manufacturing bottleneck [71]. This is achieved by encapsulating modified messenger RNAs (mRNAs) within LNPs which, after administration, can induce the production of CAR T cells in vivo. Joe G and colleagues engineered mRNA containing modified nucleosides that encode a CAR targeting fibroblast activation protein (FAP), which is a marker of activated fibroblasts, and enclosed it within CD5-targeted LNPs [71]. CD5, primarily expressed on T cells and a subset of B cells, is not essential for T cell effector function. Yet the same data illuminate trade-offs: CD5 accessibility simplifies targeting but risks bystander transfection of non-T cells; ligand density and chemistry improve tropism but may increase opsonization and narrow the therapeutic window; and transient mRNA expression enhances controllability but may limit durability [96–98]. As a result, the field's immediate priority is rational tropism engineering (balancing potency against specificity) rather than further incremental changes to baseline compositions. Antibody-functionalized LNPs directed to T-cell receptors (e.g., CD3, CD5, CD7, CD28) are promising, but their value will ultimately hinge on quantitative on-target transfection rates in vivo, head-to-head safety profiles at therapeutic doses, and manufacturability at scale [96–98].

3.2.2. Ligand-functionalized polymer for T cell nano-engineering

While lipid nanoparticles are leading vectors for nucleic-acid transfer, their patent landscape and cold-chain logistics can constrain broad deployment. Ligand-functionalized cationic polymers offer complementary advantages: ambient-temperature stability, modular chemistry, and facile conjugation of targeting ligands. The central trade-off is not simply "polycation = delivery," but how polymer architecture tunes the potency–toxicity–specificity triangle. Increasing charge density and molecular weight raise nucleic-acid condensation and uptake yet steepen cytotoxicity and hamper timely cargo release; conversely, lower-MW or degradable backbones improve tolerability but risk underdelivery. Poly(β-amino esters) (PβAEs) have provided a useful design space—readily synthesized from accessible monomers, tailorable by monomer selection/stoichiometry/curing time, and generally biocompatible for gene delivery [99]. Beyond simple polyplexes, polymer vesicles/polymersomes enable multi-functional constructs for catalysis, sensing, and therapy, expanding how we program cell interactions and pharmacology [100].

For T-cell programming in vivo, a landmark example [73] used PβAE carriers functionalized with nuclear-localization and microtubule-associated motifs to deliver a CD19 CAR plasmid together with a transposase (iPB7) and decorated the surface with anti-CD3ε F(ab')$_2$ to bias uptake into T cells. The study demonstrated on-host conversion of T cells into CAR-T cells and disease control in a leukemia model—importantly, without ex vivo manufacturing [73]. Read alongside LNP work, these data clarify the design levers and failure modes for polymer systems: (i) Tropism arises more from ligand presentation (affinity, valency, spatial density) and corona management than from the backbone per se; (ii) Safety hinges on degradability and transient exposure to high cationic charge, as well as payload choice (DNA + transposase implies insertional risk and durable expression; mRNA/circRNA improves controllability but may require redosing); (iii) Manufacturability depends on batch-consistent polymer MW/distribution, ligand-to-polymer ratios, and robust QC for residual catalysts/solvents. In short, ligand-functionalized polymers are compelling when logistics, tunability, and combination agility dominate, but they demand explicit strategies to mitigate cytotoxicity and ensure quantitative on-target transfection at therapeutic doses.

3.2.3. Ligand-functionalized extracellular vesicles for T cell nano-engineering

Extracellular vesicles (EVs) are endogenous nanocarriers that package proteins and nucleic acids during biogenesis [101,102]. Beyond descriptive features, their decision-relevant properties for immune engineering are: (i) intrinsically low reactogenicity and immunogenicity, aided by self-derived membranes [102,103]. (ii) compatibility with diverse cargo classes; and (iii) the ability to "pre-load" payloads through producer-cell biology rather than solely post-formulation chemistry [103,104]. These attributes motivate EVs as alternatives to fully synthetic systems when repeated dosing or multi-tissue distribution is required. By contrast, LNPs—while clinically proven—can show composition- and dose-dependent cytotoxicity, limited tropism, and circulation instability in certain contexts, and are often optimized for hepatotropic delivery [105]. Thus, the strategic question is where EVs create net value, not whether they can carry cargo.

Genetic control over producer cells enables programmable tropism. As illustrated in Fig. 3c, engineering cells to express mRNAs encoding scFvs or fusogens yields EVs with surface-displayed binders and enhanced uptake pathways. In the GEMINI framework, Stranford et al. used the PDGFR transmembrane domain to display anti-CD2 scFv, achieving T-cell–biased EV interactions without downstream chemical conjugation—simplifying manufacturing and preserving membrane integrity [106]. Adding viral glycoproteins further increased T-cell uptake, highlighting that entry biology, not just ligand identity, governs performance. By displaying the linked anti-CD2 scFv on the PDGFR transmembrane domain, EVs can achieve T cell targeting. In addition, they found that EVs fused with viral glycoproteins could significantly enhance the uptake of EVs by T cells [106]. Prior work similarly leveraged the PDGFR TM domain to present anti-CD3 and anti-EGFR scFvs, supporting the generality of this display scaffold [107]. Looking forward, in vivo EV bioreactors—implantable or transiently engrafted producer cells—could supply sustained, functionalized vesicles, potentially reducing redosing burden; however, this shifts risk/complexity toward cell containment, durability control, and release specifications.

Compared with mature platforms like LNPs, the real bottlenecks for EVs cluster into three trade-offs: first, tropism vs. standardization—because EVs are cell-derived, batch-to-batch biological variability is high, so release criteria must quantify ligand density and corona features to convert "which cells they target" into a reproducible process; second, potency vs. safety—adding fusogens or other entry enhancers can boost T-cell uptake/transfection yet also widen off-target uptake and narrow safety margins; and third, scalability vs. platform maturity—cell-based manufacturing must meet GMP requirements for identity, purity, potency, and adventitious agents at vaccine-scale volumes, an area that remains less mature than LNP CMC. Consequently, EV

development should shift from asking "can they carry cargo?" to "how do we achieve high, specific, and scalable delivery with tight batch control?"

4. Organ-level targeting: spleen as a key location

The spleen (the largest secondary lymphoid organ) offers a pragmatic exposure platform for in situ T cell engineering because organ-scale deposition precedes any cell level partitioning [108,109] (Fig. 4). Its distinctive microanatomy and hemodynamics (splenic cords/sinusoids, a macrophage-rich marginal zone, and filtration recirculation loops) set boundary conditions for nanoparticle retention and redistribution [110,111]. At this level, apparent pKa/charge and interfacial chemistry control plasma-protein interactions and thus the biocorona; size/shape/mechanics tune collisions with the reticuloendothelial system and traversal of sinusoids [112,113]. Small compositional shifts that reprogram the corona can at equal doses reallocate exposure among liver, spleen, and lung [114,115]. Therefore, spleen targeting should not be regarded as a simple "on/off ligand switch," but rather as a systems-level property emerging from the interplay of microanatomy, protein corona formation, and mechanical dynamics.

Most recent efforts move beyond "which lipid to use" toward programmable organ tropism and expression context. SORT-LNPs exemplify this shift: by tuning component ratios, they alter the global apparent pKa and corona to steer RNA selectively to liver, lung, or spleen—without heavy ligand decoration [116]. Spleen-directed LNPs therefore enable in-situ CAR-T by leveraging immune-organ microanatomy and RNA-level control of expression rather than particle homing alone. Consistent with this logic, cardiolipin-mimic phosphoramide lipids (CAMP; e.g., PL40) bias functional expression to spleen and transfect T cells without antibodies, with circRNA further amplifying on-organ expression and mitigating T-cell exhaustion seen with CD3-Fab strategies [117]. Non-cationic thiourea NPs (NC-TNPs) reach the same goal by replacing electrostatic complexation with hydrogen-bond encapsulation, reducing reactogenicity while preserving spleen-selective delivery [118]. Collectively, these data argue that mechanics, interfacial bonding, and RNA format are co-equal levers with chemistry and ligands, supporting ligand-minimal, redose-ready designs.

Polymer vesicles extend these principles and clarify a key pitfall: distribution ≠ expression. A poly(L-glutamic acid) phosphatidyl-polymer library (no ligands) yielded dominant splenic protein output despite mixed liver/spleen localization, with performance linked to pKa ~6.2–6.7, tail length, endosomal escape, and assembly stability [119]. A topology series spanning spheres and rods preserved splenic avidity and functional myeloid reprogramming in NHP, highlighting size/shape and route as translational knobs [120] (see also polymersome advantages in stability/loading [121,122]). More broadly, corona programming can encode organ bias: nudging complement (C3) engagement achieves spleen selectivity while maintaining biocompatibility and potentiates nanovaccination/antitumor immunity after i.v. dosing [109,123]. The unifying principle is that the destination is co-governed by nanochemistry and the adsorbed corona—not size/charge alone; these rules port back to LNPs and polymers alike [123]. For in situ CAR-T, the most pragmatic path is corona-aware, expression-centric, ligand-minimal carriers that tolerate repeat dosing, converting the spleen from an uptake sink into a productive CAR factory. However, spleen-tropic delivery of CAR-encoding mRNA may generate different types of engineered immune cell such as CAR-T, CAR-NK, CAR-macrophage, etc, in contrast to T cell-specific delivery stategy.

5. Controlling CAR expression levels for in vivo immune cell engineering

In vivo T cell engineering goes beyond delivering genetic cargo, it demands precise spatiotemporal control of transgene expression. Unlike

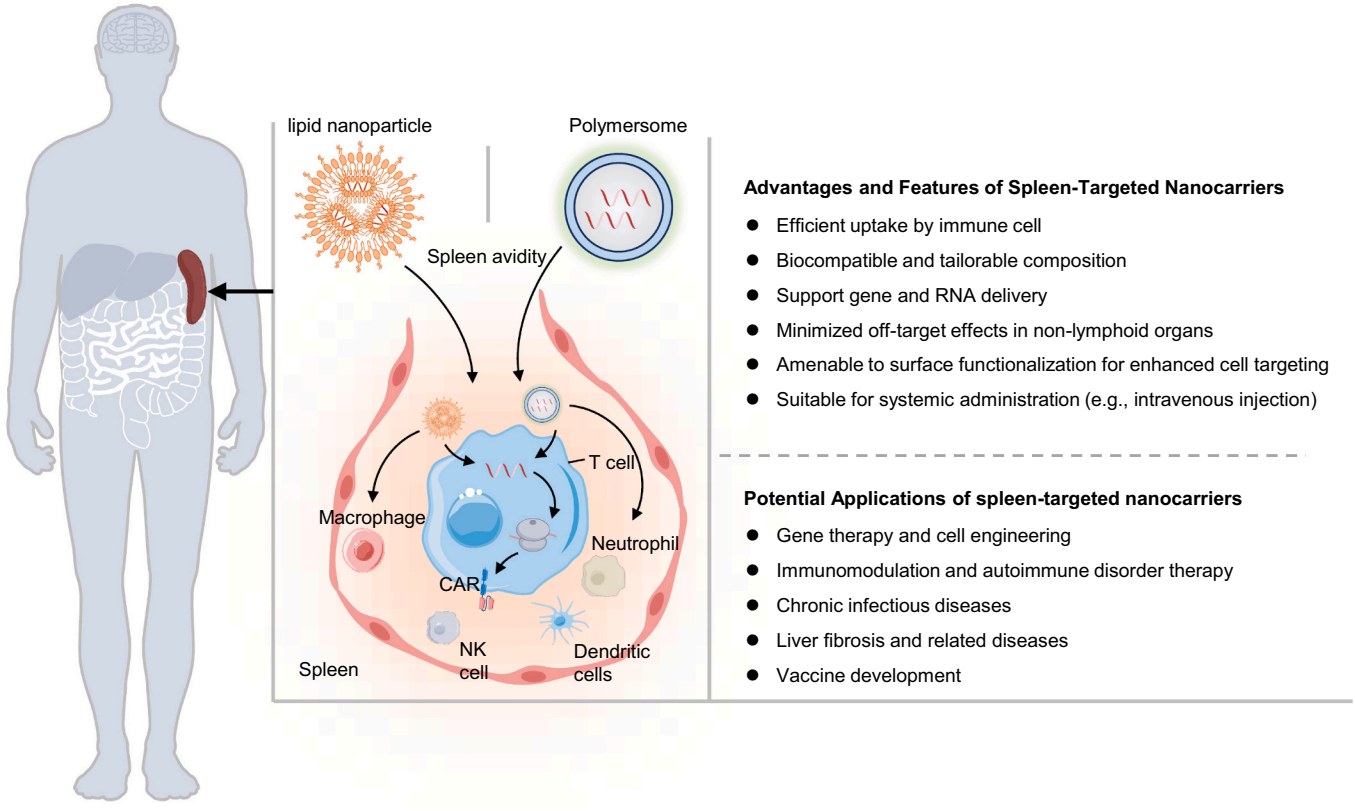

Fig. 4. Overview of spleen-tropic nanocarriers for in vivo immune cell modulation.

ex vivo settings where gene-modified cells are screened and expanded under controlled conditions, in vivo expression occurs within complex, dynamic physiological environments. This requires multi-layered regulatory mechanisms to balance efficacy with safety. At the transcriptional level, promoters such as CD2, CD3, or CD4 offer T-cell-restricted expression and mitigate ectopic activation [124–126]. These promoters can also be designed to be activation-dependent, providing dynamic regulation in response to immune signals. Compared with ubiquitous promoters (e.g., EF1α, CMV), tissue-specific elements offer improved selectivity and safety [127,128]. These lineage-specific promoters outperform ubiquitous options like EF1α or CMV in reducing off-target risks and are increasingly engineered to be activation-responsive, ensuring expression only in immunologically relevant contexts.

Post-transcriptional controls including optimized 5′/3′ UTRs and microRNA (miRNA) detargeting, provide an added layer of precision by allowing payload delivery to broad cell types while restricting translation to intended lineages. This "arrive-but-do-not-express" logic is especially critical in preventing CAR expression in antigen-expressing tumor or myeloid cells, where ectopic expression could be harmful. Protein-level switches, such as degron tags or suicide cassettes, provide pharmacological levers for managing dose escalation and adverse events [129]. These mechanisms offer reversible control during early clinical trials where safety margins are uncertain. Notably, logic-gated circuits, such as SynNotch-to-CAR cascades, represent a frontier in conditional gene expression [130]. These designs require co-expression of two or more antigens before activation, significantly reducing off-tumor risks. In murine models, such dual-input systems have demonstrated reduced toxicity without sacrificing efficacy—even when delivery tropism remained unchanged [131]. However, increasing logic complexity presents practical challenges: larger genetic payloads can exceed packaging limits of certain vectors (e.g., AAV), and co-expression requirements may strain manufacturing platforms. Thus, engineering specificity at the payload level must be balanced against vector constraints (see Table 2: Payload–Delivery Compatibility Matrix). When durable reprogramming is the goal (e.g., TCR knock-ins or metabolic rewiring), transient expression of editors—delivered via mRNA or non-integrating vectors—can be paired with donor templates. Safe harbor integration into loci such as TRAC not only ensures controlled expression but also reduces TCR–CAR cross-talk and tonic signaling. Overall, in vivo control of gene expression must evolve from binary transduction efficiency to nuanced, programmable regulation of timing, location, and intensity, a shift critical for translating CAR-T therapy into broader clinical use.

The efficacy of in vivo CAR-T programming depends critically on the compatibility between the delivery vector and the genetic payload. Although vector and payload can, in theory, be designed independently, their functional interdependence in practice necessitates a coordinated strategy. The physicochemical properties of the vector—such as size, charge, degradation kinetics, and immune profile—directly affect the bioavailability, stability, and functional readout of the delivered transgene.

Transient payloads such as mRNA and circRNA are best suited to non-viral delivery systems, including LNPs and polymeric nanocarriers. These platforms enable cytoplasmic release without nuclear entry or integration, allowing for transient CAR expression and repeated dosing. They are particularly suitable for indications requiring short-term intervention or dynamic modulation, including early-phase trials, autoimmune diseases, or solid tumors [132,133]. However, these systems typically require higher delivery efficiency and are constrained by the limited duration of expression. In contrast, durable payloads, such as plasmid DNA or transposon-based systems, require nuclear access and long-term retention, and are therefore more compatible with viral vectors such as lentivirus or AAV [134].

For complex payloads requiring genomic editing or multi-locus integration—such as TRAC knock-ins, dual-target CARs, or multiplex edits—vector selection must accommodate the payload size, nuclear access, and editing window. Transposon systems and virus-like particles (VLPs) are well-suited for these applications due to their high packaging capacity and transient expression kinetics [135,136]. Payload selection should be guided primarily by the therapeutic context, including expression duration, reversibility, and regulatory constraints. Once the expression profile is defined, a suitable delivery system can be selected to match those parameters. In summary, vector–payload compatibility is a central determinant of therapeutic performance in in vivo CAR-T engineering. Rational pairing of delivery platforms with genetic payloads, based on expression goals and clinical demands, enables the design of safe, effective, and scalable immunotherapies.

Table 2
| Payload–delivery compatibility matrix.

Question	Nanoengineering (delivery)	Genetic engineering (payload)	Typical choices & trade-offs
How to reach T cells?	Ligands (CD3/CD4/CD5/CD8), spleen-tropism (SORT, polymersomes)	–	Ligand accuracy vs repeat-dose immunogenicity; ligand-minimal spleen designs for scalability
How long should the expression last?	Dosing cadence, re-dosing feasibility	mRNA/circRNA (transient) vs LV/AAV/transposon (durable)	Transient expression boosts safety; durable expression aids persistence
How to prevent off-target biology?	Cell access control (organ/cell targeting)	Promoters/UTRs, miRNA detargeting, logic gates, kill switches	Separate "who gets cargo" from "who can express it" (payload gating)

6. In vivo T cell nano-engineering in clinical development

Current clinical development of in vivo CAR-T cell therapy is advancing through two main technical approaches: lentiviral vector-based gene therapy and mRNA-LNP-based transient expression systems (Table 3). Companies like Interius BioTherapeutics, Umoja Biopharma, and Kelonia utilize lentiviral vectors for efficient, durable CAR gene transduction, directly converting immune cells into CAR-T cells within the body. In contrast, companies such as Capstan Therapeutics, Myeloid Therapeutics, Orna Therapeutics, and Carisma Therapeutics employ mRNA-LNP systems to induce rapid but transient CAR expression in target cells, offering flexibility and safety advantages. Both methods exhibit unique strengths to developing in vivo CAR-T therapies.

One of the most promising advancements in in vivo CAR T therapy involves surface-engineered lentiviral vectors, which allow for precise targeting and modification of T cells within the body. EXUMA Biotech's innovative lentiviral vector, which encodes a CD19 CAR, exemplifies this progress [137]. Designed to target $CD3^+$ T cells, this vector was tested in a humanized NSG-SGM3 mouse model. Preclinical results revealed that in vivo editing of $CD3^+$ T cells produced functional CAR T cells and simultaneously eliminated pre-existing B cells, demonstrating both efficacy and efficiency in blood cancer treatment [137]. Based on this success, EXUMA Biotech has developed CCT303–406, a therapeutic targeting HER2-positive relapsed or refractory metastatic solid tumors [138]. Currently undergoing phase I clinical trials, this development highlights the potential for lentiviral vectors to treat hematological and solid tumors. EXUMA's work showcases how lentiviral technology expands the applicability and scope of in vivo CAR T therapies beyond blood cancers, addressing some of the most aggressive and treatment-resistant forms of cancer. Umoja Biopharma is developing an equally groundbreaking approach with its VivoVec™ platform, which leverages surface-engineered lentiviral particles to generate CAR T cells directly within the patient's body [139]. Unlike EXUMA's approach, which targets specific cancer types, VivoVec™ focuses on broader immune modulation. After a single dose of these modified lentiviral particles in

Table 3
| Clinical trials of in vivo CAR T therapy.

Pipeline	Company	Vector	CAR	Disease	Phase	Clinical trial ID
INT2104	Interius	Lentivirus	CD20	B-cell Cancer	Phase 1	NCT06539338
UB-VV111	Umoja/AbbVie	Lentivirus	CD19	Large B-cell lymphoma	Phase 1	NCT06528301
MT-303/MT-302	Myeloid	LNP	GPC3	Solid tumor and HCC	Phase 1	NCT06478693 NCT05969041
CPTX2309	Capstan	LNP	CD19	AID	Phase 1	NCT06917742
HN2301	MagicRNA	LNP	CD19	SLE	IIT	NCT06801119

NHP, the platform demonstrated potent in vivo CAR T cell generation, with functional CAR T cells peaking at multiple intervals between day 7 and day 51. Notably, the treatment-maintained B cell aplasia for up to 76 days and showed no signs of toxicity, making it both effective and safe. This result suggests the platform's potential as an off-the-shelf therapy, bypassing the labor-intensive processes required for ex vivo therapies. The combination of long-lasting efficacy and tolerability in preclinical models positions VivoVec™ as a game-changer in CAR T therapies, offering the potential for scalable, widely accessible treatments.

In parallel with lentiviral vector advancements, nanoparticle-based systems are revolutionizing in vivo CAR T therapy. Ensoma's Engenious™ platform, for example, utilizes virus-like particles to deliver gene materials up to 35 kilobases in size—far surpassing the capacity of AAV vectors [140]. This expanded payload capacity enables more sophisticated therapeutic designs and could lead to novel treatments for complex diseases beyond the capabilities of existing vector technologies. Capstan Therapeutics has taken a different approach by utilizing lipid nanoparticles to deliver CAR mRNA directly into T cells [71]. In a mouse model of heart disease, Capstan's CD5-targeting lipid nanoparticles (CD5/LNP) successfully encapsulated and delivered CAR mRNA molecules, forming CAR T cells within 48 h of injection. These advances in nanoparticle-based technologies demonstrate the versatility of in vivo CAR T generation, broadening the range of diseases that can be treated with these therapies, from cancer to cardiovascular conditions. The synergy between viral and nanoparticle platforms offers exciting possibilities for more effective and personalized treatments. Most notably, first-in-human data now demonstrate the clinical feasibility of cell-targeted LNPs in autoimmune disease: an engineered CD8-targeted LNP (HN2301) delivering CD19 CAR mRNA produced detectable $CD8^+$ CAR T cells in peripheral blood as early as 6 h after infusion, with CAR transcripts and CAR T frequencies peaking at ~6 h and returning to baseline within 2–3 days (i.e., a controllable "pulse–decay" exposure) [141]. Safety signals were mainly low-grade inflammatory responses (some managed with single-dose tocilizumab) without ICANS, but the small sample size, lack of controls, and short follow-up mean durability and optimal dosing regimens remain uncertain.

Collectively, these advances highlight the versatility of viral and non-viral platforms—and suggest pragmatic development paths: VLPs for large, durable payloads; LNP mRNA for rapid, repeatable, and integration-free dosing that can be titrated to the therapeutic window. Human-relevant preclinical systems (tumor–immune organoids, ex vivo lymph-node slices, immune organoids) remain essential to de-risk tropism and off-target immunostimulation prior to NHP bridging, while early clinical experience with LNP-CARs helps align pharmacology and manufacturability for trial planning.

Collectively, the advancements in viral and nanoparticle-based in vivo CAR T therapies represent a transformative shift in the industrial landscape. Companies such as EXUMA Biotech, Umoja Biopharma, Ensoma, and Capstan Therapeutics are leading the charge, each contributing unique approaches that tackle the limitations of current CAR T therapies, including manufacturing complexity and high costs. These innovations not only promise more accessible and scalable treatments but also expand the range of diseases that CAR T therapies can target. As these therapies progress through clinical trials, the future of CAR T treatments appears poised for breakthroughs that could revolutionize personalized medicine.

7. Challenges

Despite the promising advances in in vivo T cell nano-engineering, several significant challenges remain to be addressed to unleash the full potential of this approach for clinical application. These challenges span various aspects, including targeting specificity, safety, scalability, and the complexity of immune system interactions [142].

7.1. Targeting specificity and efficiency

Why targeting precision matters. Nonspecific delivery poses unique risks in the CAR setting. If vectors ectopically express a CAR in malignant or bystander leukocytes, acquired resistance can arise—e.g., CAR gene transfer into leukemic cells causing antigen masking and immune escape, as reported in clinical experience [143]. Hence, precision targeting is not an incremental convenience but a safety-critical requirement for in vivo T-cell engineering.

While nanocarriers can be functionalized with ligands, antibodies, or aptamers to enhance the specificity to target T cell subsets, in vivo off-target effects remain a significant concern [71]. Circulating nanoparticles in vivo face multiple hurdles before navigating to target tissue, including biological barriers, such as the endothelium, and non-specific uptake by other immune cells, tissues, or organs like the liver and lung [54,144,145]. The lack of precise targeting not only reduces the efficiency of T cell modification but also raises safety concerns, as off-target genetic modifications dilute the therapeutic effect and potentially cause unintended functional alterations in non-target cells, leading to adverse outcomes such as immune activation, inflammation, or even tumorigenesis [73]. Recently, Li et al. demonstrated that a significant proportion of CAR-positive B cells could be detected in vivo following transfection [72]. Although the anti-tumor and pro-tumor roles of B cells in immunotherapy are still controversial, it has been shown that B cell depletion reduces T cell counts and impacts effector memory T cell induction [146,147]. To reduce this risk, nanoparticle-delivered CAR transgenes can be expressed under the control of T-cell-specific promoters, which are less transcriptionally active. However, improved vector systems that allow tighter control over T-cell gene expression are being developed due to the increasing use of adoptive T-cell therapy [148–150]. Optimizing nanocarrier surface modifications for differential targeting remains a significant challenge in ensuring effective and precise delivery.

7.2. Cell modulation efficiency and durability

The persistence of modulatory effects in vivo is another significant hurdle. While non-viral delivery systems, such as LNPs carrying mRNA, offer advantages in terms of reduced immunogenicity and transient expression, their therapeutic effects are often short-lived [151]. This transient nature is a double-edged sword: on the one hand, it limits potential long-term toxicity and off-target effects, on the other hand, it necessitates repeated administration to achieve sustained therapeutic outcomes [152,153]. In contrast, CRISPR-Cas9 can induce permanent

genetic alterations, offering a potentially curative approach for certain conditions [154,155]. However, the challenge lies in controlling off-target effects, ensuring precise edits, and managing the immune response against the Cas9 protein [156]. Viral vectors, such as lentivirus or AAV, are highly efficient at delivering gene-editing tools but associated with safety concerns about permanent integration into the host genome, insertional mutagenesis, and long-term safety [157,158]. As research progresses, the trade-offs between transient and persistent gene-editing strategies in T cell engineering must be carefully considered. Novel delivery strategies that balance safety and efficacy while allowing controlled gene expression will be critical to advancing these technologies.

7.3. Regulatory and ethical considerations

The translation of in vivo T-cell engineering technologies into clinical applications entails complex regulatory and ethical considerations that extend beyond technical feasibility. Unlike ex vivo CAR-T therapies, where manipulation occurs in controlled manufacturing environments, direct in vivo modification of T cells introduces unique challenges in defining product classification, monitoring treatment risks, and protecting patient autonomy.

The rapid evolution of in vivo immunoengineering has outpaced existing regulatory frameworks. Delivery platforms such as LNPs, synthetic polymers, and virus-like particles blur the conventional boundaries between small-molecule drugs, biologics, and gene therapies. This ambiguity complicates the regulatory classification of in vivo-engineered CAR-T therapies as either advanced therapy medicinal products (ATMPs), gene transfer agents, or combination products [159]. Furthermore, transient and non-integrating platforms (e.g., mRNA or circRNA) may not meet traditional definitions of gene therapy but nevertheless induce significant immune modulation. Regulatory agencies must therefore adopt novel criteria for product characterization, including real-time assessment of transgene expression, vector biodistribution, and pharmacodynamic effects within host tissues.

In vivo CAR-T programming involves systemic or localized administration of gene-delivery vehicles, raising concerns about off-target gene transfer and immune activation. Even with T cell–specific promoters or miRNA-based detargeting strategies, a risk of ectopic CAR expression in non-target immune subsets remains, potentially leading to uncontrolled cytotoxicity or immune dysregulation [160]. For integrating vectors, such as lentiviruses or transposons, the risk of insertional mutagenesis persists and is more difficult to quantify in vivo than in ex vivo systems. Regulatory guidelines should therefore mandate longitudinal monitoring, vector shedding analysis, and functional integration site mapping to assess clonal expansion and potential oncogenic transformation.

In vivo T-cell engineering alters the behavior of immune cells within the patient's body, often irreversibly. Unlike cell products that can be tested, validated, and characterized ex vivo before infusion, in vivo approaches offer limited post-delivery control. This uncertainty imposes substantial ethical obligations regarding patient education, consent, and follow-up [161]. Informed consent documents must explicitly describe the risks of unpredictable biodistribution, the inability to remove or deactivate modified cells post-infusion, and the lack of long-term outcome data—particularly for pediatric or reproductive-age participants. Ethical review boards must consider whether current frameworks sufficiently address the challenges posed by in vivo gene editing and propose updated guidelines for trial oversight.

As in vivo immunoengineering approaches progress toward clinical viability, issues of accessibility and global equity must be addressed. Compared to ex vivo CAR-T products, which require centralized manufacturing, in vivo therapies hold promise for decentralized, scalable delivery. However, the cost of development, proprietary vector platforms, and lack of infrastructure in low-resource settings may exacerbate disparities in access. Additionally, the dual-use potential of in vivo gene modulation technologies raises ethical concerns about non-therapeutic applications. Misuse for immunoenhancement, performance augmentation, or gene editing beyond therapeutic scope must be considered in future policy development.

8. Conclusion

The field of in vivo T cell nano-engineering holds immense promise for immunotherapies and cancer treatment, but it is currently confronted with several significant challenges. Addressing these barriers will require multidisciplinary collaboration, innovative material science, and continued refinement of gene-editing tools and delivery methods. Overcoming these hurdles is critical to realizing the full potential of this approach and translating it into safe and effective therapies for patients.

CRediT authorship contribution statement

Tianyu Shi: Writing – original draft, Software, Data curation. **Yao Li:** Writing – review & editing, Validation. **Changchang Deng:** Software, Methodology. **Qiongzhe Ren:** Data curation. **Congcong Xu:** Writing – review & editing, Validation, Funding acquisition. **Zhiyuan Zhong:** Validation, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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