

Forum

Clinical technology
advances driving *in vivo*
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***In vivo* chimeric antigen receptor (CAR) engineering has advanced into clinical trials with encouraging safety and efficacy. Technology breakthroughs, including targeted lipid nanoparticles, genetically modified lentiviruses, and optimized CAR constructs, have enabled precise, efficient, and safe gene delivery. This forum summarizes innovations accelerating the clinical translation of *in vivo* CAR engineering for immunotherapy.**

***In vivo* CAR engineering enters the clinic**

In vivo CAR engineering represents a transformative approach in immunotherapy, involving the direct genetic modification of immune cells within the patient to express CARs. Unlike traditional *ex vivo* methods, which require cell extraction, modification, and reinfusion, *in vivo* CAR engineering leverages advanced gene delivery platforms, primarily lipid nanoparticles (LNPs) and viral vectors such as lentiviruses, to reprogram immune cells directly in the body [1–3]. This strategy has recently achieved major technological milestones in preclinical models and is now advancing into clinical settings with promising results.

A notable first-in-human application of this technology involved ESO-T01, an immune-shielded, nanobody-targeted lentiviral vector encoding a B cell maturation

antigen (BCMA)-specific CAR, administered to patients with relapsed or refractory multiple myeloma [4]. Four patients received therapy, resulting in encouraging safety and efficacy outcomes. In addition, earlier efforts demonstrated that viral-based *in vivo* engineering of CD19-directed CAR-T cells in a patient with refractory B cell lymphoma could achieve partial remission with minimal toxicity [5]. In parallel, another preclinical study conducted in cynomolgus monkeys used LNP-based delivery of CD20-targeted CAR mRNA constructs [6]. This study demonstrated effective B cell depletion and a favorable safety profile, supporting the potential of LNP-mediated *in vivo* CAR engineering for both oncology and autoimmune indications.

These emerging clinical and preclinical results underscore the feasibility and growing maturity of *in vivo* CAR-T cell engineering. Several biotechnology companies are actively pursuing this approach, focusing on hematologic malignancies such as B cell lymphomas and multiple myeloma using CD19⁺, CD20⁺, or BCMA-targeting CARs. Other efforts are expanding into solid tumors, such as hepatocellular carcinoma, with GPC3-targeting CARs (Table 1). The two leading platforms, lentiviral vectors and targeted LNPs (tLNPs), are being refined to enhance specificity, efficiency, and safety through improved T cell targeting, reduced off-target transduction and immunogenicity, and optimized CAR constructs that sustain expression and function (Box 1).

Taken together, these advancements position *in vivo* CAR engineering as a next-generation modality for cancer and autoimmune disease treatment. In this forum, we review the clinical and technological progress achieved to date and discuss strategies for accelerating the clinical translation of this promising therapeutic platform.

Delivery platform design

Building on the L829-tLNPs foundation (Box 1), the platform was further adapted

for selective CD8⁺ T cell engineering, which is advantageous in indications where CD4⁺ CAR-T cell activation could exacerbate toxicity or autoimmune pathology. Conjugation of anti-CD8 antibodies to L829-tLNPs enabled preferential transfection of CD8⁺ T cells in both murine and humanized models, achieving targeted, transient CAR expression in lymphoid tissues while sparing other immune subsets [6]. CD8-L829-tLNP-engineered CAR-T cells demonstrated potent antigen-specific cytotoxicity, cytokine production, and proliferative capacity *in vitro* and *in vivo* [6].

In parallel, ESO-T01 utilizes a nanobody-targeted, immune-shielded lentiviral vector that is specifically engineered for *in vivo* T cell targeting and reduced immunogenicity [4]. The vector envelope incorporates a mutated VSV-G glycoprotein to circumvent extensive tropism to mammalian cells. Targeting specificity is further enhanced by a membrane-anchored anti-T cell receptor (TCR) nanobody, which directs the vector to the TCR complex. These design features collectively enable selective, efficient, and durable *in vivo* T cell transduction.

Optimized CAR construct design

The design of the CAR construct is a critical factor influencing both the functional potency and safety profile of CAR-T cells *in vivo*. In the preclinical study in cynomolgus monkeys [6], two CAR constructs (CAR1 and CAR2) were designed to compare CAR architectures. Both featured an scFv antigen-binding domain and CD28/CD3 ζ intracellular signaling typical of second-generation CARs. CAR1 incorporated a CD8 α hinge and transmembrane domain upstream of the CD28 costimulatory module, whereas CAR2 used a CD28-derived hinge and transmembrane domain. To optimize the expression and functionality of CAR-T cells, a panel of mRNA constructs incorporating diverse untranslated regions

Table 1. *In vivo* CAR engineering in and approaching the clinic

Company	Vector	Therapeutic payload	Lead indication
Aanastra Inc.	Peptide nanoparticle (targeting CD3/CD5)	CD19-specific CAR	B cell malignancies
Aera Therapeutics	LNP	CD19-specific CAR	Autoimmune diseases
Alaya.bio	Polymeric nanoparticle (targeting CD3)	CD19-specific CAR	B cell malignancies
Capstan Therapeutics	LNP (targeting CD8)	CD19-specific CAR; CD20-specific CAR	Autoimmune diseases
Carisma/Moderna	LNP	GPC3-specific CAR	Liver cancer
Ensoma	Virus-like particles (targeting CD46)	HER2-specific CAR	HER2 ⁺ tumors
Esobiotec	Lentivirus (targeting TCR $\alpha\beta$)	BCMA-specific CAR	Multiple myeloma
GRIT Biotechnology	LNP	CD19-specific CAR	B cell malignancies
Interius	Lentivirus (targeting CD7)	CD19-specific CAR; CD20-specific CAR	B cell malignancies and autoimmune diseases
Kelonia	Lentivirus (targeting CD3)	BCMA-specific CAR	Multiple myeloma
Kernal Biologics	LNP	Undisclosed	Autoimmune diseases and hematological malignancies
Myeloid Therapeutics	LNP	TROP2-specific CAR; GPC3-specific CAR	Colon, lung, and breast cancers; liver cancer
NanoCell	LNP	CD19-specific CAR	B cell malignancies
Nitto Denko Corporation	LNP (targeting CD8)	CD19-specific CAR	B cell malignancies
Orbital	LNP	CD19-specific CAR	Autoimmune diseases
Orna	LNP	CD19-specific CAR; BCMA-specific CAR	B cell malignancies and autoimmune diseases
RiboX Therapeutics	LNP	CD19-specific CAR	B cell malignancies
Sail Biomedicines	LNP	CD19-specific CAR	Autoimmune diseases
Sanofi	LNP (targeting CD8)	CD19-specific CAR; CD20-specific CAR	B cell malignancies and autoimmune diseases
Shenzhen MagicRNA Biotech	LNP	CD19-specific CAR	Autoimmune diseases
Strand Therapeutics	LNP	Undisclosed	Hematological malignancies
Stylus Medicine	LNP (targeting CD3)	Undisclosed	B cell malignancies
Suzhou Immunofoco Biotechnology	Lentivirus (TCM3, in-house developed T cell-targeting module)	Undisclosed	B cell malignancies
Tessera Therapeutics	LNP	CD19-specific CAR; CD20-specific CAR	B cell malignancies
Velvet Therapeutics	Polyasparagine Nanoparticles	CD19-specific CAR	B cell malignancies and undisclosed solid tumors
Virovek Incorporation	AAV (T cell-targeting moiety)	CD19-specific CAR	B cell malignancies

and codon optimization strategies were developed. Through a two-step screening process that first assessed surface CAR expression levels and then evaluated *in vitro* tumor-killing activity and activation marker expression, an optimal mRNA design that enhanced CAR expression and T cell potency following *in vivo* delivery was identified.

In the clinical trial of ESO-T01, the lentiviral vector-based *in vivo* CAR therapy targeting BCMA, the CAR construct was engineered for both high efficacy and controlled cell-type-specific expression [4]. The construct employs a synthetic, T cell-specific promoter to restrict CAR expression to T lymphocytes, minimizing off-target transduction. The CAR itself

consists of a humanized variable domain of a heavy-chain-only antibody (V_{HH}) specific for BCMA, fused to a CD8 α -derived hinge and transmembrane region. The intracellular signaling module includes a 4-1BB costimulatory domain and a CD3 ζ activation domain, consistent with a second-generation CAR design. This configuration was selected to

Box 1. Overview of *in vivo* CAR-T cell therapy strategies and design principles

Various strategies have been evaluated for *in vivo* CAR engineering (Figure 1A). LNPs are designed to encapsulate CAR-encoding mRNA and are optimized for cell-specific targeting, high biocompatibility, and transient expression without genomic integration. Polymeric nanoparticles, composed of tunable polymers, allow adjustment of charge, degradability, and surface modifications to achieve stable nucleic acid delivery and controlled release. Lentiviral vectors facilitate efficient transduction and long-term CAR expression through genomic integration in both dividing and non-dividing cells, whereas adeno-associated virus (AAV) vectors mediate stable episomal expression with reduced immunogenicity and improved safety profiles.

Two leading strategies, tLNP and lentiviral-based delivery, have advanced to clinical or near-clinical application (Figure 1B). The tLNP platform incorporates a novel ionizable lipid, L829, designed to reduce off-target liver accumulation, enhance biodegradability, and minimize reactogenicity compared to benchmark lipids used in mRNA vaccines [6,14]. When formulated with CD5-targeting antibodies and encapsulating mRNA payloads, L829-tLNPs achieved efficient delivery to immune cells while sparing the liver, as demonstrated by reduced hepatic bioluminescence, faster clearance, and lower acute phase responses in rodents and nonhuman primates.

The ESO-T01 lentivector is produced by transient transfection of MHC-I^{KO}/CD47^{hi}/TCR V_{H+H}-expressing 293T producer cells with a set of four plasmids: a Gag/Pol plasmid encoding the lentiviral structural and enzymatic proteins, a Rev plasmid providing nuclear export functions, an ENV plasmid encoding the targeting-deficient mutant VSV-G envelope protein, and a transfer plasmid carrying the PRG1801 BCMA CAR construct driven by a T cell-specific synthetic promoter [4].

minimized off-target transduction, cytokine control, and safety switches for eliminating transduced cells. Here, we focus on lentiviral vector-specific safety considerations, in contrast to LNP-based systems, which have broad clinical validation and generally favorable safety profiles.

To address the broad cellular tropism of lentiviral vectors, the ESO-T01 platform incorporates targeted mutations in pivotal residues of the VSV-G, which is commonly used for viral pseudotyping [4]. These modifications are designed to restrict tropism and preferentially transduce T cells, thereby reducing the risk of off-target gene transfer to non-immune cells such as hepatocytes or stem cells. This strategy enhances the precision and safety of *in vivo* CAR gene delivery.

Beyond ESO-T01, clinical studies have shown that *in vivo* lentiviral delivery is feasible and safe. Intracerebral injection of a lentiviral *ABCD1* vector in childhood cerebral adrenoleukodystrophy caused no serious adverse events [7], and an equine infectious anemia virus-based vector delivering endostatin and angiostatin in advanced neovascular age-related macular degeneration demonstrated both safety and sustained ocular transgene expression [8]. These results highlight the versatility, safety, and clinical potential of lentiviral platforms for *in vivo* gene delivery.

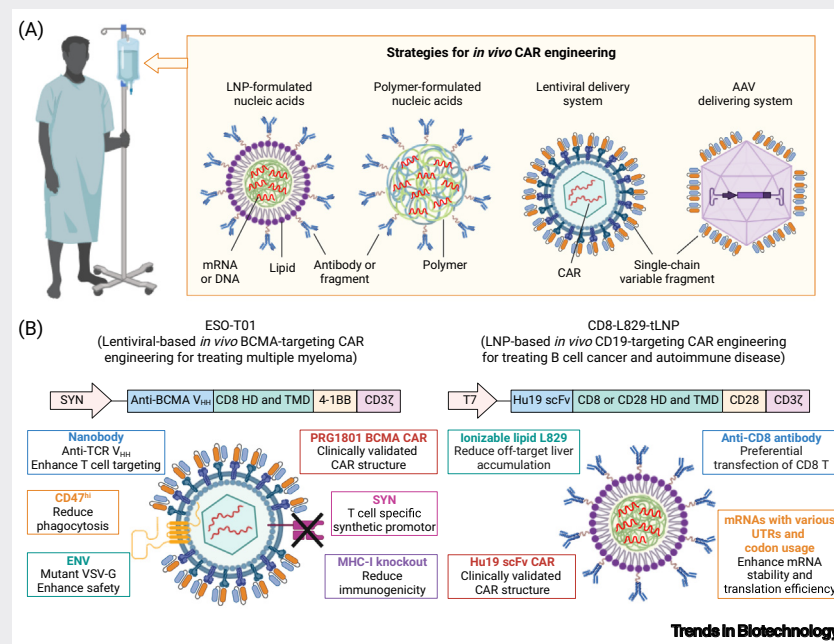


Figure 1. Various strategies for *in vivo* CAR engineering (A) and two leading strategies, targeted lipid nanoparticle (tLNP) and lentiviral-based delivery, have advanced to clinical or near-clinical application (B). See box text for details. Abbreviations: AAV, adeno-associated virus; BCMA, B cell maturation antigen; CAR, chimeric antigen receptor; MHC, major histocompatibility complex; TCR, T cell receptor; UTR, untranslated region; V_{H+H}, heavy-chain-only antibody; VSV-G, vesicular stomatitis virus G. Figure created with BioRender.

promote robust antitumor activity while mitigating potential safety risks associated with off-target expression or uncontrolled expansion.

Strategies for enhancing safety

Ensuring safety in *in vivo* CAR engineering requires cell-type-specific targeting, transient and controlled CAR expression,

Approaches to reduce immunogenicity

Although LNPs are generally considered to be less immunogenic than viral vectors, they can still be recognized by pattern-recognition receptors and elicit innate immune responses. To mitigate this response, various strategies have been employed, including modification of LNP composition, optimization of particle size, and alteration of the administration route to reduce immune activation and improve delivery efficiency [6].

During vector production, lentiviral particles incorporate host-derived membrane proteins, including HLA-I molecules, from the packaging cell line. These alloantigens can induce alloimmune reactivity, leading to rapid clearance of the vector and reduced transduction efficiency. To overcome this limitation, gene editing of packaging cells to eliminate HLA-I expression has been proposed, resulting in the generation of HLA-deficient producer cell lines that produce lentiviral vectors with reduced immunogenicity and enhanced serum stability [9].

In the ESO-T01 trial, the lentiviral vector was engineered to minimize allojection mediated by both innate and adaptive host immune responses [4]. Mutating two key residues (T214N and T352N) in the VSV-G envelope protein markedly reduced serum-mediated inactivation of the lentiviral vector. To evade clearance by the mononuclear phagocyte system, the viral membrane was modified to overexpress CD47, a ‘don’t eat me’ signal that suppresses phagocytosis by macrophages. In parallel, the MHC-I was genetically knocked out to reduce recognition and elimination by host cytotoxic T lymphocytes. These immune-evasive strategies have been extensively studied and validated in both preclinical and clinical settings for their ability to enhance the persistence of allogeneic cells and vectors by reducing rejection from host macrophages and T cells [10,11]. Additionally, incorporating nanobody-derived V_{HH} domains or employing scFv humanization represents an effective strategy to minimize anti-drug antibody formation, as demonstrated in the ESO-T01 and CD8-L829-tLNP CAR constructs.

Concluding remarks and future perspectives

Investigating the duration of *in vivo* T cell modification by different strategies is critical for both efficacy and safety. ESO-T01

CAR-T cells were first detected in patient peripheral blood between days 4 and 8, peaking at days 10–17, and remained detectable in bone marrow, tumor tissues, and pleural effusions for up to 2 months [4]. In contrast, following tLNP administration, CAR⁺ CD8 T cells in cynomolgus monkeys declined within 3 days post-dosing, necessitating multiple administrations, with CAR-T cells undetectable 1 week after the third dose [6]. These findings suggest that lentiviral-based strategies confer longer persistence of CAR-modified cells. However, the off-the-shelf nature and favorable safety profile of tLNPs allow repeated dosing, while enhancing the *in vivo* persistence of transduced CAR cells remains an important next step.

The development of Cas9-packaging enveloped delivery vehicles opens the door to precise *in vivo* genome editing, allowing for simultaneous CAR gene delivery and targeted gene knockout within the host [12]. Taken together, *in vivo* CAR engineering is rapidly evolving into a clinically viable immunotherapeutic strategy, with the potential to redefine the future of cell-based cancer therapy and extend its reach into autoimmune and other non-malignant diseases [13]. Continued integration of targeted delivery systems, synthetic biology, and genome editing will be critical to unlocking its full translational potential.

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Declaration of interests

L.Y. is a scientific advisor to AlzChem and Amberstone Biosciences, and a co-founder, stockholder, and advisory board member of Appia Bio. None of the declared

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References

- Li, Y.-R. *et al.* (2025) *In vivo* CAR engineering for immunotherapy. *Nat. Rev. Immunol.* 25, 725–744
- Mullard, A. (2024) *In vivo* CAR T cells move into clinical trials. *Nat. Rev. Drug Discov.* 23, 727–730
- Wu, J. *et al.* (2024) Chimeric antigen receptor therapy meets mRNA technology. *Trends Biotechnol.* 42, 228–240
- Xu, J. *et al.* (2025) *In vivo* B-cell maturation antigen CAR T-cell therapy for relapsed or refractory multiple myeloma. *Lancet* 406, 228–231
- Chen, X.-F. *et al.* (2024) 1482 *In vivo* CAR-T cells produced by specific virus in refractory B cell lymphoma. *J. Immunother. Cancer* Published online November 5, 2024. <https://doi.org/10.1136/jitc-2024-SITC2024.1482>
- Hunter, T.L. *et al.* (2025) *In vivo* CAR T cell generation to treat cancer and autoimmune disease. *Science* 388, 1311–1317
- Wang, Q.-H. *et al.* (2024) Phase I clinical trial of intracerebral injection of lentiviral-ABCD1 for the treatment of cerebral adrenoleukodystrophy. *Sci. Bull.* 69, 2596–2603
- Campochiaro, P.A. *et al.* (2017) Lentiviral vector gene transfer of endostatin/angiostatin for macular degeneration (GEM) study. *Hum. Gene Ther.* 28, 99–111
- Milani, M. *et al.* (2017) Genome editing for scalable production of alloantigen-free lentiviral vectors for *in vivo* gene therapy. *EMBO Mol. Med.* 9, 1558–1573
- Yamada-Hunter, S.A. *et al.* (2024) Engineered CD47 protects T cells for enhanced antitumour immunity. *Nature* 630, 457–465
- Li, Y.-R. *et al.* (2024) Engineering allojection-resistant CAR-NKT cells from hematopoietic stem cells for off-the-shelf cancer immunotherapy. *Mol. Ther.* 32, 1849–1874
- Hamilton, J.R. *et al.* (2024) *In vivo* human T cell engineering with enveloped delivery vehicles. *Nat. Biotechnol.* 42, 1684–1692
- Wang, Q. *et al.* (2025) *In vivo* CD19 CAR T-cell therapy for refractory systemic lupus erythematosus. *N. Engl. J. Med.* 393, 1542–1544
- Zhang, L. *et al.* (2023) Effect of mRNA-LNP components of two globally-marketed COVID-19 vaccines on efficacy and stability. *npj Vaccines* 8, 156