

Commentary

From bench to body: *In vivo* CAR engineering in the clinicYan-Ruide Li^{1,2,*} and Lili Yang^{1,2,3,4,5,6,*}¹Department of Microbiology, Immunology & Molecular Genetics, University of California, Los Angeles, Los Angeles, CA 90095, USA²Department of Bioengineering, University of California, Los Angeles, Los Angeles, CA 90095, USA³Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA 90095, USA⁴Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, Los Angeles, CA 90095, USA⁵Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA⁶Parker Institute for Cancer Immunotherapy, University of California, Los Angeles, Los Angeles, CA 90095, USA*Correspondence: charlie.li@ucla.edu (Y.-R.L.), liliyang@ucla.edu (L.Y.)<https://doi.org/10.1016/j.xcrm.2025.102337>

In vivo CAR engineering is an emerging therapeutic platform for cancer and autoimmune diseases. Enabled by advanced delivery systems such as immune-evasive lentiviral vectors and targeted lipid nanoparticles, this strategy has progressed to early clinical testing, showing initial safety and efficacy. Here, key opportunities and translational hurdles are critically discussed.

Chimeric antigen receptor (CAR)-engineered T cell (CAR-T) therapy has demonstrated remarkable efficacy in the treatment of both cancer and autoimmune diseases. However, its broad clinical application remains limited by the complexity of manufacturing, logistical hurdles, prolonged production timelines, and high costs. *In vivo* CAR engineering presents a transformative alternative by delivering CAR transgenes directly into endogenous T cells within the patient, reprogramming them into CAR-T cells *in situ*.¹ This strategy shifts the paradigm from a patient-specific, customized therapy to an off-the-shelf, ready-to-use platform, thereby eliminating the need for apheresis, *ex vivo* cell manufacturing, and preconditioning regimens such as lymphodepletion.

A range of preclinical approaches have been developed to optimize gene transfer tools that enable efficient and specific delivery of CAR constructs to targeted immune cell populations, while minimizing off-target effects and ensuring safety (Figure 1). These technologies include lipid-nanoparticle (LNP)-formulated nucleic acids,^{2,3} polymer-based nanoparticles,⁴ and viral vectors such as lentivirus and adeno-associated virus (AAV),^{5,6} as well as hybrid and emerging platforms including bioinstructive implantable scaffolds,⁷ Cas9-packaging-enveloped delivery vehicles (Cas9-EDVs),⁸ and virus-mimetic fusogenic nanovesicles.⁹

Concurrently, clinical translation of *in vivo* CAR engineering has begun to show promising outcomes, moving this approach from bench to bedside. In this commentary, we highlight the clinical advancements of *in vivo* CAR engineering, with a particular focus on the two leading strategies—lentiviral vectors and LNPs. We further discuss key technological opportunities and translational challenges that will shape the future of this rapidly advancing field.

Lentiviral-vector-mediated *in vivo* CAR engineering in the treatment of patients with multiple myeloma

A recent first-in-human clinical trial of *in vivo* CAR-T cell engineering using ESO-T01—a nanobody-targeted, immune-shielded lentiviral vector—demonstrated promising outcomes in patients with relapsed or refractory B cell maturation antigen (BCMA)-positive multiple myeloma (ClinicalTrials.gov NCT06691685).¹⁰ This approach was tested in four patients and showed encouraging safety and efficacy profiles.

The ESO-T01 lentiviral vector was rationally engineered with multiple features to enhance specificity, safety, and persistence of *in vivo* CAR-T cell generation. Key mutations in the vesicular stomatitis virus glycoprotein G envelope limited the vector's natural broad tropism, thereby reducing off-target transduction and

improving safety. To evade immune clearance, the vector surface was modified to overexpress CD47, a “don't eat me” signal that inhibits phagocytosis by the mononuclear phagocyte system. To further reduce immunogenicity, the major histocompatibility complex class I (MHC class I) molecules were knocked out, minimizing recognition by host immune surveillance. For targeted delivery, an anti-T cell receptor (TCR) nanobody was incorporated to ensure T cell-specific transduction. Finally, the vector carried a BCMA-targeting CAR transgene driven by a T cell-specific synthetic promoter and was equipped with a human CD8 α hinge and transmembrane domain, a 4-1BB costimulatory domain, and a CD3 ζ activation domain. Collectively, these modifications enabled precise T cell targeting, minimized off-target effects, and promoted durable CAR expression *in vivo*.¹⁰

Safety outcomes were manageable and consistent with those observed in autologous CAR-T therapy.¹⁰ Cytokine release syndrome (CRS) occurred in all patients—grade 3 in patients 1, 2, and 4, and grade 1 in patient 3—and was controlled effectively with glucocorticoids. Grade 1 immune-effector cell-associated neurotoxicity syndrome (ICANS) was observed in patient 4 and resolved with treatment. Transient grade 3–4 hematologic toxicities, including neutropenia, leukopenia, thrombocytopenia, and



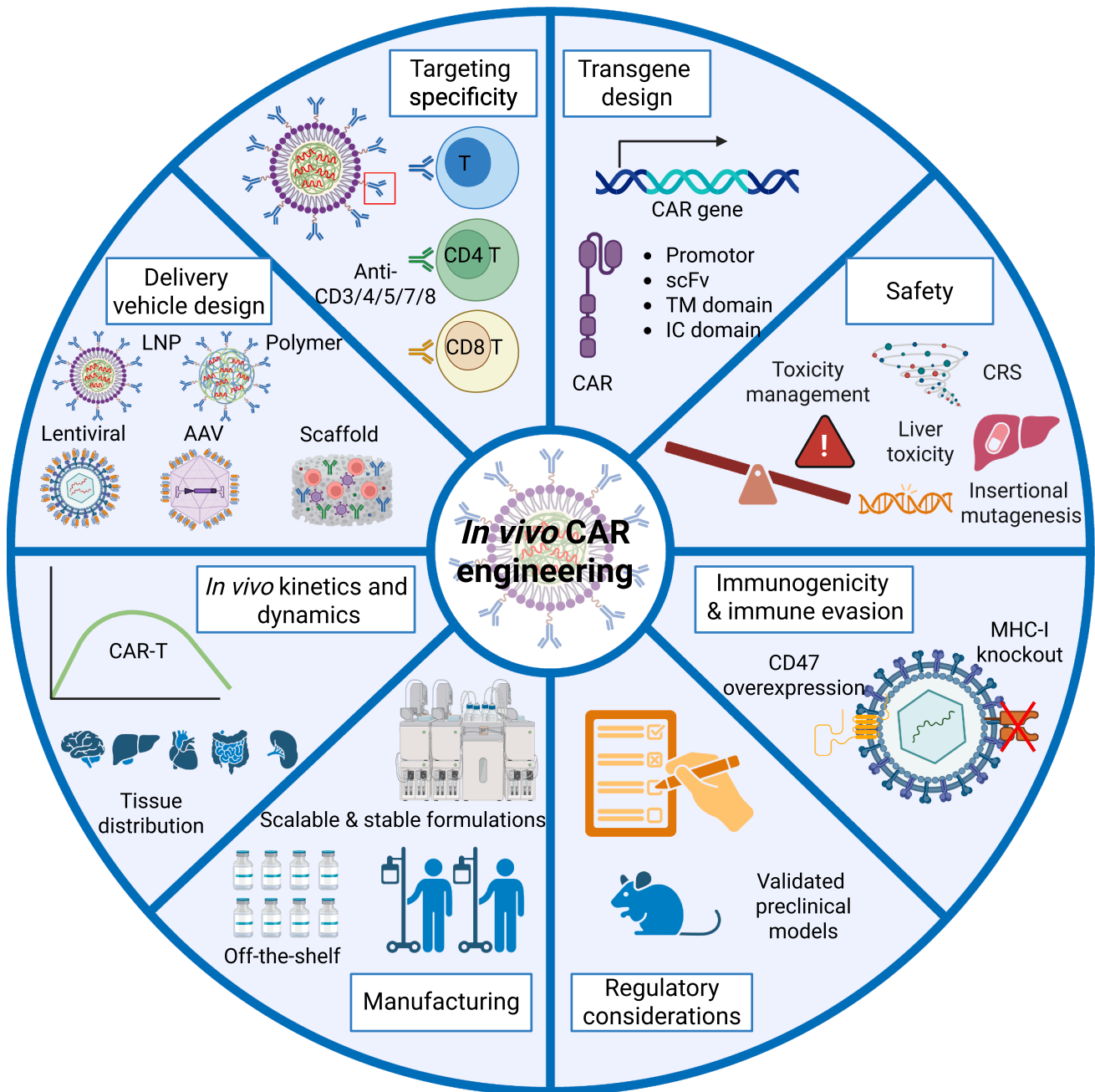


Figure 1. Key considerations for *in vivo* CAR engineering in the clinic

Targeting specificity is essential, with delivery systems designed to transduce only desired immune cell subsets (e.g., T cells) using receptor-mediated strategies such as anti-CD3 or anti-TCR ligands. The choice and design of delivery vehicles—lentiviral vectors, adeno-associated virus (AAV), and lipid or polymeric nanoparticles—must align with the therapeutic context, accounting for payload type (e.g., mRNA, DNA, and Cas9) and biodistribution. Transgene design further influences safety and efficacy, requiring immune-cell-specific promoters and optimized CAR architectures. Immunogenicity must be minimized through strategies such as MHC class I knockout and CD47 overexpression to evade immune detection and prolong persistence. Safety monitoring is critical, especially for cytokine release syndrome (CRS), immune-effector cell-associated neurotoxicity syndrome (ICANS), and potential insertional mutagenesis. Understanding *in vivo* kinetics, including CAR-T cell expansion, tissue distribution, and durability, informs dosing and therapeutic window. Scalable, stable formulations support off-the-shelf feasibility. Lastly, rigorous regulatory and translational planning, including validated preclinical models, quality control, and clinical trial endpoints, is vital. Together, these interdependent factors shape the success of *in vivo* CAR-T strategies, guiding their evolution toward broad, safe, and effective clinical use. LNP, lipid nanoparticle; scFv, single-chain variable fragment; TM, transmembrane; IC, intracellular.

lymphopenia, were noted but generally resolved during follow-up. No lentiviral particles were detected in urine, saliva, or cerebrospinal fluid, and viremia peaked within 12 h post-infusion, becoming undetectable by 48 h.

Efficacy outcomes were notable. Patient 1 achieved a stringent complete response (sCR) with resolution of all medullary and extramedullary lesions by month 2. Patient 2 also achieved sCR by day 28. Patients 3 and 4 showed partial responses, with significant tumor burden reduction and minimal residual disease negativity in bone marrow by day 28. CAR-T cells were detectable in peripheral blood between days 4 and 8, peaking at days 10–17, and were also identified in bone marrow, tumor tissue, pleural effusion, and cerebrospinal fluid.

In summary, this first-in-human trial demonstrates the clinical feasibility of *in vivo* CAR engineering using a lentiviral delivery system. The approach shows a well-controlled safety profile and encouraging therapeutic efficacy, offering a potential paradigm shift toward off-the-shelf, *in situ* CAR-T therapies for hematologic malignancies. Further clinical investigation is warranted to validate and expand the application of this promising platform.

Lentiviral-vector-mediated *in vivo* CAR engineering in the treatment of patients with B cell lymphoma

Another clinical study exploring *in vivo* CAR engineering involved the development of a targeted lentiviral vector designed to transduce T cells and direct them against CD19⁺ B cell non-Hodgkin's lymphoma.¹¹ This approach was tested in a single patient, with limited publicly available data. However, preliminary safety and efficacy outcomes have been reported. The engineered lentiviral vector was functionalized with an anti-CD3 single-chain variable fragment (scFv) to facilitate T cell recognition, along with other key modifications that have not yet been disclosed. The vector encoded a CD19-specific CAR transgene driven by an EF1 α promoter and was composed of a human CD8 α hinge and transmembrane domain, a 4-1BB costimulatory domain, and a CD3 ζ activation domain.

Following administration, *in vivo* expansion of CAR-T cells peaked around day 17. The patient experienced grade 1

CRS and transient myelotoxicity, with no observed neurotoxicity or infections.¹¹ While still in its nascent stages, this study provides an important proof of concept for *in vivo* CAR-T generation and highlights the potential of this approach to simplify manufacturing, reduce treatment timelines, and broaden accessibility in the clinical setting. Further investigation is warranted to validate its safety, efficacy, and scalability.

LNP-mediated *in vivo* CAR engineering in the treatment of cancer and autoimmune disease

A recent preclinical study demonstrated the potential of LNP-mediated *in vivo* CAR engineering for the treatment of cancer and autoimmune diseases.¹² Using human peripheral blood samples from both healthy donors and patients with autoimmune disorders, researchers successfully reprogrammed CD8⁺ T cells via mRNA-loaded, CD8-targeting LNPs. *In vivo* administration of these LNPs resulted in effective tumor control in humanized mouse models and robust B cell depletion in cynomolgus monkeys, providing compelling evidence of feasibility, efficacy, and safety in a non-human primate model.¹² Although not yet tested in human clinical trials, these findings lay a strong translational foundation and support the clinical advancement of *in vivo* CAR-T strategies.

The LNP formulation was composed of five lipid components, including an ionizable lipid and a targeting moiety (e.g., an antibody fragment) conjugated to the LNP surface, and encapsulated an mRNA payload encoding the CAR. Key design goals included minimizing off-target delivery to the liver, enhancing mRNA transfection efficiency in target immune cells, improving biodegradability, reducing innate immune activation (reactogenicity), and maximizing tolerability.¹² Importantly, the LNPs were engineered to preferentially target CD8⁺ T cells in order to avoid unintended CAR expression in CD4⁺ T cells—given that CD4⁺ CAR-T cells have been implicated in CRS and may exacerbate autoimmune pathology.

In efficacy studies involving 22 cynomolgus monkeys, animals received intravenous infusions of CD8-targeting LNPs without prior corticosteroid or antihistamine premedication. Treatment was

generally well tolerated.¹² Clinical and biomarker analyses indicated features consistent with an immune-effector cell-associated hemophagocytic lymphohistiocytosis-like response, but no central nervous system symptoms or seizures were observed. Peripheral blood B cell levels dropped sharply within 6 h of the first dose and became nearly undetectable within 24 h. CAR expression was dose dependent with up to 85% of CD8⁺ T cells and 95% of CD8⁺ natural killer cells expressing the CAR following the third dose, while CD4⁺ T cell expression remained minimal. Notably, a two-dose regimen achieved comparable B cell depletion in blood and tissue to that of the three-dose regimen, and re-emerging B cells were predominantly naive, suggesting a reset of the B cell compartment.¹²

Overall, this study represents a significant step forward in validating the safety and translational potential of LNP-mediated *in vivo* CAR-T therapy. Its demonstration of efficacy and tolerability in non-human primates provides essential preclinical support for clinical development, especially within regulatory frameworks such as those in the United States.

Conclusions

In vivo CAR engineering represents a paradigm shift in cellular immunotherapy, addressing key limitations associated with conventional autologous CAR-T cell therapies—namely, complex manufacturing processes, high costs, and limited accessibility. Recent preclinical and early clinical studies have demonstrated the feasibility, safety, and therapeutic efficacy of *in vivo* CAR generation using both lentiviral vectors and LNP-mediated platforms. Notably, the first-in-human trial utilizing the ESO-T01 lentiviral system in patients with relapsed or refractory multiple myeloma provides compelling clinical validation for this approach, while additional investigations in B cell lymphoma and non-human primates further highlight the broad applicability of *in vivo* CAR strategies across disease contexts.

Although these studies are promising, safety concerns remain and warrant careful consideration. In the ESO-T01 trial, all patients experienced CRS, with three cases reaching grade 3 severity that required vasopressor support and/or supplemental oxygen; in addition, two patients

developed pneumonia. Moreover, long-term safety data are currently limited to only 2–3 months of follow-up. Additionally, the observation of a hemophagocytic lymphohistiocytosis-like syndrome in non-human primates following LNP-mediated *in vivo* CAR engineering raises further safety concerns that need to be addressed in future studies. To mitigate these safety challenges, several strategies are under active investigation. These include the development of immune-evasive and tissue-targeted delivery platforms to enhance cellular specificity, as well as the incorporation of built-in safety mechanisms such as suicide switches or drug-inducible elimination systems that allow for the controlled removal of CAR-expressing cells. Approaches using transient expression technologies—such as mRNA or non-integrating viral vectors—are also being employed to reduce the risk of prolonged or uncontrolled CAR activity. Furthermore, real-time monitoring of CAR expression and immune responses through non-invasive imaging and biomarker analysis could provide early warning of emerging toxicities. Robust preclinical testing in humanized models and extended clinical follow-up will be critical to fully assess safety risks and therapeutic durability. Successfully addressing these complex safety issues will be key to realizing the full potential of *in vivo* CAR therapies and guiding the development of safer, more effective next-generation platforms.

A growing number of biotechnology companies and academic centers are actively developing *in vivo* CAR platforms, with a primary focus on B cell malignancies and autoimmune diseases.¹³ This emphasis is largely informed by the extensive clinical experience and success of CD19-targeting CAR-T therapies. Given the systemic nature and accessibility of circulating malignant B cells and autoreactive lymphocytes, hematologic malignancies and autoimmune disorders present favorable entry points for *in vivo* CAR translation. Furthermore, the intrinsic liver tropism of LNPs offers a strategic advantage for targeting hepatic malignancies, such as Glypican-3-positive liver cancer, suggesting that organ-preferential delivery may be leveraged to expand the therapeutic reach of LNP-mediated CAR engineering.

Although still in the early stages of development, these emerging technologies strongly support the core concept of *in situ* immune cell reprogramming. Lentiviral vectors provide durable CAR expression and robust T cell activation, while LNPs offer a non-viral, scalable, and tunable alternative with promising implications, especially in contexts where CD4⁺ CAR-T activity must be minimized, such as autoimmune disease. Additionally, next-generation platforms such as Cas9-EDVs offer a potential pathway to site-specific, durable CAR integration without the risks associated with random genome insertion, although such tools will require rigorous safety and efficacy evaluation prior to clinical application.⁸

Moving forward, the successful clinical translation of *in vivo* CAR technologies will depend on addressing key challenges, including achieving precise immune cell targeting, minimizing off-target effects and immunogenicity, and ensuring durable therapeutic responses. Continued innovation in delivery system design, comprehensive preclinical safety assessment, and alignment with evolving regulatory standards will be essential. Nevertheless, the accelerating progress in this field positions *in vivo* CAR engineering as a promising next-generation modality, with the potential to democratize CAR-T therapy and significantly expand its impact across oncology and autoimmune medicine.

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AUTHOR CONTRIBUTIONS

All authors contributed to the design and writing of this commentary and approved the final version of the commentary.

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 companies contributed to or directed any of the research reported in this article.

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