Narrative Review Article

In-vivo CAR-T Cell Engineering: A Narrative Review of Emerging Delivery Strategies and Their Potential Future Impact on Accessibility

Lia R. Bhatia

Brentwood School, 201 Avondale Avenue, Los Angeles, CA 90049, United States

ABSTRACT

Chimeric antigen receptor T-cell (CAR-T) therapy has emerged as a groundbreaking advancement in cancer immunotherapy, progressively improving over decades and producing remarkable clinical outcomes. This review examines the evolution of CAR-T cell therapy by comparing ex vivo and newer in-vivo approaches, evaluating the four primary delivery vehicles used for in-vivo CAR-T therapy, and discussing their implications for safety, scalability, accessibility and future development. Although ex vivo CAR-T therapy has demonstrated substantial clinical success, its complex manufacturing process, high cost, prolonged production time, and reliance on specialized facilities limit patient access worldwide. In contrast, in-vivo CAR-T therapy genetically reprograms T cells directly within the body, eliminating external manipulation of T cells and thus many of the logistical barriers associated with ex vivo manufacturing. Four primary delivery vehicles have shown immense progress within-vivo CAR-T cell therapy: lentiviral vectors, lipid nanoparticles, polymeric nanoparticles, and virus-like particles. Each delivery platform offers distinct advantages and limitations in terms of efficacy, safety, and scalability. Most evidence for in-vivo CAR-T therapy to date comes from preclinical and early-phase studies; if these approaches continue to mature, they may offer a more scalable, cost-effective, and accessible alternative to traditional ex vivo CAR-T therapy, though this remains a projected rather than demonstrated benefit.

Keywords: CAR-T cell therapy; in-vivo gene delivery; lentiviral vectors; lipid nanoparticles; polymeric nanoparticles; virus-like particles; cancer immunotherapy; accessibility

INTRODUCTION

Chimeric antigen receptor (CAR) engineered T cell therapy represents a remarkable breakthrough in cancer immunotherapy, evolving through scientific progress and research advancements. Burnet's Clonal Selection theory, discovered in 1957, was among the first frameworks to establish a direct connection between the immune system and cancer. Burnet proposed that the immune system continuously surveys the body for abnormal cells, including cancer cells. During a viral infection, a virus infects a cell and the cell then processes viral proteins and presents viral peptide fragments on major histocompatibility complex (MHC) molecules. T cells recognize these viral peptide–MHC complexes through their T-cell receptors (TCRs), triggering immune activation and elimination of infected cells. Burnet suggested that this process also enables the immune system to recognize and eliminate malignant cells,

Corresponding author: Lia R. Bhatia, E-mail: liareddybhatia@gmail.com.

Accepted July 31, 2026
https://doi.org/10.70251/HYJR2348.44586596

establishing the conceptual foundation for modern cancer immunotherapy (1,2). Unlike normal T-cells, CAR-T cells use engineered chimeric antigen receptors to recognize specific cell surface antigens without requiring MHC presentation, allowing targeted recognition of cancer cells (3). Another pivotal advancement toward developing CAR-T cell therapy was tumor-infiltrating lymphocyte (TIL) research in the late 1980s, which demonstrated that endogenous T cells could combat cancer. TILs were tested as early as 1987 in experiments with mice. Cells extracted from a tumor were transferred to other mice with lung tumors, resulting in tumor elimination. These findings confirmed that T cells possess the capacity to destroy malignant cells (4).

Building on this foundation, the first-generation CAR-T cell was developed during the 20th century. Eshhar described this early CAR-T construct as the "T Body". The first-generation CAR model established the basic structural framework: an antibody-based targeting domain, the single-chain variable fragment (scFv), and activation signaling domains derived from the T-cell receptor (TCR) and CD3 zeta (CD3ζ) complex. Researchers also demonstrated that these modified T cells could be reinfused into the bloodstream to target malignant cells. This initial model provided essential insight into how T cells can be engineered to recognize and attack cancer cells (3).

While this model established a foundation, the second generation of CAR-T cells demonstrated greater sustainability and persistence. Second-generation CAR-T cells integrated a costimulatory domain, most commonly CD28 or 4-1BB, along with a signaling domain within a receptor. This enabled T cells to kill cancer cells while continuing to expand in response to ongoing antigen exposure. The added costimulatory signaling protected the cells from early exhaustion and promoted persistence. Consequently, CAR-T cells persisted longer in the body and exhibited evidence of immune memory, representing a critical technological advancement (5).

In the third generation of CAR-T cell therapy, a second costimulatory domain was added, most often combining CD28 and 4-1BB with CD3ζ. The CD28 component promoted targeted cell elimination, whereas 4-1BB enhanced persistence. By integrating two costimulatory domains, researchers aimed to strengthen efficacy and extend the duration of the CAR-T cell activity (6).

Fourth-generation CAR-T cells, also known as TRUCKs (T cells Redirected for Universal Cytokine-mediated Killing), are engineered on a second-generation backbone with an additional transgene encoding a cytokine, commonly interleukin-12 (IL-12), that is released upon CAR engagement This local cytokine release is intended to recruit and activate additional endogenous immune cells within the tumor microenvironment, making it more difficult for tumors to evade immune destruction. (6).

Fifth-generation CAR-T cells build on the third-generation design by incorporating an additional intracellular domain to engage cytokine-receptor signaling pathways, rather than through general 'stability' modifications alone. One example fuses a truncated cytokine receptor tail from the interleukin-2 (IL-2) receptor β-chain to STAT3/5-binding motifs, enabling ligand-induced or constitutive cytokine signaling alongside conventional CAR activation, with the goal of improved persistence and reduced toxicity (6).

Collectively, these developments illustrate the ongoing evolution of CAR-T cell therapy and its increasing precision and effectiveness over time. While numerous publications describe CAR-T cell biology and clinical applications, fewer reviews focus specifically on emerging in-vivo delivery strategies and their potential to improve global accessibility. This review examines ex vivo and in-vivo CAR-T cell approaches, summarizes the major delivery vehicles, and evaluates their mechanisms, advantages, limitations, and potential for improving the accessibility of cellular immunotherapy in the future. A summary of current ex vivo CAR-T cell therapy and emerging strategies for in-vivo CAR-T cell therapy are summarized in Figure 1.

EX-VIVO AND IN-VIVO CAR-T CELL THERAPY

Ex-Vivo CAR-T Cell Therapy

In ex vivo CAR-T cell therapy, the initial step involves leukapheresis, during which T cells are collected from the patient's bloodstream. This can be performed through two primary approaches: autologous or allogeneic. The autologous approach uses the patient's own cells, reducing the risk of immune rejection, whereas the allogeneic approach uses donor-derived T cells. Although this reduces processing time, it increases the likelihood of immune complications (7).

Following leukapheresis, the T cells are genetically engineered, typically in a laboratory, by introducing a chimeric antigen receptor onto the cell surface. This CAR protein consists of four principal components. The extracellular domain enables the T cell to recognize and bind its target cell via the scFv. The hinge region

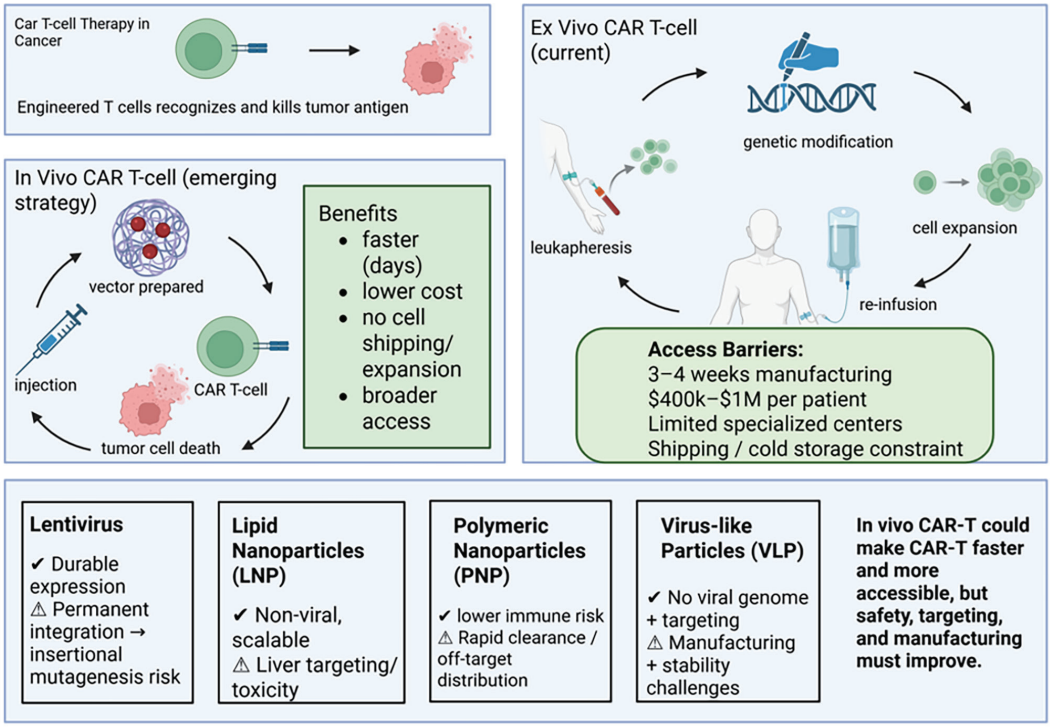

Figure 1. *Overview of CAR-T cell therapy and in-vivo delivery vehicles. CAR-T cell therapy works by engineering T cells to recognize and destroy tumor antigens. The current ex vivo approach, while effective, faces significant access barriers that limit its widespread use. In-vivo CAR-T represents an emerging strategy that could address these limitations by genetically reprogramming cells within the patient. Four delivery vehicle platforms, such as lentivirus, lipid nanoparticles (LNPs), polymeric nanoparticles (PNPs), and virus-like particles (VLPs), have high potential, each with different benefits and tradeoffs in safety, targeting, and manufacturability.*

connects the extracellular domain to the transmembrane domain, which anchors the CAR within the cell membrane. The intracellular domain transmits activation signals that trigger cancer cell destruction (8). CD3ζ, part of the intracellular domain, initiates T cell activation, proliferation, cytotoxicity, and cytokine release. Costimulatory domains such as CD28 or 4-1BB further enhance T-cell effectiveness. Additional elements, including IL-12 inducers or IL-2 receptor β chains, further improve tumor growth suppression.

After modification, CAR-T cells are expanded over several weeks in the laboratory. Once sufficient cell numbers are achieved, they are reinfused into the patient in the hope that they will locate and eliminate cancer cells (9). Throughout this process, patients are closely monitored for signs of immune rejection or severe adverse effects. While this procedure is time-consuming and costly, it has demonstrated substantial success and continues to show significant promise for the future of immunotherapy.

In-vivo CAR-T Cell Therapy

In contrast, in-vivo CAR-T cell therapy bypasses external cell manipulation by genetically reprogramming T cells directly within the patient's body. Rather than extracting cells and reprogramming cells externally in a laboratory, in-vivo therapy delivers genetic material into the patient to alter cells in situ. Delivery methods such as lipid nanoparticles, virus-like particles, polymeric nanoparticles, and lentiviruses are commonly used to deliver the CAR gene to T cells. By eliminating ex vivo cell manipulation, this approach has the potential to reduce cost, shorten preparation time, and increase accessibility, although these benefits remain primarily projected rather than clinically demonstrated. Unlike 'off-the-shelf' allogeneic CAR-T products, which use standardized donor-derived cells manufactured in advance, in-vivo CAR-T therapy instead involves direct administration of a gene-delivery vehicle to the patient; use of the term 'off-the-shelf' here refers to the potential for a standardized, pre-manufactured delivery product

rather than to a banked cellular product. However, challenges remain, including the risk of off-target effects and immune rejection. Despite these challenges, in-vivo CAR-T cell therapy remains an area of active preclinical and early clinical investigation with promising, though not yet fully validated, potential (10). It remains to be determined which delivery vehicles will be most effective for in-vivo CAR-T therapy. The key features of each vehicle are summarized in Table 1.

Table 1. *Comparison of in-vivo CAR-T delivery vehicles. Summary of four delivery platforms: lentiviral vectors, lipid nanoparticles (LNPs), polymeric nanoparticles (PNPs), and virus-like particles (VLPs). Summarizes mechanisms, advantages, limitations, and accessibility and scalability potential. LNP, lipid nanoparticle; PNP, polymeric nanoparticle; VLP, virus-like particle. Accessibility and Scalability Potential ratings (Projected Moderate, Projected Moderate to High, Projected High) are the authors' qualitative synthesis of the manufacturing complexity, existing clinical/manufacturing precedent, and targeting requirements discussed in the corresponding sections and cited sources for each platform, rather than a validated scoring instrument; ratings reflect projected potential and do not imply established clinical accessibility. Established clinical experience (e.g., lentiviral vectors in ex vivo manufacturing, mRNA-LNP platforms) is distinguished in the text from speculative future potential specific to vivo CAR-T applications.*

Delivery Vehicle	Mechanism	Advantages	Limitations	Accessibility and Scalability Potential
Lentiviral Vectors Sources: (11-19)	Viral vectors integrate CAR genes directly into T-cell DNA, resulting in long-term CAR expression.	Durable gene expression; effective transduction of resting and dividing cells; potential for long-lasting immune memory.	Risk of insertional mutagenesis; irreversible genomic integration; difficult to discontinue if severe toxicity occurs; manufacturing complexity.	Projected Moderate. Proven technology with strong efficacy, but safety concerns and manufacturing Requirements may limit widespread use.
Lipid Nanoparticles (LNPs) Sources: (20-26)	Nonviral lipid carriers deliver mRNA encoding, CAR constructs are directly delivered to T cells in-vivo.	Nonviral; scalable manufacturing; lower immunogenicity; avoids permanent genomic modification; adaptable for repeated dosing.	Liver accumulation and potential hepatotoxicity; transient expression requiring repeat administration; storage challenges.	Projected High. Scalable production and existing clinical experience with mRNA-LNP platforms make this one of the most promising approaches for broad implementation.
Polymeric Nanoparticles (PNPs) Sources: (27-35)	Biodegradable polymer-based carriers deliver genetic material or signaling molecules to target cells.	Biodegradable; tunable degradation rates; reduced long-term toxicity; capable of carrying diverse cargo.	Rapid clearance from circulation; potential off target distribution; variable delivery efficiency; limited clinical experience.	Projected Moderate to High. Attractive due to the safety profile, but optimization of targeting and persistence remains necessary.
Virus-like Particles (VLPs) Sources: (36-43)	Engineered viral shells lacking viral genomes deliver genetic cargo without causing infection.	Highly targeted delivery; no risk of viral replication; Efficient intracellular delivery; potential for precise gene editing.	Manufacturing complexity; stability concerns; high production costs; limited large-scale experience.	Projected High. Manufacturing challenges need to be overcome. Offers strong safety and targeting advantages.
Overall Sources: (8-10, 29, 44-47)	All delivery systems aim to generate CAR-T cells directly within the patient, eliminating ex vivo manufacturing.	Reduced treatment time, lower costs, broader geographic access, and potential "off-the-shelf" administration.	Safety, targeting precision, persistence, and manufacturing remain active areas of research.	In-vivo CAR-T therapy may improve global access to cellular immunotherapy relative to current ex vivo approaches, pending further clinical validation.

DELIVERY VEHICLES FOR IN-VIVO CAR-T CELL THERAPY

Lentiviral Vectors

Lentivirus is one of the primary delivery vehicles for CAR-T cell therapy. Although it is currently the state-of-the-art method for ex vivo CAR-T cell therapy, researchers are working to adapt the virus for in-vivo applications. Historically, lentivirus use has been restricted to ex vivo cell therapy, in which a patient's T cells are removed, genetically modified, and reinfused as engineered CAR-T cells. More recently, lentiviral vectors have been engineered for systemic administration, enabling target-cell modification without prior cell extraction (11).

Normally, the VSV-G protein binds LDLR, enabling targeting of many cell types. Researchers have modified this by incorporating a different envelope protein, cocal, and added receptors such as anti-CD3 scFv to activate the T cells. Costimulatory ligands, such as CD80 and CD58, further enhance cellular strength and persistence. At the beginning of the process, anti-CD3 scFv enables particle binding and T-cell activation, after which the cocal protein directs the T cell to its target (12). Lentivirus is widely used as a viral vector because it is safe, effective, and long-lasting. It can insert desired genes into both resting and dividing cells, which is a major advantage because many other viral vectors can only transduce dividing cells (13).

Despite these advantages, immune rejection remains a challenge. Immune reactions occur when the body detects a foreign gene, such as green fluorescent protein (GFP). In an experiment with mice, lentivirus carrying GFP was initially expressed within cells but was later recognized as foreign, triggering immune clearance. GFP was subsequently eliminated from the body, demonstrating a limitation of lentiviral delivery. Researchers are attempting to address this by using antigen-presenting cells to promote immune tolerance, targeting genes to specific sites, and controlling gene expression levels (14).

In general, CAR-T-cells expand rapidly, reach peak concentration during initial tumor burden reduction, and subsequently decline while persisting long-term, providing years of immune memory (15). However, lentiviral use carries risks. Because the virus integrates into the patient's DNA, it cannot be turned off or removed if complications arise (16).

Severe adverse effects, such as cytokine release syndrome, are therefore difficult to reverse. Insertional mutagenesis also poses a serious threat, as viral gene insertion may activate oncogenes or disrupt critical genes. Researchers are addressing this through structure quality control to prevent replication-competent viruses, careful dosing, and awareness of endogenous retroviruses that may recombine with therapeutic vectors (17). Lentivirus is also a versatile delivery vehicle capable of targeting not only hematologic malignancies but also solid tumors and immune cells such as dendritic cells (18).

Advances are improving accessibility by combining CAR-T cell and lentiviral technology to increase infection efficiency while reducing vector dose requirements, thereby lowering cost. Concentrating T cells and lentivirus particles enhances efficiency and may reduce dosing frequency (19). Overall, lentivirus is an effective delivery vehicle that originated in ex vivo therapy but is increasingly being adapted for in-vivo use, potentially enabling long-lasting treatment that continues to advance.

Lipid Nanoparticles

Lipid Nanoparticles (LNPs) are a key delivery vehicle that plays a major role in-vivo CAR-T cell therapy. As LNP production improves, they are becoming safer, more effective, and a longer-term solution. Compared with other delivery vehicles, LNPs cause less immune reaction because they are composed of naturally occurring lipids rather than viral vectors. They can carry larger genetic material, are easier to manufacture, and can be produced at a larger scale. LNPs are ionizable, meaning they can change charge and alter their structure to improve delivery effectiveness. Polyethylene glycol (PEG)-lipids may also prolong LNP circulation time in the body; however, they may induce anti-PEG antibodies that clear LNPs, reducing effectiveness while increasing the likelihood of side effects. There is also a focus on organ targeting to direct LNPs to specific tissues (20). However, T-cell-specific targeting for CAR delivery has not been solved to the same degree as organ-level targeting, and minimizing transfection of non-target cell types remains an open engineering challenge for LNP-based in-vivo CAR-T approaches. Current approaches include decorating delivery vehicles with T-cell-targeting ligands or antibodies (for example anti-CD3 or anti-CD5), although optimal specificity remains an active area of investigation.

Despite these advances, challenges remain. LNPs tend to accumulate in the liver. Liver toxicity occurs when liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), increase

indicating liver damage. PEGylated LNPs and ionizable lipids can still bind apolipoprotein E (ApoE) in circulation. ApoE naturally directs lipoprotein particles to LDL receptors on hepatocytes, leading to liver uptake and potential toxicity (21).

To address this limitation, a method called Selective Organ Targeting (SORT) has been developed. SORT lipids direct nanoparticles to different organs: cationic lipids target the lungs, anionic lipids target the spleen, and ionizable lipids can also target the lungs if needed (22). This improves tissue specificity and delivery accuracy. Additionally, N-acetylgalactosamine (GalNAc)-conjugated delivery systems have been used to guide LNPs specifically to hepatocytes rather than other cell types; this evidence comes from hepatocyte-directed RNAi therapeutics rather than from CAR-T-specific engineering, and the strategy is discussed here as a general LNP organ-targeting approach that has not yet been demonstrated for T-cell-directed CAR delivery (23).

Other challenges include mRNA loss during delivery, unpredictability of immune response, and limited stability, which may require the administration of multiple doses. The therapeutic goal is for LNPs to enter cells, release mRNA, and enable protein expression; however, mRNA degradation during this process reduces effectiveness. In vitro testing does not always predict in-vivo outcomes. In one study comparing four common LNP formulations (SM-201, ALC-0315, MC3, and C12-200), laboratory results did not fully correlate with mouse model outcomes, and increased cellular uptake did not necessarily correspond to greater effectiveness, as immune responses were similar across formulations (24).

mRNA has a short half-life, resulting in transient expression and the need for repeat dosing. Storage also poses accessibility challenges, as mRNA-LNP therapies often require very low temperatures. Lyophilization is being explored to improve stability. With this method, LNPs can remain stable for 12 weeks at room temperature and 24 weeks at 4 °C while maintaining effectiveness. The addition of sugars during the freeze-drying process protects the LNP structure (25).

Beyond current in-vivo CAR-T applications, LNPs have also been used to deliver gene-editing tools such as Cas9, CFTR genes for cystic fibrosis, CAR-mRNA for cancer therapy, and siRNA for amyloidosis (26). Overall, while LNPs represent a promising delivery vehicle for in-vivo CAR-T cell therapy and other treatments, challenges such as liver toxicity, immune response, and delivery efficiency remain. Advances, including SORT targeting, GalNAc ligands, and improved storage methods, continue to enhance their safety, effectiveness, and accessibility.

Polymeric Nanoparticles

Polymeric nanoparticles (PNPs) are another delivery vehicle that has advanced CAR-T cell therapy. They are microscopic particles composed of polymers such as polylactic-co-glycolic acid (PLGA) or PEG-based materials. They can be used in-vivo to deliver drugs to cells and regulate CAR-T cell activity. During nanoparticle formation, the drug is mixed with polymer using solvent evaporation or nanoprecipitation. The resulting particles circulate through the bloodstream to reach target cells. Cells internalize nanoparticles via endocytosis, after which the PNP degrades and releases the drug (27).

To circumvent the transient nature of RNA delivery by PNPs, scientists have employed transposon systems that permanently integrate DNA sequences into the genome. However, this approach carries risks of unintended genomic changes and long-term effects. Alternatively, PNPs can deliver synthetic mRNA, which does not alter DNA but instead instructs cytoplasmic protein production without entering the nucleus (28). If complications occur, biodegradable PNPs naturally degrade within the body (29, 27).

Compared with LNPs, PNPs degrade more rapidly because polymers break down faster than the lipid structure. They can be administered at higher doses, engineered for variable degradation rates, and break down into relatively safe byproducts. In-vivo studies in mice demonstrated a half-life of approximately 1-7 hours. Although PNPs persist briefly, they deliver genetic instructions rapidly before degrading. In murine studies, polymeric nanoparticles demonstrated a half-life of approximately 1-7 hours (29). In a brain tumor trial, it was effective in gene delivery (27).

PNPs also protect CAR-T cells during delivery, facilitate cellular entry, deliver signaling molecules, protect genetic material, increase CAR-T cell numbers, and enhance function by carrying immune-modeling agents (30).

However, limitations exist. Reported adverse effects include extrapyramidal symptoms due to dopamine receptor disruption, cytotoxicity, apoptosis, DNA damage, and structural cell injury (31); these findings come from general polymeric nanodrug toxicology studies rather than CAR-T-specific trials, and their direct relevance to CAR-T engineering warrants further study.

Despite toxicity concerns, in a trial, there was no sign of toxicity when using PNP in CAR-T cell therapy. It was a trial with mice, and 6% of cells were modified with CAR after 6 days and 20% after 12 days. In 7/10 mice, their tumors were removed, and they lived an average of 58 days longer (32). Off-target delivery and rapid clearance also pose challenges. The mononuclear phagocytic system (MPS) removes nanoparticles via macrophage-mediated clearance. Opsonization by plasma proteins forms a protein corona on nanoparticles, which can either aid targeting or promote immune elimination and misdirection (33, 34).

Storage is another limitation. Liquid PNP formulations require preservatives; therefore, lyophilization or spray-drying is used to stabilize particles. Freeze-drying removes water by sublimation, whereas spray-drying converts liquid into dry powder (35).

A newer approach involves injectable mRNA-loaded nanoparticles capable of reprogramming cells in-vivo. Because mRNA is translated directly in the cytoplasm rather than requiring nuclear entry, this reduces processing time relative to DNA-based approaches, since no nuclear transport or genomic integration step is required. Overall, polymeric nanoparticles are an effective CAR-T delivery system with advantages including biodegradability, targeted delivery, and reduced immune rejection. While challenges such as off-target effects, rapid clearance, and potential toxicity are present, ongoing research continues to improve their safety and efficiency.

Virus-like Particles

Virus-like particles (VLPs) are another major delivery vehicle for in-vivo CAR-T cell therapy. VLPs structurally resemble viruses and mimic viral outer components, allowing immune recognition, but they lack viral genomes and therefore do not cause infection (36). VLPs can be engineered to carry DNA or RNA encoding CAR constructs. Surface receptors are incorporated to target T cells specifically, enabling binding, intracellular delivery of mRNA, and subsequent therapeutic activity. VLPs are effective in both ex vivo and in-vivo applications, with recent advances emphasizing in-vivo use. Engineered VLPs efficiently deliver ribonucleoproteins (RNPs), including gene-editing components such as base editors, demonstrating high targeting accuracy and reduced off-target effects due to the short lifespan of RNPs (37).

VLPs achieve specific targeting by incorporating antibodies on their surface to bind designated cells. They deliver drugs directly into cells without lysosomal degradation. After entry via endosomes, VLPs escape before lysosomal fusion, preventing destruction and enabling intracellular delivery (38).

VLPs can be engineered to deliver both DNA and RNA cargo. RNA delivery is more challenging due to molecular size and polarity, but VLP-mediated DNA delivery has been more extensively studied in nanocarrier and gene-delivery contexts rather than specifically as a clinically tested CAR-T platform (39); most clinical experience with VLPs to date comes from their use as vaccines rather than as vehicles for CAR gene delivery, and this distinction is important when evaluating their readiness for CAR-T applications. Delivery activates both humoral and cellular immune responses: antibody production by B cells and activation of T-cell cytotoxic responses (40).

Manufacturing challenges include assembly inefficiency, aggregation, sensitivity to environmental factors such as oxygen, temperature, and pH, and high testing costs. Proper storage conditions are essential for maintaining VLP stability (41). VLPs may induce long-term immune protection or require repeated administration depending on the specific VLP platform and therapeutic application. Animal studies suggest long-term tumor immunity, with no recurrence at the original tumor site indicating immune memory, although further validation is needed (42). However, long-term immune protection observed in preclinical studies does not necessarily imply that a single administration will be sufficient in future clinical use. Certain VLP-based applications, such as vaccines, may require multiple doses (43).

Overall, VLPs provide a promising CAR-T delivery platform with precise targeting, efficient intracellular delivery, and potential long-term immune protection without live-virus risk. Despite manufacturing and stability challenges, continued advancements may establish VLP-based therapies as major tools in disease treatment.

COST AND ACCESSIBILITY

One of the major limitations of ex vivo CAR-T cell therapy is low accessibility due to high cost and limited treatment centers. Ex vivo treatment is complex. Cells are extracted via leukapheresis, isolated, expanded, genetically modified with CAR engineering, stored, transported, and reinfused. Each step is time-intensive, requiring approximately three to four weeks for preparation, and extremely costly, ranging from $400,000

to $1,000,000 per patient, excluding hospitalization (44).

The high cost largely reflects laboratory infrastructure, specialized equipment, personnel, and storage requirements. Storage itself restricts accessibility because CAR-T cells have limited shelf life outside the body, making widespread distribution difficult. In-vivo therapy addresses several of these barriers. CAR-T cells are generated directly within the body, eliminating many complex ex-vivo steps. This reduces cost and processing time, potentially shortening therapy preparation from weeks to less than one week. If successfully translated clinically, in-vivo CAR-T therapy could eliminate the need for ex vivo cell expansion, cryopreservation, storage, and transport. Internal modification also increases sterility, speed, and direct patient delivery. However, the durability of treatment depends on the delivery platform. Integrating viral vectors are designed to support longer-lasting CAR expression following successful gene delivery, whereas mRNA-based platforms such as lipid nanoparticles generally produce transient CAR expression and may therefore require repeated administration (45).

Delivery vehicles reprogram cells in-vivo, reducing reliance on chemotherapy and improving patient comfort and safety (46). Because laboratory manipulation is unnecessary, treatment can be administered at more locations, including clinics rather than specialized centers. This expands access, particularly for underserved populations distant from advanced medical facilities. Ex vivo therapy requires individualized cell processing, whereas in-vivo CAR-T therapy could resemble vaccine-like standardized production. Although benefits are substantial, side effects may still occur, including immune rejection, fever, chills, dyspnea, headache, nausea, and fatigue (47).

Studies indicate that repeated in-vivo dosing can achieve outcomes comparable to multiple ex-vivo treatment cycles in diseases such as leukemia, prostate cancer, and hepatitis B (28). Another advancement is VivoVec, a platform engineered to produce CAR-modified cells in Vivo. VivoVec is a modified lentiviral vector containing enhanced signaling components. In nonhuman primate studies, all B cells were eliminated within 10 weeks through anti-CD20 CAR-T production, representing an important preclinical advance in in-vivo therapy development (12).

In summary, although ex vivo CAR-T therapy is effective, its cost and logistical complexity limit accessibility. In- vivo CAR-T therapy addresses these challenges by reducing laboratory dependence, preparation time, and storage constraints, offering the potential for globally accessible treatment.

CONCLUSION

Advancements in CAR-T cell therapy have transformed cancer treatment but continue to face challenges related to cost, accessibility, effectiveness, and efficiency. Ex vivo therapies have achieved major clinical success but remain limited by technological requirements, high cost, and long processing times, all of which restrict patient access. These limitations have driven the development of in-vivo CAR-T therapy, which genetically modifies cells directly within the patient's body, eliminating many steps required in ex vivo therapy, including cell extraction, modification, storage, and reinfusion. Consequently, it reduces cost, time, and technological requirements while expanding treatment accessibility to patients who would otherwise lack access.

In-vivo CAR-T therapy is most commonly delivered using four vehicles: lentiviral vectors, lipid nanoparticles, polymeric nanoparticles, and virus-like particles. Each delivery vehicle has distinct advantages and limitations. Lentiviral vectors enable stable gene delivery but may cause insertional mutagenesis or permanent genomic alteration. Lipid nanoparticles effectively deliver mRNA but may cause liver-associated toxicity. Polymeric nanoparticles are biodegradable and less immunogenic but are rapidly cleared and may exhibit targeting inaccuracies. Virus-like particles provide precise targeting but may lack consistency and stability. In-vivo therapy also presents broader complications, including immune rejection and off-target modification. Although these limitations reduce therapeutic efficacy, continued development is improving in each system.

Although substantial progress has been made, several challenges must still be addressed before in-vivo CAR-T cell therapy can become widely accessible. Future research should prioritize improving targeting precision, safety, scalable manufacturing, and global accessibility. Targeting precision remains a major challenge because delivery vehicles often accumulate in non-target organs, such as the liver, leading to increased toxicity and reduced efficacy. Emerging strategies such as Selective Organ Targeting (SORT) lipids and GalNAc ligands have shown promise in reducing off-target effects, while enhancing efficacy and minimizing side effects. To improve the safety of integrating delivery platforms, the risk of insertional mutagenesis can be reduced through

controlled CAR gene integration, and programmable safety off-switch mechanisms can be integrated to deactivate engineered cells if they cause serious adverse effects. Finally, storage challenges create a barrier to scalability as strict temperature control is often required for manufacturing, storage, and transport. Stabilization strategies, such as lyophilization and spray-drying, are being explored to improve transportability and global distribution. Importantly, clinical evidence for in-vivo CAR-T therapy remains limited relative to the ex vivo field: most findings summarized here derive from preclinical, animal, or early-phase studies; long-term safety and durability data in humans are not yet available, and head-to-head comparisons among the four delivery platforms have not been conducted. Priorities for future research therefore include developing robust T-cell-specific targeting strategies, controllable and reversible CAR expression systems, standardized biodistribution and immunogenicity assays that allow direct comparison across platforms, repeat-dosing studies, large-animal and clinical-phase testing, formal cost-effectiveness analyses, and dedicated evaluation of implementation strategies for low-resource settings.

While all four delivery platforms demonstrate promise, lipid nanoparticles and virus-like particles have demonstrated several characteristics that may support future clinical translation since they pair strong safety profiles with the potential for scalable manufacturing. Lipid nanoparticles benefit from scalable manufacturing, established clinical experience through mRNA therapeutics, and avoidance of permanent genomic modification. Virus-like particles provide highly targeted delivery without the risk of viral replication and may have more precise cellular engineering. Since this is a narrative review, the discussion synthesizes findings across studies that vary substantially in maturity, ranging from established clinical CAR-T therapies to preclinical investigations of emerging delivery systems. Furthermore, differences in experimental models, CAR constructs, delivery methods, and outcome measures currently limit direct comparison among delivery platforms.

Continued advances in targeting specificity, safety mechanisms, manufacturing processes, and clinical validation will ultimately determine which platform emerges as the preferred strategy for large-scale implementation of in-vivo CAR-T cell therapy. As these technologies mature, they have the potential to transform cancer treatment towards faster, safer, more effective, and more accessible therapies for patients worldwide.

ACKNOWLEDGEMENTS

The author wishes to thank Luke Riggans, PhD, for providing guidance and insight that contributed to this review.

CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to this work.

REFERENCES

1. Sir Frank Macfarlane Burnet [Internet]. Melbourne: Walter and Eliza Hall Institute of Medical Research; 2023. Available from: https://www.wehi.edu.au/about/history/past-directors/sir-frank-macfarlane-burnet/#block_e7f0e9f9609e850cfd587511f33643a9-11 (accessed on 2025-05-04)
2. Burnet M. Cancer—a biological approach. *Br Med J.* 1957; 1 (5023): 841–7. doi: 10.1136/bmj.1.5023.841
3. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci U S A*. 1993 Jan 15; 90 (2): 720–4. doi:10.1073/pnas.90.2.720
4. Spiess PJ, Yang JC, Rosenberg SA. In-vivo antitumor activity of tumor-infiltrating lymphocytes expanded in recombinant interleukin-2. *J Natl Cancer Inst*. 1987 Nov; 79 (5): 1067–75. doi:10.1093/jnci/79.5.1067
5. Feucht J, Sadelain M. Function and evolution of the prototypic CD28ζ and 4-1BBζ chimeric antigen receptors. *Immunooncol Technol*. 2020 Dec; 8: 2–11. doi:10.1016/j.iotech.2020.09.001
6. Tomasik J, Jasiński M, Basak GW. Next generations of CAR-T cells – new therapeutic opportunities in hematology? *Front Immunol*. 2022 Oct 28; 13: 1034707. doi:10.3389/fimmu.2022.1034707
7. Song F, Hu Y, Zhang Y, Zhang M, Yang T, Wu W, *et al*. Safety and efficacy of autologous and allogeneic humanized CD19-targeted CAR-T cell therapy for patients with relapsed/refractory B-ALL. *J Immunother Cancer.* 2023 Feb; 11 (2): e005701. doi:10.1136/jitc-2022-005701
8. Bui TA, Mei H, Sang R, Ortega DG, Deng W. Advancements and challenges in developing in-vivo CAR T cell therapies for cancer treatment. *EBioMedicine*. 2024 Aug; 106: 105266. doi:10.1016/j.ebiom.2024.105266
9. Car-T cells: engineering immune cells to treat cancer

[Internet]. Bethesda: National Cancer Institute. Available from: https://www.cancer.gov/about-cancer/treatment/research/car-t-cells (accessed on 2025-05-07).

10. Song Z, Zhou Y, Wang B, Geng Y, Tang G, Wang Y, *et al*. In-vivo production of CAR T cell: opportunities and challenges. *Genes Dis*. 2025 Nov; 12 (6): 101612. doi:10.1016/j.gendis.2025.101612 11.
11. Tuerxunyiming M, Yin JM, Zhu P, Liu M, Fu Z, Zhao Q. A targeting lentiviral vector for generation of CAR-T cells in-vivo. *Sci Rep*. 2026 Mar 17; 16 (1). doi:10.1038/s41598-025-17342-1
12. Nicolai CJ, Parker MH, Qin J, Tang W, Ulrich-Lewis JT, Gottschalk RJ, *et al*. In-vivo CAR-T cell generation in nonhuman primates using lentiviral vectors displaying a multidomain fusion ligand. *Blood*. 2024 Aug 29; 144 (9): 977–87. doi:10.1182/blood.2024024523
13. Milone MC, O'Doherty U. Clinical use of lentiviral vectors. *Leukemia*. 2018 Mar 22; 32 (7): 1529–41. doi:10.1038/s41375-018-0106-0
14. Annoni A, Battaglia M, Follenzi A, Lombardo A, Sergi-Sergi L, Naldini L, *et al*. The immune response to lentiviral-delivered transgene is modulated in-vivo by transgene-expressing antigen-presenting cells but not by CD4+CD25+ regulatory T cells. *Blood*. 2007 Sept 15; 110 (6): 1788–96. doi:10.1182/blood-2006-11-059873
15. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. *Nat Rev Clin Oncol*. 2023 Apr 13; 20 (6): 359–71. doi:10.1038/s41571-023-00754-1
16. Kriesel F, Stegmann L, Ciesek S, Kaderali L, Baldauf H-M. Efficient inactivation of pseudotyped HIV-based lentiviral vectors and infectious HIV. *J Virol Methods*. 2020 Feb; 276: 113768. doi:10.1016/j.jviromet.2019.113768
17. Connolly JB. Lentiviruses in gene therapy clinical research. *Gene Ther*. 2002 Nov 29; 9 (24): 1730–4. doi:10.1038/sj.gt.3301893
18. Andorko JI, Russell RM, Schnepp BC, Grubaugh D, Mullen KF, Wakabayashi A, *et al*. Targeted in-vivo delivery of genetic medicines utilizing an engineered lentiviral vector platform results in CAR-T and NK cell generation. *Mol Ther*. 2025 Oct; 33 (10): 4937–52. doi:10.1016/j.ymthe.2025.06.036
19. Charles River Laboratories. Leveraging lentiviral vectors [Internet]. Wilmington: Charles River; 2023. Available from: https://www.criver.com/eureka/leveraging-lentivirus (accessed on 2025-05-18).
20. Wang J, Ding Y, Chong K, Cui M, Cao Z, Tang C, *et al*. Recent advances in lipid nanoparticles and their safety concerns for mRNA delivery. *Vaccines (Basel)*. 2024 Oct 8; 12 (10): 1148. doi:10.3390/vaccines12101148
21. Hosseini-Kharat M, Bremmell KE, Prestidge CA. Why do lipid nanoparticles target the liver? Understanding of biodistribution and liver-specific tropism. *Mol Ther Methods Clin Dev*. 2025 Mar; 33 (1): 101436. doi:10.1016/j.omtm.2025.101436
22. Wang X, Liu S, Sun Y, Yu X, Lee SM, Cheng Q, *et al*. Preparation of selective organ-targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. *Nat Protoc*. 2022 Oct 31; 18 (1): 265–91. doi:10.1038/s41596-022-00755-x
23. Janas MM, Harbison CE, Perry VK, Carito B, Sutherland JE, Vaishnaw AK, *et al*. The nonclinical safety profile of GalNAc-conjugated RNAi therapeutics in subacute studies. *Toxicol Pathol*. 2018 Aug 23; 46 (7): 735–45. doi:10.1177/0192623318792537
24. Lindsay S, Hussain M, Binici B, Perrie Y. Exploring the challenges of lipid nanoparticle development: the in vitro–in-vivo correlation gap. *Vaccines (Basel)*. 2025 Mar 21; 13 (4): 339. doi:10.3390/vaccines13040339
25. Young RE, Hofbauer SI, Riley RS. Overcoming the challenge of long-term storage of mRNA-lipid nanoparticle vaccines. *Mol Ther*. 2022 May; 30 (5): 1792–3. doi:10.1016/j.ymthe.2022.04.004
26. Nature Research Custom Media. Beyond COVID vaccines: what's next for lipid nanoparticles? [Internet]. London: Nature Research; 2022. Available from: https://www.nature.com/articles/d42473-022-00270-3 (accessed on 2025-07-17).
27. Mangraviti A, Tzeng SY, Kozielski KL, Wang Y, Jin Y, Gullotti D, *et al*. Polymeric nanoparticles for nonviral gene therapy extend brain tumor survival *in-vivo*. *ACS Nano*. 2015 Feb 10; 9 (2): 1236–49. doi:10.1021/nn504905q
28. Parayath NN, Stephan SB, Koehne AL, Nelson PS, Stephan MT. In vitro-transcribed antigen receptor mRNA nanocarriers for transient expression in circulating T cells in-vivo. *Nat Commun*. 2020 Nov 27; 11 (1). doi:10.1038/s41467-020-19486-2
29. Li Y-R, Zhu Y, Halladay T, Yang L. In-vivo CAR engineering for immunotherapy. *Nat Rev Immunol*. 2025 May 16; 25 (10): 725–44. doi:10.1038/s41577-025-01174-1
30. Abdalla AM, Xiao L, Miao Y, Huang L, Fadlallah GM, Gauthier M, *et al*. Nanotechnology promotes genetic and functional modifications of therapeutic T cells against cancer. *Adv Sci (Weinh)*. 2020 Feb 20; 7 (10). doi:10.1002/advs.201903164
31. Sairam AB, Sanmugam A, Pushparaj A, Mahesh Kumar G, Sundarapandian N, Balaji S, *et al*. Toxicity of polymeric nanodrugs as drug carriers. *ACS Chem Health Saf*. 2023 Jun 23; 30 (5): 236–50. doi:10.1021/acs.chas.3c00008
32. Shin S, Lee P, Han J, Kim S-N, Lim J, Park D-H, *et al*.

Nanoparticle-based chimeric antigen receptor therapy for cancer immunotherapy. *Tissue Eng Regen Med*. 2023 Mar 3; 20 (3): 371–87. doi:10.1007/s13770-022-00515-8

33. Parodi A, Quattrocchi N, van de Ven AL, Chiappini C, Evangelopoulos M, Martinez JO, *et al*. Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions. *Nat Nanotechnol*. 2012 Dec 16; 8 (1): 61–8. doi:10.1038/nnano.2012.212
34. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. *Nat Rev Drug Discov*. 2020 Dec 4; 20 (2): 101–24. doi:10.1038/s41573-020-0090-8
35. Zielińska A, Carreiró F, Oliveira AM, Neves A, Pires B, Venkatesh DN, *et al*. Polymeric nanoparticles: production, characterization, toxicology and ecotoxicology. *Molecules*. 2020 Aug 15; 25 (16): 3731. doi:10.3390/molecules25163731
36. Zepeda-Cervantes J, Ramírez-Jarquín JO, Vaca L. Interaction between virus-like particles (VLPs) and pattern recognition receptors (PRRs) from dendritic cells (DCs): toward better engineering of VLPs. *Front Immunol*. 2020 Jun 9; 11: 1100. doi:10.3389/fimmu.2020.01100
37. Banskota S, Raguram A, Suh S, Du SW, Davis JR, Choi EH, *et al*. Engineered virus-like particles for efficient in-vivo delivery of therapeutic proteins. *Cell*. 2022 Jan; 185 (2): 250-265. doi:10.1016/j.cell.2021.12.021
38. Zdanowicz M, Chroboczek J. Virus-like particles as drug delivery vectors. *Acta Biochimica Polonica*. 2016 Jul 30; 63 (3). doi:10.18388/abp.2016_1275
39. He J, Yu L, Lin X, Liu X, Zhang Y, Yang F, *et al*. Virus-like particles as nanocarriers for intracellular delivery of biomolecules and compounds. *Viruses*. 2022 Aug 28; 14 (9): 1905. doi:10.3390/v14091905
40. Ruedl C, Schwarz K, Jegerlehner A, Storni T, Manolova V, Bachmann MF. Virus-like particles as carriers for T-cell epitopes: limited inhibition of T-cell priming by carrier-specific antibodies. *J Virol*. 2005 Jan 15; 79 (2): 717–24. doi:10.1128/jvi.79.2.717-724.2005
41. Van Rosmalen MGM, Li C, Zlotnick A, Wuite GJL, Roos WH. Effect of dsDNA on the assembly pathway and mechanical strength of SV40 VP1 virus-like particles. *Biophys J*. 2018 Nov; 115 (9): 1656–65. doi:10.1016/j.bpj.2018.07.044
42. Kines RC, Thompson CD, Spring S, Li Z, de los Pinos E, Monks S, Schiller JT. Virus-like particle-drug conjugates induce protective, long-lasting adaptive antitumor immunity in the absence of specifically targeted tumor antigens. *Cancer Immunol Res*. 2021 Jun; 9 (6): 693–706. doi:10.1158/2326-6066.CIR-19-0974
43. Vicente T, Roldão A, Peixoto C, Carrondo MJT, Alves PM. Large-scale production and purification of VLP-based vaccines. *J Invertebr Pathol*. 2011 Jul; 107 Suppl: S42–8. doi:10.1016/j.jip.2011.05.004
44. Di M, Long JB, Isufi I, Foss FM, Seropian S, Gross CP, *et al*. Total costs of care during chimeric antigen receptor T-cell therapy in patients with relapsed/refractory B-cell non-Hodgkin lymphoma: a large private insurance claim-based analysis. *Blood*. 2022 Nov 15; 140 (Supplement 1): 10818–9. doi:10.1182/blood-2022-164915
45. Wang X, Rivière I. Clinical manufacturing of CAR T cells: foundation of a promising therapy. *Mol Ther Oncolytics*. 2016; 3: 16015. doi:10.1038/mto.2016.15
46. Fontana D. In-vivo CAR-T: faster, cheaper, and more effective cancer care [Internet]. London: Drug Target Review; 2026. Available from: https://www.drugtargetreview.com/in-vivo-car-t-faster-cheaper-and-more-effective-cancer-care/64485 8 (accessed on 2025-08-02).
47. Mansouri V, Yazdanpanah N, Rezaei N. The immunologic aspects of cytokine release syndrome and graft versus host disease following CAR T-cell therapy. *Int Rev Immunol*. 2021 Oct 5; 41 (6): 649–68. doi:10.1080/08830185.2021.1984449