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Lipid nanoparticles for engineering next generation CAR T cell immunotherapy

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Lipid nanoparticles are a burgeoning technology which has vast potential to improve chimeric antigen receptor (CAR) T cell immunotherapy. This focused review provides an overview of CAR T cell therapy – highlighting its promises, limitations, and challenges – and describes ways in which lipid nanoparticles (LNPs) can be rationally designed to circumvent some of the challenges. Of particular note are antigen presenting cell-mimetic LNPs, which have the potential to streamline the CAR T cell production process by activating T cells and delivering the CAR transgene in a single step. Although the current clinical standard is *ex vivo* CAR T cell production, *in vivo* CAR T cell production represents a potentially transformative alternative. Recent innovations in each production method are described, with a particular emphasis on ways in which LNPs may enable *in vivo* CAR T cell production. The review concludes with a discussion of safety, immunogenicity, scalability, manufacturing, and regulatory factors which will be essential as LNP-based CAR T cell immunotherapies move toward clinical translation.

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1. Introduction

1.1. CAR T cell therapy: promises, limitations, and challenges

Chimeric antigen receptor (CAR) T cell therapy is emerging as a breakthrough approach in cancer treatment. Mechanistically, this method relies on the genetic modification of T cells to express synthetic CAR receptors that particularly bind to tumour-associated antigens (TAA), therefore directing their cytotoxic activity towards cancer cells. Clinical studies of CAR T cell therapy have found favourable results in hematological malignancies such as aggressive lymphoma, with some cases of relapsed or refractory acute lymphoblastic leukaemia (ALL) demonstrating complete and sustained remission.[1–5] To produce CAR T immunotherapy, T cells are collected and separated from the patient, modified *ex vivo* to express CARs, and then re-infused into the patient. The transmembrane CAR design allows T cells to recognize and bind to malignant cells and trigger an intracellular cascade that potentiates cytotoxic functions, thereby inducing apoptosis and eradicating cancer *via* the immune system of the patient.[6]

Despite their success in treating certain forms of leukaemia and lymphoma, CAR T cell therapies face substantial limitations when applied more broadly. In solid tumours, their efficacy is often poor due to insufficient trafficking of T cells into tumour sites, highly immunosuppressive microenvironments, and the emergence of antigen escape variants that evade immune recognition.[1,2] These shortcomings severely limit curative potential outside of hematological malignancies. Furthermore, CAR T activation can trigger severe systemic toxicity, most notably cytokine release syndrome (CRS), which in some cases escalates to life-threatening conditions. Neurotoxicity is another major safety concern, with manifestations such as encephalopathy, seizures, and cerebral edema. While CRS can be partially mitigated using agents such as the IL-6 blocking antibody Tocilizumab, regulating these adverse events remains difficult and continues to shape the overall safety profile.[7] Additionally, some patients relapse because CAR T cells do not persist long enough or lose efficacy over time. Prolonged exposure to antigen and suppressive signals in the tumour microenvironment (TME) drives T cells into exhaustion and senescence, further limiting cytotoxicity and durability.[8–10] Collectively, these biological, safety, and durability issues underscore the current limitations of CAR T therapy, particularly in solid tumours (Scheme 1).

Beyond biological constraints, the broader application of CAR T therapy is hindered by major translational and logistical challenges. Manufacturing remains highly complex, requiring individualized *ex vivo* T cell engineering, often with viral vectors, making the process costly, time-consuming, and difficult to scale. These hurdles delay treatment for patients with rapidly progressing disease and limit accessibility across large patient populations. Tumour heterogeneity adds another

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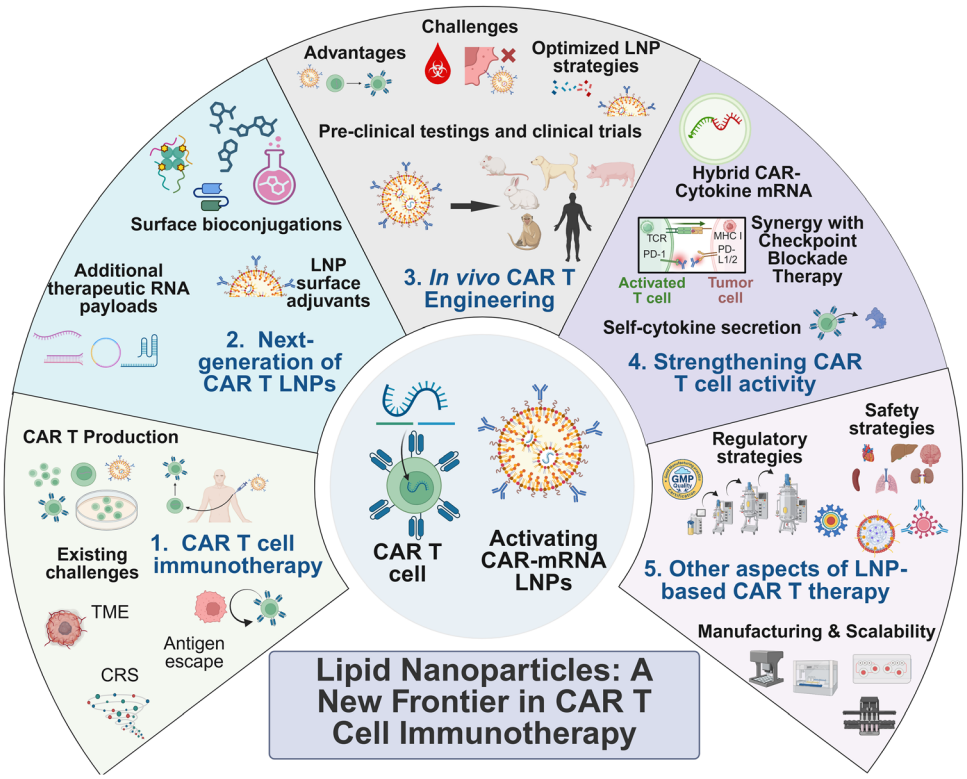

Scheme 1 Schematic illustration of lipid nanoparticle (LNP)-mediated strategies in advancing CAR T cell immunotherapy. The scheme highlights key aspects of CAR T cell therapy, comparing traditional *ex vivo* production methods with emerging *in vivo* engineering strategies enabled by activating CAR-mRNA LNPs. Detailed are the current and next-generation chemistries employed for surface functionalization (*e.g.*, click chemistry and affinity-based conjugations) and versatile RNA payloads (cytokines, circular RNA, saRNA, siRNA, CRISPR/Cas9 systems) for tailored therapeutic outcomes. Further, optimized LNP formulations to overcome delivery challenges such as off-target toxicity and suboptimal targeting efficiency are depicted. The synergy between CAR T cells and checkpoint blockade therapies is also illustrated, emphasizing combined modalities for enhanced immune activation. Lastly, critical considerations for safety, manufacturing scalability, and regulatory compliance essential for clinical translation of LNP-based CAR T therapies are summarized.

challenge, as differential or dynamic antigen expression can result in immune escape and disease relapse, as highlighted in early clinical experiences such as refractory large B-cell lymphoma.[1] In solid tumours, chronic antigen exposure and competition for metabolic resources within the TME exacerbate functional decline, making durable responses difficult to achieve. Current reliance on autologous production further complicates scalability, as the massive doses required for clinical benefit exceed preclinical models and risk additional toxicities.[2,5,8] Consequently, "off-the-shelf" allogeneic CAR T approaches are being investigated to streamline manufacturing and broaden accessibility. Addressing these multifaceted challenges will require innovations in CAR design, strategies to counteract immunosuppressive TMEs, and improved manufacturing protocols. These efforts are essential to expand the therapeutic reach of CAR T therapies beyond hematologic malignancies and toward more diverse cancers and clinical indications. Thus, in this focused review, we address the state-of-the-art as well as offer a critical evaluation of the advantages and difficulties of CAR T therapy, focusing on the ways in which lipid nanoparticles (LNPs), one of the most clinically advanced delivery platforms, could be applied to overcome existing difficulties.

1.2. Cellular activation requirements for CAR T cell production and the use of CAR mRNA-LNPs

T cells must be activated in order to perform their effector functions in the body. Antigen-presenting cells (APCs) mediate T cell activation by capturing, processing, and presenting antigenic peptides to T cells. APCs – most notably dendritic cells (DCs) – internalize pathogens *via* mechanisms including phagocytosis, receptor-mediated endocytosis, or macropinocytosis.[11,12] Upon antigen capture and recognition of danger signals, DCs undergo maturation, characterized by the upregulation of co-stimulatory molecules CD80 and CD86 and chemokine receptor CCR7. These changes facilitate the migration of DCs to secondary lymphoid organs, where they activate naive T cells. Complete T cell activation requires multiple signals from DCs: (1) processed antigen presented on the DC surface (*via* major histocompatibility complexes (MHCs)) must engage with T cell receptor (TCR)/CD3 complexes on the T cell. This is known as "Signal 1," and is antigen specific. (2) CD80 or CD86 on the DC surface must engage with CD28 on the T cell surface. This is known as "Signal 2," and is antigen nonspecific.[13–15] Apart from DCs, other professional APCs, such as macrophages and B cells, also greatly help to coordinate adaptive immunity. Often abundant in sites of infection and inflammation, macrophages continually phagocytose pathogens and preserve

antigen presentation throughout an immune response.[16,17] Mostly known for their function in antibody synthesis, B cells can also internalize antigens *via* their particular B cell receptors and provide processed peptides to helper T cells, thus strengthening the humoral response.[18] Taken together, these coordinated events ensure that T cells obtain the exact signals required for activation, growth, and the development of long-lasting immunity.

In current *ex vivo* CAR T cell manufacturing processes, the isolated patient T cells must be activated prior to viral gene transfer. Because it would be exceedingly difficult to exactly recapitulate APC-T cell interaction *ex vivo*, antibodies binding to CD3 and CD28, often attached to magnetic beads for easy removal, are used as artificial APCs.[19] This step, followed by viral gene transfer and expansion, is a tedious multi-step process that often takes 1–2 weeks. Manufacturing also must be completed in specialized good manufacturing practices (GMP) facilities, which is labor-intensive and expensive. Further, prolonged activation and expansion drive T cells to differentiate, which can diminish their persistence and antitumour potency. These logistical and biological challenges have constrained the scalability of CAR T therapies.[20–23]

In recent years, researchers have explored innovative approaches to streamline CAR T production by combining or even eliminating steps. A key goal is to simultaneously activate T cells and deliver CAR genes, thereby avoiding lengthy expansion. Several breakthrough strategies have been reported in the past five years. One strategic approach is rapid *ex vivo* manufacturing. For instance, it was demonstrated that functional CAR T cells can be created in less than 24 hours from peripheral blood without any dedicated T cell activation step.[20] By optimizing culture conditions such as cytokines, media formulation, and surface area to volume ratio, efficient viral transduction of resting T cells can be achieved to bypass the usual bead activation and expansion. Remarkably, this overnight production of CAR T cells retained a less differentiated phenotype and demonstrated potent anti-leukaemia activity in mice, even surpassing conventionally expanded CAR T cells in efficacy on a per-cell comparison basis. This finding suggests that avoiding prolonged *ex vivo* stimulation can produce a more potent T cell product, addressing one of the drawbacks of the standard protocol: over-differentiation of T cells. While this method still relies on viral vectors, it proves that the time from vein to product can be dramatically reduced, laying the groundwork for point-of-care CAR T therapies.

Moreover, the current dependence on viral vectors for gene delivery raises safety and regulatory concerns such as insertional mutagenesis and further drives up cost and manufacturing time.[24]

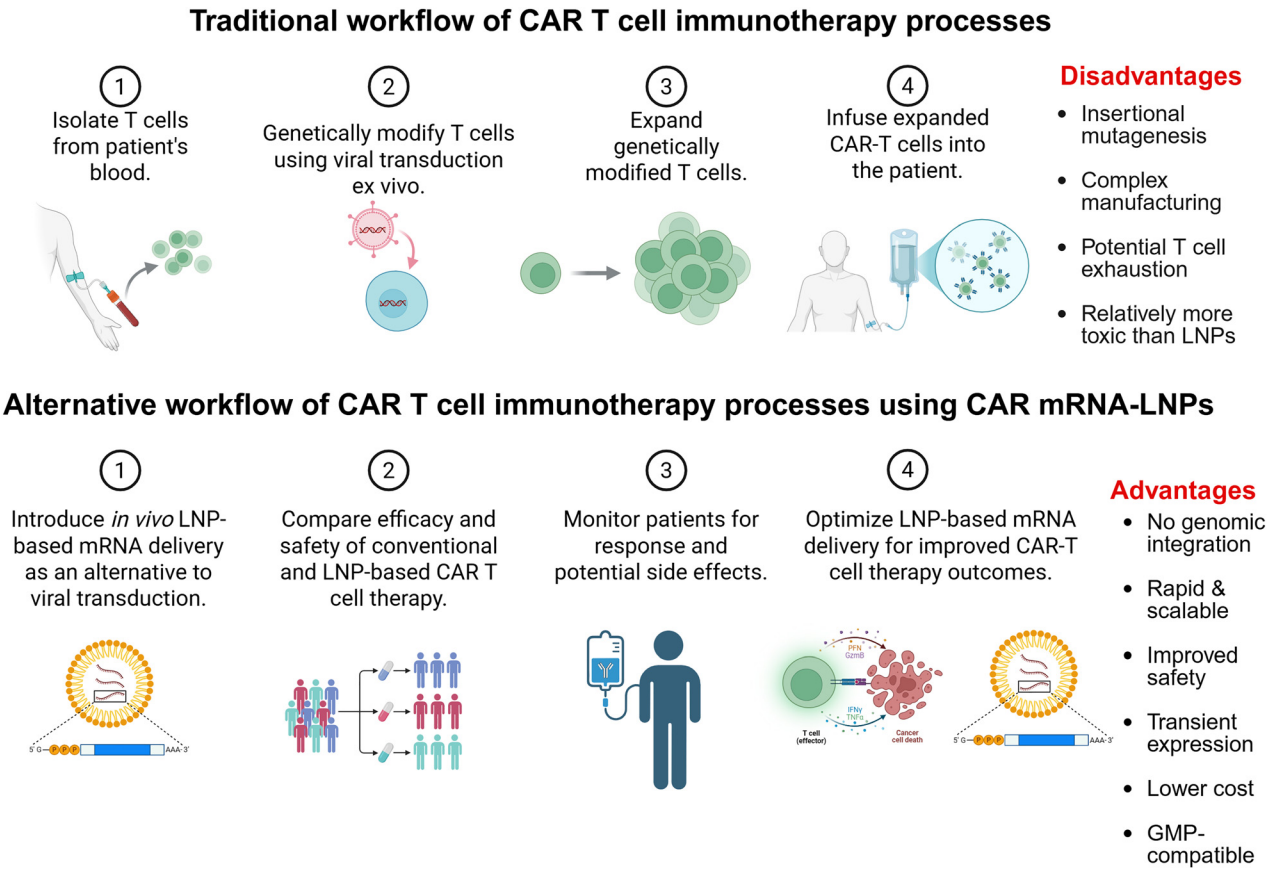

Fig. 1 Comparison of traditional CAR T cell immunotherapy workflow *versus* mRNA-LNP based approaches. The traditional process involves viral transduction of patient-derived T cells *ex vivo*, expansion, and reinfusion, but carries disadvantages such as insertional mutagenesis risk, complex manufacturing, T cell exhaustion, and higher toxicity. In contrast, mRNA-LNP strategies enable rapid, non-integrating, and scalable delivery of CAR constructs, offering transient expression, improved safety, reduced cost, and compatibility with automated GMP manufacturing.

Thus, there is a strong motivation to develop faster, simpler, nonviral strategies for CAR T cell activation and genetic modification. Nanoparticles – particularly lipid nanoparticles (LNPs) – have emerged as a promising nonviral delivery system. LNPs can deliver CAR-encoding mRNA without requiring viral vectors, avoiding insertional mutagenesis and potentially reducing manufacturing complexity. Notably, CAR T cells engineered *via* mRNA-LNP transfection exhibit prolonged CAR expression and function *in vitro* with less toxicity and T cell exhaustion than those made by electroporation. LNP-based approaches can also enable novel strategies such as the *in vivo* generation of CAR T cells inside the patient, obviating lengthy *ex vivo* culture (Fig. 1). We have therefore focused this review on integrating LNP technology into next-generation CAR T cell therapy. Importantly, the LNP platform is highly versatile and can be modularly assembled with various surface antibodies to mimic APCs. Such APC-mimetic LNPs can facilitate T cell uptake and activation for CAR T immunotherapy. Examples of these targeting antibodies include anti-CD3, anti-CD28, anti-CD4, anti-CD5, and anti-CD7.[25–30]

2. Design and engineering of APC-mimetic LNPs

2.1. Rational design of APC-mimetic LNPs

Thus, an initial research focus in the field was to develop and optimize lipid nanoparticles (LNPs) with the ability to deliver CAR-encoding messenger RNA (mRNA), *ex vivo*, to primary human T cells. Typically, LNPs are formulated from four components: an ionizable lipid which maintains a neutral charge at physiological pH but assumes a positive charge once in the acidic endosomes to facilitate cytosolic mRNA delivery; a helper phospholipid to improve LNP structure and molecular arrangement; cholesterol to promote LNP stability; and lipid-anchored polyethylene glycol (PEG) to support LNP assembly and reduce aggregation.[31,32] These components are suspended in an ethanolic phase and combined with aqueous mRNA in a microfluidic mixing device to produce mRNA-LNPs.[33,34] Toward the goal of using LNPs to deliver mRNA *ex vivo* to human T cells, LNPs formulated with different ionizable lipids were screened to identify an ionizable lipid that resulted in robust CAR mRNA delivery to primary human T cells,[35] before using a design of experiments approach to optimize the proportions of helper phospholipid, cholesterol, and lipid-PEG included in the LNPs for maximal CAR mRNA delivery.[36]

The self-assembled nanostructure of an LNP encapsulating mRNA is typically an amorphous lipid matrix with the mRNA strands electrostatically complexed in the core (Fig. 2). Despite extensive study, the precise internal structure of mRNA-LNPs remains an area of ongoing research due to their dynamic and fluid nature. High-resolution analyses such as cryo-EM and NMR indicate that ionizable lipids and phospholipids likely form an inverted micellar structure or lipid bilayer fragments surrounding the nucleic acid, with cholesterol dispersed throughout and PEG-lipids predominantly decorating the surface.[31,33,36] The ratio and arrangement of these components can subtly shift depending on formulation parameters, which in turn affects biological performance. Particle size, typically 50

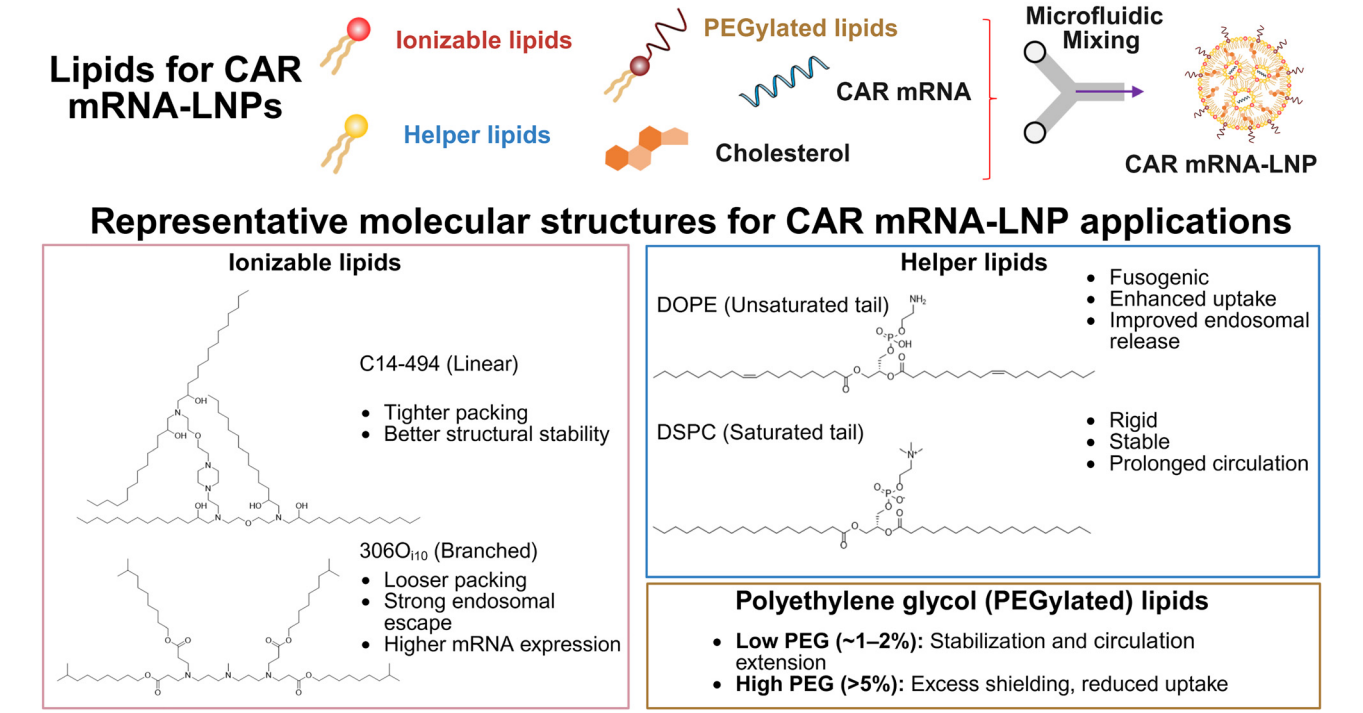

Fig. 2 Key lipid components of CAR mRNA-LNPs. Ionizable lipids (linear *vs.* branched) dictate packing, stability, and mRNA expression. Helper lipids such as DOPE (fusogenic) and DSPC (rigid) tune uptake and circulation, while PEGylated lipids balance stability with cellular uptake depending on PEG density.

to 150 nm for mRNA-LNPs, is another critical structural attribute. Studies have found that smaller nanoparticles tend to penetrate tissues and cell membranes more readily, whereas larger ones may carry more payload but risk clearance by macrophages. In the context of CAR-T cell engineering, tuning the LNP size and surface composition can influence whether nanoparticles preferentially accumulate in organs like the spleen, where many T cells reside, or remain in circulation.

The material choices above impart LNPs with a finely balanced profile of stability and delivery efficiency. The combination of helper lipids and cholesterol grants structural robustness, ensuring the mRNA is protected from RNases and mechanical stress during circulation. At the same time, the inclusion of ionizable lipids, as opposed to permanently charged lipids and optimized PEG content allows the nanoparticle to shed its stealth coating when needed.[31,33,36] This facilitates and promotes cellular uptake and endosomal escape once the LNP reaches target cells. Studies have shown that using different ionizable lipid chemistries can dramatically alter transfection outcomes and toxicity profiles. For instance, LNPs incorporating the lipid SM-102 generally elicited higher inflammatory cytokine release than those incorporating the lipid MC3, indicating that lipid structure can nuance the immune response and uptake efficiency of LNPs. These findings reinforce that materials chemistry at the nanoscale, such as the choice of lipid tail saturation, headgroup pK_a, PEG density, or inclusion of targeting moieties, directly translates to biological differences in how well the LNPs deliver to T cells, how efficiently they release mRNA inside those cells, and how stable the mRNA payload remains *en route*.[31] Going forward, continued research on novel lipids and nanoparticle architectures, including one-component systems that integrate multiple functions, will further refine the stability and targeting of mRNA-LNPs, accelerating their application in next-generation CAR-T cell therapies.

Separately, there has been much recent interest in conjugating antibodies or peptides covalently to the surface of LNPs to direct them to endocytic receptors on target cell populations.[37,38] Such strategies improve on-target mRNA delivery while decreasing off-target mRNA delivery. Currently, several bioconjugation strategies are under exploration for functionalization of the surface of mRNA-LNPs. LNPs can be formulated with a portion of the lipid-PEG replaced by lipid-PEG-maleimide for a ''click'' chemistry reaction with a thiol functional group for Michael-addition.[39–43] The thiol group can be added to the antibodies by reacting them with N-succinimidyl S-acetylthioacetate (SATA),[41,42] or by adding a reducing agent to convert endogenous disulfide bonds within the antibodies to thiols.[39,40,43] Similarly, LNPs can be formulated with a portion of the lipid-PEG replaced by lipid-PEG-azide, and the azide can undergo strain-promoted ''click'' chemistry reaction with an antibody which has been labeled with a DBCO moiety.[44]

Recently, the use of lipid-PEG-TCO (*trans*-cyclooctene) have also expanded the click chemistry bioconjugation reactions. The cyclopropene functional group, such as cyclopropene derivative of lysine (CypK) can be easily incorporated into the antibody through genetic-code expansion using noncanonical amino acids. The bioconjugation proceeds by an inverse electron demand Diels–Alder reaction that has been shown to be rapid, efficient, stable in serum and adopted in antibody–drug conjugates.[45] Other strategies have also been developed, which involve the use of protein engineering to create antibody and protein constructs with the ability to directly fuse with the LNP surface. This type of bioconjugation involves non-covalent reactions. Two approaches have been established on this front. The ASSET system (Anchored secondary scFv enabling targeting) utilizes a lipid-anchored single-chain antibody fragment inserted into LNPs to rapidly and efficiently bind the Fc regions of diverse targeting antibodies in a versatile, plug-and-play manner, enabling highly specific immune-cell targeting *in vivo* without altering antibody function.[46] The biotin–streptavidin approach similarly leverages affinity-based conjugation, linking biotinylated antibodies to biotin-functionalized LNPs *via* streptavidin, facilitating quick and flexible ligand attachment. Although streptavidin–biotin conjugation demonstrates strong binding and rapid surface decoration, its practical use *in vivo* is somewhat limited by potential immunogenicity and steric complexity.[25]

Overall, current work in this space generally relies on antibodies against an endocytic receptor.[37,38] Thus, while the antibody conjugation aids with LNP uptake, it does not directly influence the phenotype of the target cell. However, T cells are a unique target because they must be activated prior to *ex vivo* engineering with LNPs:[35,36,47] thus, simply targeting an endocytic receptor would not be sufficient. As noted above, in the body, T cell activation is facilitated by APCs through numerous precisely regulated receptor–ligand interactions. The two most essential activation signals are provided when MHCs (displaying antigenic peptides) on an APC bind to the TCR/CD3 complex on a T cell, and when CD80 or CD86 molecules on the APC engage with CD28 on the T cell.[14,15] For *ex vivo* engineering of T cells, this process is mimicked with antibodies against CD3 and CD28, which are used either in soluble form or conjugated to magnetic beads for enhanced stimulation and easy removal.[19] In a standard T cell LNP administration workflow, isolated primary T cells are incubated with activating beads in culture for 24 hours. Then, the beads are removed with a magnet and mRNA-LNPs are added.[35,36]

While effective, this strategy is expensive due to the high cost of the beads. It is also time consuming due to its multiple steps, and results in loss of isolated T cells because a portion of the cells remain attached to the beads during the removal process. Therefore, a recent study aimed to develop mRNA-LNPs with CD3 and CD28 antibodies directly conjugated to their surface.[48] Different from previous antibody-LNP strategies which have sought only to target an endocytic receptor, the goal of this LNP was to directly modulate the phenotype of the T cell. By mimicking antigen-presenting cells (APCs), these ''activating LNPs'' (aLNPs) provide both the primary TCR/CD3 signal and the secondary CD28 costimulatory signal while simultaneously delivering CAR mRNA. This design enabled one-step T cell

activation and RNA delivery, bypassing the need for activating beads.[48] In preclinical studies, aLNPs efficiently activated primary human T cells, produced functional CAR T cells in a single step, and reduced tumour burden *in vivo*. This APC-mimetic strategy illustrates the potential of engineering nanoparticle surfaces to direct T cell fate during CAR T cell production.

2.2. Key innovations and next directions for the APC-mimetic LNP platform

In the above study, aLNPs were used to deliver CD19-directed CAR mRNA to primary human T cells *ex vivo*, and the resulting CAR T cells were administered in a xenograft mouse model of acute lymphoblastic leukaemia resulting in reduced tumour burden.[48] Moving forward, aLNPs could be applied in numerous other ways. For example, because aLNPs activate T cells, perhaps they could be intratumourally injected to turn ''cold'' immunosuppressive tumours into ''hot'' immunologically active tumours. Additionally, the aLNP cargo would not need to be restricted to CAR mRNA – aLNPs could be used to deliver mRNA encoding proteins such as cytokines or chemokines to further bolster T cell activation. Since multiple mRNAs can be formulated into a single LNP, these mRNAs could be delivered alone, or in combination with CAR mRNA – or multiple CARs could be delivered to protect against antigen escape. Furthermore, with slight changes to the base LNP formulation, aLNPs could be used to deliver circular RNA or self-amplifying RNA for protein expression that would remain transient but be more durable than with standard mRNA. Also, aLNPs could encapsulate a combination of mRNA and siRNA. Such a strategy was recently used to deliver CAR mRNA and PD-1 siRNA to create PD-1-knockout, checkpoint blockade resistant, CAR T cells.[49] Alternatively, the LNP base formulation could be altered to enable the encapsulation of transposon systems or CRISPR-Cas9 systems. As specific examples, a transposon system could be employed as a nonviral strategy to produce permanently transduced CAR T cells, and CRISPR-Cas9 systems could be used to knock out MHC expression for rejection-resistant ''off-the-shelf'' allogeneic cell therapy products.

Furthermore, CD3 and CD28 represent two of many T cell receptors that could be targeted by aLNPs. For instance, *in vivo*, after T cells are initially activated by CD3/28 stimulation, the engagement of additional T cell surface receptors helps to maintain activation and promote overall T cell wellness. As examples, CD2 engagement increases cytokine signaling and proliferation, CD27 engagement promotes longevity and memory formation, 4-1BB engagement boosts effector function and overall survival, and OX40 engagement prevents apoptosis.[15] Similarly, stimulation of several T cell interleukin receptors (IL-Rs) further bolsters activation. IL-2R and IL-7R binding promotes proliferation and long-term survival; IL-15R binding prevents exhaustion and maintains a memory cell population; and IL-12R and IL-18R binding increase the activity of cytotoxic T cells.[50] Combinations of antibodies against the aforementioned targets and recombinant cytokines could be conjugated to the aLNP surface in addition to the CD3 and CD28 antibody fragments to create aLNPs that not only activate T cells and deliver CAR mRNA, but also promote and protect the health of the resulting CAR T cells to create infusion products with highly desirable phenotypes. Finally, we provide a comparison of current approaches and summarize the key APC-mimetic LNP strategies in Table 1, outlining their principal advantages and limitations. This includes the prototypical anti-CD3/CD28 aLNP design as well as potential variant strategies that incorporate alternative ligands or payloads.

3. LNP applications in rapid CAR T cell engineering and cancer immunotherapy

3.1. *In vivo* CAR T cell engineering

Due to the challenges involved with *ex vivo* engineering, *in vivo* engineering presents a potentially promising advancement in the development of CAR T cell therapies – and LNPs represent a promising enabling technology.[48] *In vivo* CAR T refers to the direct genetic modification and activation of T cells within the patient's body bypassing the traditional *ex vivo* cell manipulation steps. One of the most notable benefits of *in vivo* CAR T cell

Table 1 Strategies for APC-mimetic LNPs, with key advantages and limitations

APC-mimetic LNP strategy	Advantages	Limitations
Activating LNP (aLNP) with anti-CD3 and anti-CD28	• One-step T cell activation and transfection, eliminating bead step	• Transient expression (mRNA)
LNP decorated with T-cell activating antibodies, delivering CAR mRNA	• Streamlined manufacturing and reduced cost	• Antibody conjugation adds formulation complexity
	• ~6.5-fold higher mRNA delivery efficiency	• Preclinical proof-of-concept stage
	• Retains CAR T cell cytotoxicity	• Possible immunogenicity from antibody fragments
	• Tunable CD3:CD28 ratio to adjust T cell phenotype	
Variant aLNP designs (alternative or additional ligands)	• Flexible activation by incorporating other ligands (*e.g.*, 4-1BB, OX40)	• Limited validation; largely conceptual
	• Targeted delivery to specific T cell subsets	• Balancing multiple stimulatory signals requires optimization
	• Potential for diverse payloads (DNA, siRNA, proteins)	• If mRNA-based, expression remains transient

engineering is the substantial reduction in time required for therapy preparation. Traditional *ex vivo* methods can take 2–3 weeks to harvest, engineer, and reinfuse CAR T cells into the patient. In contrast, the *in vivo* approach skips the time-consuming steps of leukapheresis and external engineering, leading to a significant reduction in overall treatment timelines. This also significantly reduces the overall cost of the therapy, making it accessible to a larger patient population. Furthermore, *in vivo* engineering removes the risk of external variability in the biological CAR T cell product being engineered. With *ex vivo* engineering, variability arises in the manipulation of biological materials, particularly with respect to the T cells themselves. In contrast, by bypassing *ex vivo* cell manipulation, *in vivo* CAR T cell engineering reduces variability and improves the overall consistency of the final product. Finally, *in vivo* CAR T cell generation can also bypass the need for allogeneic cell sources for CAR T cell therapy. Allogeneic cell sources have gained interest in CAR T cell therapy due to the difficulty in obtaining and culturing autologous cells to reach therapeutic levels. However, the use of allogeneic cells often raises concerns about graft-versus-host disease (GVHD), an immune complication that results in damage to the recipient from the transplanted T cells.[51] *In vivo* CAR T cell generation circumvents this issue by enabling delivery of CAR constructs directly to the patient's own T cells, thus eliminating the need for an allogeneic cell source and mitigating the risk of GVHD.

Despite its advantages, *in vivo* CAR T cell engineering also presents a few challenges.[5] One major concern is the potential for off-target delivery resulting from the chosen delivery vehicle. Off-target delivery of CAR constructs can result in unwanted side-effects and unexpected toxicity. Thus, the delivery vehicle utilized for *in vivo* CAR delivery to T cells must be carefully selected and optimized to minimize off-target delivery. Additionally, achieving optimal delivery efficiency to ensure that the engineered CAR T cells reach sufficient therapeutic thresholds in the body remains an ongoing challenge. While *in vivo* delivery may bypass some of the limitations associated with *ex vivo* culture, the efficiency of the delivery vehicle in targeting T cells remains crucial. Current research efforts are focused on improving delivery methods, with particular attention to optimizing targeting to the spleen and specifically T cells. Thus, ensuring the effective delivery and subsequent generation of CAR T cells is key to maximizing therapeutic outcomes.[48]

Overall, *in vivo* CAR T cell engineering offers a promising alternative to traditional *ex vivo* methods, addressing issues related to time, cost, and variability – which could greatly increase access to CAR T cell therapy. However, further advancements are needed to refine delivery vehicles and optimize cell generation efficiency to fully realize the potential of *in vivo* CAR T cell therapies. The modularity of the antibody-conjugated LNP platform means that it may be well-poised to support the development of *in situ* CAR T cell therapy. For instance, ionizable lipids can be formulated to be biodegradable which may improve the tolerability of intravenous LNP administration. And, because mRNA sequences can be precisely specified, microRNA target sequences could easily be added to the mRNA transcripts as a strategy to decrease unwanted off-target delivery. Further, careful selection of the ligands on the LNP surface might be able to promote T cell activation, proliferation, and survival without producing adverse systemic reactions.

3.2. Preclinical and clinical studies of *in vivo* CAR T cell engineering

To that end, there have already been several preclinical and clinical studies exploring *in vivo* CAR T cell therapies. These studies, using a variety of delivery vectors, highlight the breadth and promise of this burgeoning field – to which LNPs are already being applied. Successful *in vivo* CAR T cell engineering hinges on the effective delivery of the CAR construct specifically to T cells, the translation of the CAR protein within the T cells, and the subsequent functional activity of the CAR against the chosen antigen. There are various methodologies at the preclinical stage that are currently being explored to facilitate CAR delivery to T cells *in vivo*. These strategies can be broadly classified into viral and non-viral delivery approaches. Furthermore, several of these strategies are currently being tested in clinical trials. A summary of these emerging *in vivo* CAR therapies is shown in Table 2. These trials, employing both viral and non-viral delivery technologies, are exploring the use of CAR T cells in liquid tumours, solid tumours, as well as B cell-driven autoimmune diseases.

Viral delivery of CAR constructs. Viral delivery has been the traditional approach for introducing CAR constructs into T cells. Among the seven FDA-approved CAR T cell therapies, five (Abecma, Aucatzyl, Breyanzi, Carvykti, and Kymriah) utilize lentiviruses, while two (Tecartus and Yescarta) utilize retroviruses to deliver the CAR transgene. These viruses have been engineered to enable potent intracellular delivery of the CAR construct. They generally possess viral glycoproteins, such as vesicular stomatitis virus glycoprotein (VSVG), to promote fusion with the cell membrane and enable intracellular delivery.[52] Moreover, viral surface proteins can further be engineered to also display cell-selective targeting molecules alongside the fusogenic proteins, thereby enabling cell-specific delivery. One study leverages these properties to display anti-CD3/anti-CD28 single chain variable fragments (scFvs) on the surface of virus-like enveloped delivery vehicles (EDVs) to achieve T cell activation and deliver lentiviral CAR constructs specifically to T cells *in vivo*.[53] They demonstrate efficient targeting of human T cells *in vivo* in humanized mouse models and show complete B cell aplasia when delivering anti-CD19 CAR to T cells. Another study engineers a replication-incompetent lentiviral vector to display anti-CD3 scFv, enabling targeting of T cells *in vivo*.[54] This system, termed VivoVec, delivers anti-CD19 CAR while simultaneously delivering Rapamycin Activated Cytokine receptor (RACR), which triggers IL-2/IL-15 signaling upon interactions with the common cancer treatment, rapamycin.[54]

However, viral delivery methods are not without significant drawbacks. One of the major concerns is the risk of insertional mutagenesis, where the viral genome integrates into the host

Table 2 Current *in vivo* CAR T cell therapies in the clinical pipeline. R/R, Relapsed/Refractory

Drug name	Company/sponsor	Vector (cell-targeting mechanism)	Therapeutic payload (CAR target/type)	Lead indication (Category)	Planned status	Trial phase
INT2104	Interius BioTherapeutics (acquired by Gilead)	Lentivirus (CD7 scFv)	Anti-CD20 CAR transgene (drives *de novo* CAR-T and CAR-NK cell production)	R/R B-cell malignancies (liquid tumour)	Phase I initiated October 2024 (Australia)	Phase 1 (dose escalation active)
UB-VV111	Umoja Biopharma (AbbVie opt-in)	Lentivirus (CD3 scFv, CD80 and CD58)	Transgene encoding an anti-CD19 CAR plus a rapamycin-activated cytokine receptor (RACR for *in vivo* expansion)	R/R large B-cell lymphoma & CLL (liquid tumour)	Phase I initiated March 2025 (United States of America, Australia)	Phase 1 (dose escalation active)
ESO-T01	EsoBiotec SA (acquired by AstraZeneca)	Lentivirus (T cell-tropic)	Anti-BCMA CAR transgene (*in vivo* CAR-T programming)	R/R Multiple Myeloma (liquid tumour)	Phase I initiated November 2024 (China)	Phase 1 (dose escalation active)
MT-302	Myeloid therapeutics	LNP (Myeloid cell Fcα receptor)	mRNA encoding an anti-TROP2 CAR (expressed selectively in myeloid cells)	TROP2-expressing solid tumours (*e.g.* triple-negative breast, lung, colon) (solid tumour)	Phase I initiated August 2023 (Australia)	Phase 1 (dose escalation active)
MT-303	Myeloid therapeutics	LNP (Myeloid cell Fcα receptor)	mRNA encoding an anti-GPC3 CAR (expressed in myeloid cells)	Hepatocellular carcinoma (HCC) (solid tumour)	Phase I initiated July 2024 (Australia, South Korea)	Phase 1 (dose escalation active)
CPTX2309	Capstan therapeutics (acquired by Abbvie)	LNP (CD8 antibody)	mRNA encoding an anti-CD19 CAR (transient *in vivo* CAR-T programming in CD8 T cells)	B cell–mediated autoimmune diseases (*e.g.* lupus, myositis, RA, MS)	Phase I initiated April 2025 (Australia)	Phase 1 (dose escalation active)

genome, potentially leading to unintended genetic alterations that could cause adverse effects such as cancer.[55] Additionally, viral delivery systems typically result in persistent CAR expression, which could lead to toxicity even after the desired therapeutic effect has been achieved. Viral vectors also have a limited cargo capacity, restricting the size of the CAR construct that can be delivered.[56] Furthermore, the immune system may recognize the viral vector as foreign, triggering an immune response and preventing re-dosing of the therapy.[56,57] As a result of these limitations, non-viral delivery systems have gained traction as a safer and more flexible alternative.

Non-viral delivery of CAR constructs. Non-viral delivery systems do not pose the same risks of insertional mutagenesis as viral methods, nor do they generally induce long-term immunogenicity.[35] These systems typically result in transient expression of the CAR, which is advantageous in preventing long-term toxicity and allowing for controlled CAR expression. Nucleic acids such as mRNA, circular RNA (circRNA), and plasmid DNA (pDNA) that do not integrate into the genome and result in transient CAR expression are central to non-viral delivery approaches. Several types of nanoparticles have shown promise as non-viral delivery vehicles for delivering CAR-encoding nucleic acids. Poly beta-amino ester nanoparticles (PBAE NPs), in particular, have been utilized to achieve targeted delivery of anti-CD19 CAR-encoding mRNA to CD8 T cells *in vivo*. This targeted delivery enhanced the efficiency of CD8+ CAR+ T cells and improved the therapeutic outcome in preclinical Raji cell lymphoma mouse models.[21]

Similarly, lipid nanoparticles (LNPs) – the focus of this article – have also gained attention as non-viral delivery vehicles for *in vivo* CAR T cell engineering. As noted above, lipid nanoparticles can also be modified on their surface with targeting ligands to enable cell-specific delivery. One study employed anti-CD3 $F(ab')_2$-conjugated LNPs to demonstrate proof-of-concept mRNA delivery to T cells *in situ*.[58] Another study utilized anti-CD5 targeted LNPs to deliver CAR mRNA encoding for fibroblast activation protein (FAP) to T cells.[59] During cardiac injury, the engineered T cells were able to target fibroblasts, trogocytose FAP from the fibroblast surface, and improve cardiac function in injured mice. Another more exploratory study explored various targeting moieties, specifically anti-CD3/CD5/CD7 antibody fragments, to target T cells to engineer CAR T cells *in vivo*.[35,39] These LNPs were able to achieve extrahepatic delivery to T cell targets of interest, generate anti-CD19 CAR T cells, and effectively induce B cell aplasia in healthy mice.

Inspired by viral vectors, virus-like particles (VLPs) have emerged as a promising non-viral delivery system for CAR mRNA. VLPs mimic viral particles but lack viral genomic material, reducing the risk of genotoxicity. In one study, the fusogenic and T cell-tropic nature of human immunodeficiency virus (HIV) was employed to create T cell-targeting VLPs that were able to deliver CAR mRNA to T cells in humanized mice.[60] These *in vivo* engineered CAR T cells were able to reduce tumour burden and promote survival in mouse models of B cell lymphoma. Like their polymeric NP and LNP counterparts,

VLPs were also able to result in transient delivery of CAR, with diminishing numbers of CAR-expressing T cells observed up to 72 hours post-VLP treatment.

In conclusion, while viral delivery remains the cornerstone of CAR T cell engineering, the rise of non-viral strategies such as LNPs offers exciting new avenues for safer, more versatile therapies. With ongoing preclinical and clinical investigations, *in vivo* CAR T cell engineering holds great promise for advancing cancer immunotherapies and extending the benefits of CAR T cells to a broader range of patients.

3.3. Enhancing CAR T cell activity

CAR T cells have demonstrated remarkable efficacy in treating B cell-driven cancers, particularly in haematological malignancies such as leukaemia and lymphoma. However, their success in treating solid tumours has been more limited. This is due to several factors, including poor tumour infiltration, immunosuppressive signals in the tumour microenvironment, and interactions that lead to rapid T cell exhaustion and a subsequent decline in CAR T cell activity. As a result, there is a critical need to develop strategies that can enhance the activity and persistence of CAR T cells without the side effects of long-term toxicity in the context of solid tumours. Several approaches are being explored to strengthen CAR T cells, equipping them with better constructs to overcome these barriers and enhance their efficacy in both solid and liquid tumours.

Synergy with checkpoint blockade therapy. One of the most promising strategies to improve CAR T cell function is the use of checkpoint blockade therapy. Immune checkpoints, such as PD-1 and CTLA-4 on the T cell surface, bind to PD-L1, CD80 and CD86 on tumour cells and act as negative regulators of T cell activity. Thus, tumours often exploit these pathways to dampen immune responses, leading to T cell exhaustion and reduced efficacy of CAR T cells. By combining CAR T cell therapy with checkpoint blockade on tumour cells, it is possible to enhance the activity of CAR T cells and improve their ability to target and eliminate tumour cells. A key preclinical study demonstrated that tyrosine kinase-like orphan receptor 1 (ROR1)-targeted CAR T cells combined with anti-PD-L1 checkpoint blockade antibodies was able to enhance CAR T cell accumulation in the tumour and subsequent antitumour activity in a mouse model of lung adenocarcinoma.[61]

Another potential strategy to enhance CAR T cell activity is co-delivering the CAR construct while simultaneously inhibiting checkpoint receptors on T cells. A recent Phase I clinical trial attempted intrapleural administration of mesothelin-targeted CAR T cells to combat malignant pleural mesothelioma. This trial highlighted that anti-PD1 (Pembrolizumab) following CAR T administration was safe and demonstrated sufficient antitumour efficacy in the treated patients.[62] Another preclinical study utilized LNPs to knock down programmed death 1 (PD1) expression using siRNA and deliver CAR mRNA to T cells, thereby engineering CAR T cells that demonstrated reduced exhaustion, extended T cell activity, and enhanced CAR expression kinetics.[49] These engineered CAR T cells were able to effectively induce tumour cell lysis of Raji B cell lymphoma cells *ex vivo*. Although done *ex vivo* in the proof-of-concept study, this LNP-based approach holds significant promise and can be further explored as an *in vivo* LNP platform.

Exploiting cytokine support for stronger CAR T cells. An exciting frontier in CAR T cell engineering is the use of cytokine support to strengthen CAR T cells. Cytokines play a crucial role in regulating immune responses, and enhancing the responsiveness of CAR T cells to these signals can improve their persistence and function.[63] One strategy is to have CAR T cells express engineered cytokine receptors on their surfaces, making them more responsive to specific cytokine cues. One study designed chimeric receptors that fused an orthogonal IL-2 receptor extracellular domain with common γ-chain cytokine receptor intracellular domains.[64] Of these, an IL-9 receptor intracellular domain resulted in activity that allowed for CAR T cells to have enhanced effector T cell function as well as characteristics of stem cell memory. Specifically, this IL-9R-driven signaling was able to enhance the tumour-killing activity of CAR T cells in solid tumour models of melanoma and pancreatic ductal adenocarcinoma and was able to prevent tumour burden upon rechallenge, indicating T cell memory.[64] A follow-up study also showed that natural IL-9R on CAR T cells was able to demonstrate similar enhanced activity, reduction in tumour burden and retention of the central memory phenotype.[65] Looking forward, the potential of both synthetic and endogenous IL-9R can be harnessed in CAR T cell engineering with the use of LNPs. For example, co-delivering nucleic acids encoding both CAR and IL-9R to enhance CAR T cell activity represents a promising next step in leveraging this cytokine receptor to strengthen CAR T cell function.

In addition to receptor engineering, another approach involves modifying CAR T cells to secrete cytokines themselves. These engineered CAR T cells can secrete cytokines that modulate the immune response, supporting not only the CAR T cells themselves but also other immune cells that contribute to the antitumour response. For example, the pleitropic cytokine IL-9 has been shown to control T cell-driven tumour cell killing. One study showed that sequestration of IL-9 led to enhanced tumour growth in a mouse model of pulmonary melanoma.[66] In contrast, treatment with tumour-specific Th9 cells, or helper T cells with high IL-9 signaling, led to decreased tumour burden. These T cells retained their IL-9 secretion and signaling even after adoptive transfer and resulted in efficient priming of CD8+ cytotoxic T cells for coordinated tumour cell killing. Another exciting study employed cytokine support from IL-7 while engineering CAR T cells *in vivo*. This study used an LNP system to deliver mRNA encoding for anti-tyrosinase related protein 1 (TRP1) CAR along with IL-7 to T cells.[25] Secretion of IL-7 by the CAR T cells enhanced proliferation of both CAR T cells as well as other intratumoural cytotoxic T cells. This also led to better tumour infiltration, enhanced tumour clearance, and improved survival in a mouse model of melanoma. Collectively, these studies highlight the transformative potential of combining cytokine support with CAR T cell engineering, offering exciting prospects for optimizing and advancing immunotherapy strategies. Furthermore, strategies

Table 3 Strategies to enhance CAR T cell therapy

Strategy	Advantages/rationale	Challenges/limitations
In vivo CAR T generation *via* targeted LNPs	• Bypasses *ex vivo* culture; scalable and off-the-shelf • Reduces cost and complexity • Transient, non-integrating mRNA improves safety	• Transient CAR expression may require repeat dosing • Risk of anti-vector immunity • Still at early clinical/preclinical stage
Armoured CAR T cells	• Secrete cytokines (*e.g.*, IL-12, IL-15) to remodel the tumour microenvironment • Support CAR T persistence and recruit other immune cells	• Risk of systemic cytokine toxicity • Added engineering complexity • Regulatory hurdles for multi-factor products
Checkpoint-modified CAR T cells	• Overcome exhaustion by PD-1 knockout or switch receptors • Improve persistence in suppressive environments • Early clinical signs of feasibility	• Autoimmunity risk if inhibitory pathways are removed • Gene-editing efficiency and safety require optimization • Permanent checkpoint removal may need safety switches
Multi-target/logic-gated CARs	• Reduce antigen escape by recognizing multiple antigens • Improve specificity and logic designs • Effective in heterogeneous tumours	• Complex constructs and co-expression may reduce yield • Potential cross-signaling interference • Risk if either antigen is expressed on healthy cells
Enhanced trafficking/persistence	• Chemokine receptor engineering improves tumour homing • Metabolic reprogramming enhances T cell fitness • Synergy with other tumour-modulating therapies	• Tumour specificity of chemokine signals may limit effect • Requires multiple modifications with regulatory complexity • Limited clinical data to date
ARMFUL Membrane-fusogenic liposome to engineer M1 macrophages	• One-step dual engineering (surface + cytoplasm) • Blocks CD47 for stronger phagocytosis • Resists M2 repolarization, sustains M1 state • Boosts antigen presentation and T cell activation • *In vivo* tumour suppression, immune memory, synergy with anti–PD-1	• Complex nanoparticle formulation • Scale-up and GMP hurdles • Possible immunogenicity of aCD47/lipids • Likely need for repeat dosing • Translation to humans untested

such as these are compatible with LNP technology, suggesting LNPs as a powerful tool for advancing the *in vivo* engineering of CAR T cells, paving the way for potentially more effective treatments.

Lipid-based modulation of macrophages to improve CAR T outcomes. In addition to direct T cell modification, lipid-mediated reprogramming of tumour-associated macrophages (TAMs) can potentially improve CAR T cell support. For example, a recent work developed a specialized core–shell liposomal system (ARMFUL) that fuses with M1 macrophage membranes and delivers an anti-CSF1R payload, all while inserting an anti-CD47 'don't-eat-me' signal blocker on the macrophage surface.[67] The result is a macrophage that resists M2 polarization and remains in a sustained pro-inflammatory (M1) state. In a melanoma mouse model, these engineered M1 macrophages effectively repolarized native TAMs to M1, remodeled the immunosuppressive microenvironment, and enhanced T cell activation and memory responses against the tumour. Such lipid-based macrophage polarization strategies could be combined with CAR T therapy. For instance, co-delivering ARMFUL-engineered macrophages alongside CAR T cells aids in improving CAR T cell infiltration and efficacy in solid tumours by creating a more permissive immune microenvironment.[67]

There have been a wide range of strategies that have been developed to enhance the efficacy and persistence of CAR T cells, each addressing different biological barriers such as tumour immune evasion, exhaustion, and trafficking. These approaches span novel manufacturing paradigms including *in vivo* CAR T generation *via* LNPs, genetic modifications such as armoured CAR T cells or checkpoint-modified constructs, and systems-level engineering that include multi-target or logic-gated designs, as well as enhancements to trafficking and metabolic fitness. A summary of these emerging strategies is shown in Table 3.

4. Challenges and future perspectives

4.1. Safety and immunogenicity considerations of LNPs

LNP-based platforms have emerged as transformative vehicles for mRNA therapeutics, as evidenced by recent advances in mRNA CAR T cell engineering. However, the clinical translation of these systems is tempered by concerns regarding off-target delivery, cytotoxicity, immunogenicity, and complement activation. A key consideration in developing future LNPs (and subclasses of LNPs, in particular, the APC-mimetic LNPs described above) is safety, which ensures a potent immune activation without off-target damage or systemic toxicity. Animal studies so far suggest that these biomimetic nanocarriers can be engineered for a favourable safety profile, but a careful and rational design is crucial. Targeted biodistribution is one strategy to mitigate toxicity. For instance, dendritic cell membrane-coated nanoparticles can naturally traffick to lymphoid organs, concentrating immune activation in lymph nodes rather than in distal tissues. Similarly, macrophage-membrane-coated nanoparticles which leverage innate homing molecules such as CCR2 or vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) can preferentially accumulate in inflamed tumours, thereby limiting exposure to healthy organs.[68] This tissue targeting strategy helps to avoid broad systemic T cell activation that could otherwise trigger cytokine-release syndrome. Nevertheless, like conventional LNPs, APC-mimetic LNP formulations could induce innate immune reactions such as complement activation, and rapid clearance by the mononuclear phagocyte system remains a concern. Cationic lipid components or immunoglobulin mimetics on the nanoparticle surface may set off hypersensitivity reactions such as complement activation-related pseudo-allergy if not properly controlled.[69] To address this, researchers are exploring stealth coatings and membrane cloaking to confer self-identity, which prolongs circulation and reduces acute inflammatory responses. Early toxicity studies in murine models have reported minimal off-target organ damage and no overt systemic autoimmunity when dosing with these biomimetic nanoparticles.[70,71] However, comprehensive safety profiling, such as cytokine release, histopathology, and long-term tolerance, is still needed before clinical translation.

On the immunogenicity front, APC-mimetic LNPs are designed to modulate both innate and adaptive immunity in a balanced and coordinated fashion. Importantly, providing the necessary signals prevents T cell anergy in nanoparticles presenting antigen alone, which could result in risk-inducing tolerance rather than immunity. Studies consistently show that incorporating co-stimulatory ligands on these mimetic particles yields robust T cell proliferation and effector functions. For example, a dendritic cell-mimetic nanovaccine displaying tumour peptide-MHC complexes alongside a co-stimulatory CD86 signal and an anti-checkpoint antibody was able to activate naive and even exhausted T cells in tumour-bearing mice.[72] This led to potent cytotoxic responses capable of rejecting established tumours while simultaneously ''reprogramming'' the immunosuppressive tumour microenvironment. In parallel, many APC-mimetic systems also engage the innate immune system to amplify adaptive responses. Some designs incorporate pathogen-associated stimuli or endogenous danger signals such as STING agonists or photodynamic therapy agents to provoke local dendritic cell maturation and cytokine release, effectively adjuvanting the T cell response. Conversely, other biomimetic nanoparticles exploit immune-evasion tactics to fine-tune immunogenicity, such as coating an LNP with leukocyte membranes, which can contain CD47 and other ''don't-eat-me'' signals that dampen immediate innate clearance, thus allowing the APC-LNPs to persist longer and distribute to lymphoid sites before unleashing their targeted immunostimulatory payload. The net effect is a carefully balanced immune engagement in which one aims to maximize antigen-specific activation in both $CD8^+$ and $CD4^+$ T cell responses while avoiding inadvertent tolerance induction or hyperinflammatory reactions.

From a precautionary viewpoint, the hybrid nature of these APC-LNPs, in which clinically validated synthetic carriers are integrated with biological components, raises unique safety

considerations. For example, the incomplete removal of cellular debris when sourcing membranes can introduce unwanted immunogens, risking anti-self-responses or unpredictable inflammation. Rigorously refining purification steps and characterizing nanoparticle composition is essential to ensure no unintended immune activation. Long-term toxicity also requires evaluation, including the fate of the nanoparticles post-delivery and any potential for autoimmunity or immunopathology upon repeated dosing. Looking ahead, the field of APC-LNPs is now moving toward more sophisticated "plug-and-play" designs in which modular nanoparticles can be easily configured with different targeting ligands, antigens, or immune modulators, depending on the clinical need. As we deepen our understanding of how these APC-mimetic LNPs interact with the immune system and learn more about their safety profiles within the non-human primate models or in the early-phase clinical trials, the insights gained will guide us towards safer, more effective immunotherapies.

4.2. Scalability, manufacturing, and regulatory considerations for APC-mimetic LNPs

The clinical translation of future APC-mimetic LNPs in cancer immunotherapy hinges on the ability to produce these nanoparticles at scale while maintaining quality and affordability. This is similar to the requirements of traditional LNPs. Traditional cell-based therapies like CAR T cells are manufactured *via* laborious, patient-specific processes, but APC-mimetic LNPs offer a more streamlined, off-the-shelf approach. By mimicking APCs and delivering activation signals, these synthetic nanoparticles could dramatically simplify production. Nonetheless, realizing this promise requires overcoming substantial chemistry, manufacturing, and control (CMC) challenges that have long impeded nanomedicine commercialization.[73] Encouragingly, LNP technology is amenable to large-scale fabrication. The recent success of mRNA vaccine LNPs demonstrated that billions of doses can be manufactured rapidly under Good Manufacturing Practice (GMP) conditions.[74] APC-mimetic LNPs leverage similar formulation principles, suggesting that batch or continuous production methods used for vaccines and RNA drugs can be adapted as well. In particular, microfluidic manufacturing has emerged as a powerful approach to ensure reproducibility when scaling up LNP synthesis. For example, advanced microfluidic platforms have achieved throughputs of $\sim$17 L hour^{-1} for mRNA-LNP production, bridging laboratory-scale formulation with industrial output.[34] Such high-throughput, automated systems can maintain tight control over particle size and composition, which is critical as scaling up LNP synthesis to GMP volumes often introduces challenges like suboptimal mixing, batch variability, and contamination risks. Innovative solutions, including AI-driven optimization of formulation parameters and design-of-experiment strategies, are being explored to fine-tune these complex manufacturing processes. Together, these advances indicate that the large-scale production of APC-mimetic LNPs is technically feasible, building on the robust nanoparticle manufacturing infrastructure established in recent years.

A major motivation for developing APC-mimetic LNPs is the potential to reduce therapy costs by replacing expensive biologic reagents and cell-culture steps with a nanoparticle platform. Current *ex vivo* T-cell activation relies on antibody-coated magnetic beads that are not only labor-intensive to use but also costly, adding significant expense to CAR T cell manufacturing. In contrast, APC-mimetic LNPs can be produced in bulk, independently of patient-specific factors, which unlock economies of scale. Key raw materials for LNP production, ionizable lipids, helper lipids, PEG-lipids and additional excipients can be synthesized at an industrial scale, and mRNA or peptide antigens can be generated *via* high-yield cell-free processes, driving down the cost per dose. Large-scale LNP manufacturing has proven to be rapid and cost-efficient in the vaccine realm, owing to continuous *in vitro* transcription and nanoparticle self-assembly methods. It should be noted that initial development and setup costs for new APC-mimetic LNP formulations could be high due to the design of customizable lipid components and specialized equipment. These are significant investments, and with even more complex APC-mimetic LNP designs, the process might require additional purification steps. Scaling up production also incurs expenses in ensuring sterile, GMP-compliant operations. When amortized over large batches, the pre-treatment cost of LNP-based immunotherapy could be substantially lower than patient-tailored cell therapies, especially as manufacturing processes mature.[74]

In another aspect, regulatory approval of APC-mimetic LNPs will demand even more rigorous demonstrations of safety, quality consistency, and clinical benefits. Generally, LNPs face scrutiny because seemingly minor changes in formulation or process can alter their biological behavior. Complex biomimetic nanoparticles such as APC-mimetic LNPs do potentially have minor batch-to-batch variations in their physicochemical properties like particle size and surface ligand density which must be tightly controlled, as even small deviations could impact efficacy or immunogenicity.[75] Developers and manufacturers will need to establish comprehensive analytical characterization and stable formulation storage to satisfy regulators that the product is well-defined and reproducible. Encouragingly, regulatory precedent for nanoparticle therapeutics is growing, with over 15 nanomedicine products having gained clinical approval, indicating that agencies are open to well-substantiated nanoparticle platforms. In fact, the expedited approval of LNP-based COVID-19 vaccines illustrated that with strong efficacy data and a robust manufacturing process, regulators can rapidly authorize novel nanoparticle modalities. Nonetheless, for APC-mimetic LNPs, sponsors will likely engage in early discussions with regulatory bodies to clarify requirements. They will need to perform extensive toxicology studies to ensure that immune activation does not lead to unacceptable off-target effects. By proactively addressing these regulatory considerations through meticulous CMC documentation, validated scalable processes, and thorough safety profiling, the path to potential clinical translation of APC-mimetic LNP immunotherapies can be expedited.

5. Conclusion

LNPs are a promising modality which has the potential to be applied to CAR T cell immunotherapy. While CAR T cell immunotherapy has resulted in extraordinary clinical results for the treatment of hematological malignancies, the technology faces several obstacles such as long manufacturing times, high costs, and potential genotoxicity from the viral vectors commonly used in production. Thus, there is much impetus to develop faster, less expensive, nonviral methods of CAR T cell production. Nanoparticle technology has recently undergone massive development, and in particular, LNPs have emerged as a promising technology with the potential to circumvent many existing challenges of CAR T cell therapy. Notably, LNPs are highly modular, versatile, and can be rationally designed to include surface-conjugated antibodies against T-cell-specific targets.

In the context of *ex vivo* CAR T cell production, APC-mimetic LNPs decorated with antibodies against CD3 and CD28 have been designed to enable simultaneous T cell activation and CAR transgene delivery, which has the potential to decrease production costs and time. In the context of *in vivo* CAR T cell production – a potentially transformative alternative to *ex vivo* production – LNPs can be decorated with antibodies to facilitate uptake into circulating T cells while minimizing delivery into off-target cell populations. Furthermore, the modularity of the LNP platform will be compatible with continued advancements in CAR T cell technology, including the incorporation of cytokine signaling and checkpoint blockade. To enable the advancement of LNP-based CAR T cell immunotherapies toward the clinic, it will be critical to bear safety, immunogenicity, scalability, manufacturing, and regulatory considerations in mind during research and development. Thus, by addressing key barriers in safety, scalability, and manufacturability, LNP-based CAR T cell immunotherapy stands poised to revolutionize cancer treatment, potentially broadening therapeutic access and affording new opportunities to benefit patients.

Conflicts of interest

M. J. M. is a Scientific Advisor to and holds equity in Capstan Therapeutics. M. J. M. is an inventor on U.S. provisional patent applications related to ionizable lipids and lipid nanoparticles for CAR mRNA delivery described in this article.

Data availability

All relevant data supporting the findings presented in this Focus Article are available within the manuscript. Additional data or materials can be made available by the corresponding author upon reasonable request.

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