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Review article

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In vivo chimeric antigen receptor (CAR)-T cell therapy

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Abstract

Chimeric antigen receptor (CAR)-T cell therapy has transformed the outcomes of patients with haematological malignancies, yet its use is limited by labour-intensive manufacturing, constrained production capacity and variable clinical performance. In vivo CAR-T cell engineering, in which CAR-T cells are generated directly inside the patient's body, seeks to overcome these challenges by eliminating the need for ex vivo cell processing and complex logistics, as well as improve clinical performance. Recent advances in virology, RNA medicines and nanotechnology have catalysed a radical overhaul of this approach, which uses targeted delivery systems such as lentiviral vectors and lipid nanoparticles to introduce CAR-encoding genetic material into endogenous T cells. Early clinical studies have shown efficient transduction, sustained CAR expression and initial signs of antitumour activity, establishing proof of concept. This Review explores the underlying technologies — including RNA delivered by lipid nanoparticles and engineered viral vectors — and discusses how they are being adapted to develop more broadly applicable, scalable, safe and effective CAR-T cell therapies. By removing the need for ex vivo manipulation and chemotherapeutic conditioning, this strategy could enable the wider application of CAR-T cell therapies not just to blood cancers but to autoimmune diseases for which ex vivo CAR-T cell therapies have shown strong promise, such as systemic lupus erythematosus.

Sections

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Introduction

Immunotherapy has undergone a major transformation during the past three decades driven by advances in monoclonal antibodies[1], antibody–drug conjugates[2], cytokines[3], immune cell engagers[4], DNA vaccination[5] and, more recently, RNA-based vaccines[6] and engineered T cell therapies[7,8]. Among these modalities, autologous chimeric antigen receptor (CAR)-T cell therapy — in which a patient's own T cells are collected, genetically modified to express a CAR and reinfused — has stood out for its potency, showing curative potential across B cell malignancies[9,10]. Despite expectations, the expansion of autologous or allogeneic CAR-T cell therapies into broader indications and patient populations has been slower than anticipated. Multiple hurdles have been identified, including manufacturing and logistical complexity, limited production capacity and the requirement for chemotherapy-based lymphodepletion conditioning, all of which limit access and restrict applicability. The field thus stands at another inflexion point, requiring innovation in delivery and engineering technologies to overcome these limitations and fully realize the potential of CAR-T cell therapy, and immunotherapy in general.

In this Review, we first discuss key insights gained from the development of conventional ex vivo engineered CAR-T cell products, which have prompted efforts to advance in vivo CAR technologies as an alternative approach. This novel strategy eliminates cell manufacturing and related access-limiting logistics, and avoids chemotherapy-based lymphodepletion conditioning, thus presenting the opportunity of unleashing the full potential of CAR technology including larger indications that present a higher safety bar. We then review the leading in vivo CAR platforms, and the preclinical or clinical proofs of concept that support their development. Finally, we discuss potential limitations, strategies to advance these therapies to clinical application and how this progress may drive a broader shift to in vivo immune cell engineering utilizing a diversified range of mechanisms and payloads.

Lessons from ex vivo engineered CAR-T cells

To date, multiple autologous CAR-T cell products engineered using retroviral or lentiviral vectors targeting CD19 or B cell maturation antigen (BCMA) have been approved for the treatment of several B cell malignancies, including B cell acute lymphoblastic leukaemia, non-Hodgkin's lymphomas and multiple myeloma[10–17]. In addition, hundreds of CAR-T cell programmes are in various stages of development, with more than 1,000 clinical trials ongoing worldwide[18]. These include off-the-shelf allogeneic CAR-T cell (allo-CAR-T cell) products that are yet to show clinical performance comparable with autologous CAR-T cells in oncology[19] but may find applicability in autoimmune diseases[20,21]. Detailed evaluation of approved CAR-T cells — including reverse translational analysis to understand the mechanisms of toxicity and treatment resistance — has provided key lessons that guided the design of next-generation cell-based and in vivo CAR therapies (Box 1).

CAR-T cell therapy as a transformational modality

Although autologous CAR-T cells have primarily been developed for cancer, emerging evidence supports their potential in treating a much broader range of disorders, including autoimmune diseases. Preclinical studies in fibrosis and autoimmunity[22,23], along with multiple clinical case studies spanning autoimmune disease indications — including lupus nephritis, myositis and systemic sclerosis[24] — have shown durable, drug-free response, ablation of autoreactive memory B cells and reduction of disease-associated biomarkers[25–34]. This mechanism, termed 'immune reset', mimics the effect of high-dose chemotherapy followed by rescue with autologous stem cell transplantation, to treat severe autoimmune disorders[35,36]. In essence, this reset results in the replacement of an immune repertoire containing pathogenic autoreactive immune cells with a normally functioning immune system leading to complete and sustained remission.

The substantial clinical data from ex vivo engineered CAR-T cell products, including more than 35,000 patients with cancer treated with commercial products, led to characterization and standardization of the

Box 1 | Key lessons learned from the development of ex vivo viral-engineered CAR-T cell products

Strengths

Chimeric antigen receptor (CAR)-T cell therapy is a potent intervention with curative potential: autologous CAR-T cell products achieve a durable response in a subset of patients with B cell malignancies[7] and may induce 'immune reset' in autoimmunity involving B cells[24–34]. Durable responses can be achieved without prolonged on-target toxicities and persisting CAR-T cells[47]. The grading and management of treatment-related adverse events are well understood and standardized[37–40].

Limitations

Many patients with cancer do not achieve durable clinical benefit owing to limited product T cell fitness, detrimental tumour characteristics and/or tumour clonal evasion[50–52]. Serious toxicities occur in some patients and may include immune inflammatory adverse events (cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity (ICANS), immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome (IEC-HS))[37–40] and conditioning-related bone marrow toxicities. From an access standpoint, only a subset (~20%) of eligible patients have access to commercial products[43] owing to cell manufacturing scalability and logistics, and confinement to accredited centres. Use of integrating viral vectors and reliance on chemotherapy conditioning may also influence applicability.

Future opportunities

Looking ahead, the field is focusing on simplifying manufacturing and logistics simplification to improve patient access, with particular emphasis on off-the-shelf formats. In the near term, there is potential to expand the role of CAR-T cell therapies in haematological malignancies beyond relapsed and refractory disease to also include first-line[196], recently diagnosed high-risk patients for curative purpose, as well as in non-B cell blood cancers. In solid tumours[250], efforts are aimed at developing products with greater efficacy and durability of remission. Beyond oncology, there is growing interest in applying CAR technologies to non-oncology indications, including autoimmune disorders, regenerative medicine and infectious diseases[251].

Box 2 | Mechanistic differences between ex vivo engineered CAR-T cell products and in vivo CAR therapy

Ex vivo viral-engineered CAR-T cells

Lymphodepletion conditioning is needed to enable activation, expansion and differentiation of infused chimeric antigen receptor (CAR)-T cells to effector cells[44,45]. This process is primarily driven by less differentiated T cells[47] such as stem cell-like memory T cells, with the highest expansion and differentiation capability. Within 7–14 days post treatment, differentiating effector cells engage and eliminate target cells directly and through co-opting other immune mechanisms[48,49]. In a subset of patients, permanently engineered CAR-T cells may show exacerbated expansion or persistence, also co-opting macrophages, and leading to cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS) and immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome (IEC-HS)[37–40]. In addition, some patients may experience very durable B cell aplasia and hypogammaglobulinaemia, requiring intravenous immunoglobulin treatment[252] to prevent infections. A version of this platform comprises mRNA rather than an integrating vector as the payload, resulting in transient CAR expression[30].

In vivo CAR therapy

Upon infusion of viral vectors or lipid nanoparticle (LNP) formulations comprising CAR-expressing payloads, immune cells — including already differentiated effector cells — are rapidly engineered leading to engagement and eradication of target cells within the microenvironment. Lymphodepletion conditioning is not compatible with this treatment modality as pre-existing immune cells are needed. Some vectors are engineered to enable cell uptake and selectivity or modulate the activity of the engineered immune cells. In contrast to lentiviral vectors, CAR mRNA formats are designed to work transiently, thereby limiting by design the duration of on-target activity. Nevertheless, they may require substantial optimization or re-dosing to achieve sufficiently high pharmacological activity. In contrast, viral-based platforms result in T cells with a permanently integrated CAR payload, that may continue to expand and/or persist in vivo, depending on the antigen.

grading and management of treatment-related adverse events[37–40], such as cytokine release syndrome (CRS), immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome (IEC-HS), immune effector cell-associated neurotoxicity syndrome (ICANS), neutropenia and B cell aplasia associated with hypogammaglobulinaemia. Altogether, this knowledge can be used to facilitate clinical development of next-generation CAR-T cell products aimed to massively expand the footprint of this treatment modality.

Access and clinical performance limitations

Despite these successes, cost, scalability and feasibility issues are severely limiting access to commercial ex vivo engineered CAR-T cell products[41,42], and could also hamper broader applicability of such products to non-oncologic and earlier-line cancer indications. In fact, about four out of five eligible patients with lymphoma in the United States do not currently have access to commercial CAR-T cell products[43]. Besides scalability, there are several other caveats of CAR-T cell products in clinical practice or in development. These include a risk profile that requires the use of chemotherapy-based lymphodepletion conditioning[44,45], typically including a DNA alkylating agent such as cyclophosphamide, plus fludarabine, a purine analogue in oncology practice. These agents add well-described toxicities to a range of other adverse events stemming from excessive CAR-T cell activity, which can require intensive, in-hospital management in a subset of patients[37–40], thereby confining this treatment modality to accredited treatment centres[46]. Collectively, all these factors contribute to access challenges and are likely to limit broader application in indications such as autoimmunity and regenerative medicine.

In addition, efficacy limitations in oncology arise from complex mechanisms of treatment resistance that affect a subset of patients. These mechanisms include poor immune cell fitness[47], tumour-intrinsic factors, such as an immunosuppressive microenvironment[48,49], and immune evasion driven by low or heterogeneous expression of target antigens[50–52]. Addressing these factors systematically is essential to expanding the benefits of CAR-T cells to a broader population of patients with haematological malignancies and to achieving comparable efficacy in solid tumours, an ambitious objective that will likely require new approaches, such as multimodal in vivo engineering of the immune system.

In vivo cell therapy: a historical perspective

In vivo CAR-T cell therapy is emerging as a scalable alternative obviating both ex vivo cell manipulation and lymphodepletion conditioning. The major mechanistic feature of this approach is direct in situ CAR engineering of immune cells with off-the-shelf vectors or formulations (Box 2). These in vivo engineered immune cells can be T cells, macrophages, natural killer cells and/or other immune cells, already differentiated to effector cells, or even haematopoietic stem cells (HSCs) with great capability to differentiate to immune effector cells. The approach carries the promise of bringing together the transformational potency of CAR molecules, with the scalability of these off-the-shelf cell-free approaches, thereby potentially addressing both access and even some clinical performance limitations of conventional therapies.

This field emerged at the intersection of several areas including nanotechnology, RNA medicines, virology and CAR therapy (Fig. 1), raising considerable biopharma interest, with several companies entering the clinical stage (Tables 1 and 2). The first preclinical reports of successful in situ programming of CAR-T cells were from Matthias Stephan's group at Fred Hutchison Cancer Center in August 2017 (ref. 53) and Christian Buchholz's team at Paul-Ehrlich Institute in September 2018 (ref. 54). The team at Fred Hutchinson Cancer Center developed biodegradable DNA-carrying polymer-based nanoparticles that target CD3 on murine T cells to deliver a therapeutic payload, a piggyBac transposon system encoding a CD19-specific CAR. In a mouse model of acute lymphoblastic leukaemia, the intravenously infused anti-CD19 CAR nanoparticles performed as well as adoptive T cell therapy, while obviating the need for cell manipulation ex vivo. The Paul-Ehrlich Institute team had already described a human CD8-targeted lentiviral

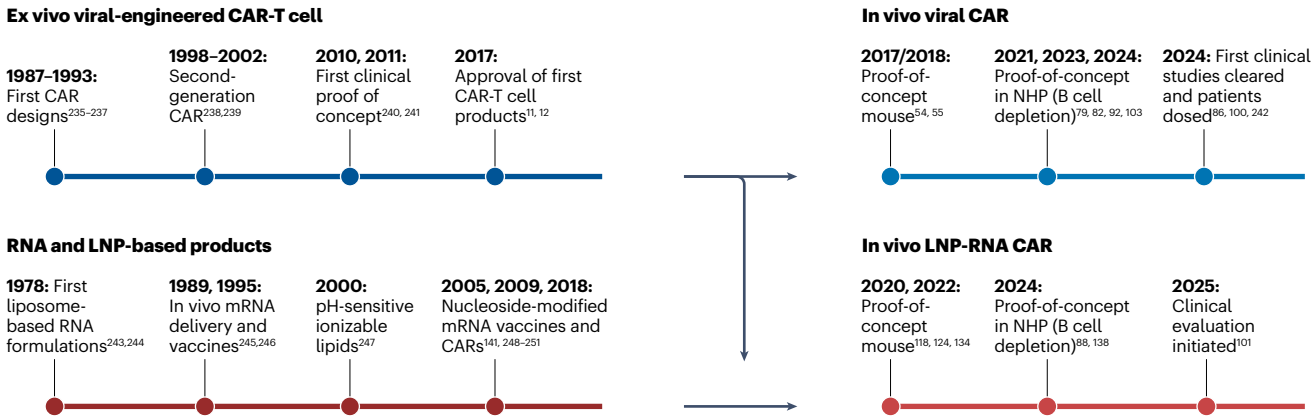

Fig. 1 | Timeline: major discoveries and milestones leading to clinical translation of in vivo CAR technologies. Development of ex vivo engineered chimeric antigen receptor (CAR)-T cell products represented a major milestone in medicine but shed light on the access and clinical performance limitations of this platform (top left). The advent of in vivo CAR technologies, aiming to overcome these limitations, was catalysed by progress in several different fields: virology, RNA therapeutics and nanomedicines (bottom left). Two major in vivo CAR platforms emerged at the intersection of these fields and are currently translated to the clinic: engineered viral (lentiviral or gamma-retroviral) vectors leading to payload integration in the genome (top right); and lipid nanoparticles (LNPs) loaded with RNA, leading to transient payload expression (bottom right)[233–249]. NHP, non-human primate.

vector in 2012 and suggested that this feature is instrumental for in vivo gene delivery of antigen receptors to cytotoxic T cells[55]. After packaging a CD19-specific CAR, the preclinical proof of concept included T cell engineering in transplanted humanized mouse models generated via infusion of human peripheral blood mononuclear cells or HSCs, which were accompanied by B cell depletion and cytokine production[54].

However, clinical translation seemed far off, for three main reasons. Firstly, these in vivo programming breakthroughs were not immediately known among venture capitalists and biopharma. Secondly, Novartis and Kite had just received US Food and Drug Administration (FDA) approvals for the first-ever CAR-T cell therapies (Kymriah and Yescarta) with Kite Pharma being acquired by Gilead in a US $11.9 billion deal, boosting investor confidence in ex vivo engineered CAR-T cell products[56]. Lastly, there were high hopes that off-the-shelf allogeneic cell-based technologies[57] would rapidly translate to a second wave of commercial products with broader applicability. Compared with the challenge of developing injectable multicomponent nanomedicines — a field most biopharma was unfamiliar with at that time — ex vivo T cell engineering technologies seemed less risky and an easier path to market and financial returns in the wake of clinical proof of concept and product approvals. Moreover, legal wrangles over intellectual property rights covering CAR-T cell technology and intellectual disputes over nanoparticle drug-delivery systems further complicated translation of in vivo CAR-T cell technologies[58]. Nevertheless, July 2018 saw the launch of Tidal Therapeutics, the first biotech company focused on in vivo CAR-T cell therapy. Others quickly followed — Umoja Biopharma, Orna Therapeutics and Sana Biotechnology in 2019, Abintus Bio in 2020 and Capstan Therapeutics in 2021 — setting the stage for rapid growth, innovation and investment in this field, in which more than 30 companies now operate. In 2021, Sanofi acquired Tidal Therapeutics and is currently developing three in vivo CAR-T cell programmes for oncologic and inflammatory diseases[59]. Recently, Astra Zeneca acquired EsoBiotec developing in vivo viral CAR therapy for multiple myeloma[60], and Abbvie acquired Capstan developing in vivo CAR products based on RNA-lipid nanoparticles (RNA-LNPs) for autoimmunity and other indications[61].

In vivo immune engineering: a mechanistic background

In vivo CAR engineering enables rapid generation of CAR-modified immune cells and their engagement with target cells, either within the same microenvironment where modification occurs or in distant tissues following CAR-T cell trafficking. Hence, lymphodepletion conditioning — which is key to facilitating CAR-T cell expansion upon cell product delivery — is not only unnecessary but is incompatible with in vivo CAR-T cell treatment. Conversely, in addition to increasing access and broadening applicability, avoidance of chemotherapy conditioning opens avenues to more effectively co-opting broader immune mechanisms, needed to augment clinical efficacy. However, some beneficial effects of lymphodepletion conditioning for CAR-T cell therapy in oncology, such as the immunomodulating effect on the tumour microenvironment (TME) or innate immunity[62], may need to be addressed, for example by co-delivery of biological response modifiers such as γ-chain receptor cytokines. In any case, to enable successful reprogramming of desired immune cells while minimizing uptake by pathogenic cells or other cells in the body, the vectors or formulations used for in vivo CAR-T cell therapy need to have remarkable tissue and cell selectivity. The two leading platforms entering clinical development differ in their payloads and delivery strategies: engineered viral vectors with integrating payloads; and LNPs carrying transiently expressed RNA. In vivo CAR technologies using integrating payloads result in immune cell populations that may self-adjust their expansion and persistence in response to antigen presence and burden, which may be an advantage in indications that demand higher potency for clinical efficacy. By contrast, technologies relying on transiently expressed, non-integrating payloads present the opportunity to control exposure (tune up or down the number and persistence of CAR-engineered immune cells) relatively independently of antigen burden, through modulating the dose regimen, which may be an advantage in indications with a higher safety bar.

Below, we describe the main in vivo CAR therapy platforms and the leading product candidates entering the clinical stage (Tables 1 and 2 and Figs. 2 and 3), categorized based on whether or not they have an integrating payload. In addition, we outline specific challenges in

translating in vivo CAR technologies from preclinical to clinical applications, and discuss how these challenges compare with those faced by traditional ex vivo CAR-T cell therapies.

Viral vectors

Early development of viral vectors for in vivo CAR-T cell therapy

This platform relies on cell-selective vector delivery and genomic integration of the CAR payload via engineered lentiviral or gamma-retroviral vectors. A critical requirement for these viral vectors is achieving remarkable selective gene transfer into the patient's T cells. Vector particles need to avoid uptake by undesired cells, ensuring safety and boosting efficiency by minimizing the sink effect. This has been achieved through engineering of viral envelope proteins used to pseudotype lentiviral vectors. The concept is based on simultaneously eliminating the interaction with natural cell surface receptors, together with displaying high-affinity binders that recognize a T cell-specific cell surface marker. Proof of concept for lentiviral vectors was provided using measles virus glycoproteins, in which the membrane fusion and receptor attachment functions were separated onto two proteins, thus facilitating the engineering process[63,64]. Engineered Nipah virus (NiV) glycoproteins made the vector particles more active for gene delivery[65], whereas the display of CD4 and CD8 specific binders enabled T cell-specific transduction, achieving in the latter case more than 99% specificity for CD8-positive T cells[66].

An important advance in lentiviral glycoprotein engineering was the adaptation of receptor-targeted delivery strategies to the G glycoprotein of the rhabdovirus vesicular stomatitis virus (VSV). VSV lentiviruses harbour the advantage of high yields and established purification and good manufacturing practice (GMP) processes. In contrast to paramyxoviral glycoproteins, which mediate highly efficient pH-independent fusion at the cell surface, the VSV-G protein combining receptor binding and membrane fusion in a single molecule requires the low pH of late endosomes to initiate fusion. Clinical testing will ultimately inform on whether different entry modes have an effect on gene delivery rates as well as off-target delivery and toxicities. Based on the principle of ablating natural receptor usage and displaying high-affinity binders either fused to VSV-G protein or as a separate transmembrane protein, various target receptors, including T cell markers, have been evaluated[67,68].

The first T cell-specific lentivirus (CD8-lentivirus) used for in vivo CAR-T cell generation was based on the NiV glycoproteins displaying a high-affinity designed ankyrin repeat protein (DARPin) that was specific for the CD8α chain[54,69]. This CD8-lentivirus enabled proof-of-concept demonstration of in vivo generation of human CD19 CAR-T cells[54]. A single systemic administration in a humanized mouse model resulted in the transduction of enough human T cells into CAR-T cells to mediate effective clearance of primary B cells or lymphoma cells from the circulation and bone marrow[54,70]. Follow-up studies relying on similar humanized mouse models using lentivirus targeted to other T cell markers resulted in relevant findings for in vivo CAR therapy. Notably, delivery of CARs exclusively into CD4-positive T cells by anti-CD4-lentiviral vectors resulted in highly efficient target cell elimination in preclinical models, comparably or exceeding the activity achieved with CD8-lentiviral vectors[71]. Targeting CD3 with agonistic single-chain antibodies allowed for selective binding to T cells while simultaneously inducing their activation[72,73]. As CD3 is specifically expressed on T cells and has a central role in T cell receptor (TCR) signalling, its engagement typically triggers internalization and receptor downregulation as part of the activation process. To prevent this loss of surface CD3 — which would limit vector binding and transduction efficiency — the tyrosine-kinase inhibitor dasatinib was used during transduction to transiently suppress TCR signalling, thereby preserving CD3 expression and enhancing gene transfer[74]. Through targeting markers on less

Table 1 | Lentiviral-based in vivo CAR-T cell platforms in development

Company	Targeting mechanism	Therapeutic payloads	Lead indications	Preclinical evidence	Development stage
Interius BioTherapeutics	Anti-CD7 scFv-decorated particles (T cell and natural killer cell engineering)	Anti-CD20 CAR, anti-CD19 CAR	B cell malignancies, autoimmunity	Proof of principle in mice and NHPs[84]	Clinical (phase I enrolling in 2024 with anti-CD20 CAR)[78,85]
Umoja Biopharma/ Abbvie	Multi-domain anti-CD3, CD80, CD58 decorate particles (T cell engineering)	Anti-CD19 CAR, anti-CD22 CAR, anti-CD20 CAR	B cell malignancies, autoimmunity	Proof of principle in mice and NHPs[81,88,91]	Clinical (phase I initiated in 2024 with anti-CD19 CAR, the others in 2024, 2026)[90]
Shenzen Genocury Ltd	Anti-CD3 decorated particles	Anti-CD19 CAR	B cell malignancies	Not disclosed	Investigator-sponsored trial, first responder in a patient with lymphoma[95,96]
EsoBiotec/Astra Zeneca	Targeted lentiviral particles	Anti-BCMA CAR, undisclosed	Multiple myeloma, autoimmunity, solid tumours	Proof of principle in mouse model[95]	Phase I initiation in 2025, first clinical response in myeloma, acquisition[98,101,148]
Kelonia	Anti-CD3 decorated particles	Anti-BCMA CAR	Multiple myeloma	Proof of principle in mice and NHPs[102,103]	Phase I initiation mid 2025
Sana	Anti-CD8 fusogen-decorated particles	Anti-CD19 CAR	Undisclosed	Proof of principle in mice and NHPs[78,109,111]	Undisclosed
Ensoma	CD46-targeted viral-like particles (multilineage)	Anti-HER2 CAR	Multiple solid tumours	Proof of principle in preclinical models[112]	Undisclosed
Exuma Biotec	CD3-targeted lentiviral vector	Anti-CD19 CAR	B cell malignancies	Proof of principle, mouse models[114]	Undisclosed

BCMA, B cell maturation antigen; CAR, chimeric antigen receptor; NHP, non-human primate.

Table 2 | LNP-RNA-based in vivo CAR-T cell platforms in development

Company	Targeting mechanism	Therapeutic payloads	Lead indications	Preclinical evidence	Development stage
Myeloid Therapeutics	LNPs – macrophage tropic	Anti-Trop2 CAR, anti-GPC3 CAR, anti-HER2 CAR, anti-gp75 CAR (CD89 and natural killer cell p44-based CAR constructs)	Multiple solid tumours and hepatocellular carcinoma	Multiple preclinical models[122,124]	Clinical (phase I initiated in 2024 with anti-TROP2 and GPC3 CARs) and first clinical response[125,127]
Capstan	Anti-CD8 monoclonal antibody-decorated LNPs (CTL engineering)	Anti-CD19 CAR and undisclosed	Autoimmunity and undisclosed	Mouse and NHP proof of principle[135–137]	Phase I initiated (NCT06917742)
Immorna	tLNPs (T cells, myeloid cells and natural killer cells)	B cell-targeted CAR (RNA format)	$CD19^+$ B cell malignancies	Undisclosed	Phase I initiated with first patient dosed[140]
Shenzen MagicRNA	CD8 T cell tLNPs	Anti-CD19 CAR (mRNA format)	Systemic lupus erythematosus	Mouse and NHP proof of principle[142]	Phase 1 initiated and evidence of activity reported[140,143]
Orna	LNPs containing immunotropic lipids (pan-T cell engineering)	Anti-CD19 CAR (circular RNA format)	B cell malignancies and autoimmunity	Mouse and NHP proof of principle[148]	Phase I initiation by 2026
Sanofi	tLNPs (T cell engineering)	Anti-CD22 and anti-CD19 CAR (mRNA)	Oncology, autoimmunity	Mouse and NHP proof of principle[152,154]	Undisclosed
Carisma/ Moderna	LNPs – macrophage tropic	Anti-GPC3 CAR and not disclosed	Hepatocellular carcinoma	Preclinical mouse models[161,162]	Preclinical stage; strategic changes announced[164]
Tessera	tLNPs	Anti-CD19, CD20, BCMA using RNA writer (integrating payload)	Oncology, autoimmunity	Preclinical modelling[171–173]	Undisclosed
Orbital	tLNPs and viral-like particles	CAR (circular RNA format) – details not disclosed	Undisclosed	Undisclosed	Undisclosed

BCMA, B cell maturation antigen; CAR, chimeric antigen receptor; CTL, cytotoxic T cell lymphocyte; GPC3, glypican 3; LNP, lipid nanoparticle; NHP, non-human primate; tLNP, targeted LNP.

differentiated T cells, such as CD62L, CAR payloads can be preferentially delivered into naive, stem cell-like memory or central memory T cells, which could be beneficial in augmenting efficacy or preventing early exhaustion[75]. One barrier for this technology is represented by macrophages that may act as a sink. Their activity in eliminating vector particles can be substantially reduced by shielding the vector surface against non-specific phagocytosis through incorporating the 'don't eat me signal' CD47 within the lentivirus envelope membrane[76].

All preclinical models for in vivo CAR-T cell engineering mentioned above used B cell-targeted CARs. However, a recent preclinical publication showed that angioblastic T cell lymphoma, comprising CD4-positive tumour cells, can be effectively addressed by in vivo CD4 CAR-T cell delivery using CD8-targeted lentivirus[77]. In addition, selective delivery of the CAR payload to $CD8^+$ T cells prevented CAR delivery into malignant cells and, thus, potential treatment resistance.

Although mouse models are ideal for generating proof of concept for in vivo CAR delivery, large animal models (such as non-human primates (NHPs)) proved crucial for translation to the clinic. NHPs have been instrumental to guide platform, product and dose-regimen optimizations, setting the stage for clinical testing of T cell-targeted lentivirus with NiV glycoproteins[78] or rhabdoviral envelope glycoproteins[79,80], with the latter just having entered phase I development (Table 1).

Below, we describe the leading engineered viral vectors for in vivo CAR therapy translated to the clinic or at an advanced preclinical development stage by Interius BioTherapeutics, Umoja Biopharma, Shenzen Genocury, EsoBiotec, Kelonia Therapeutics and Sana Biotechnology.

Engineered viral platforms in clinical development

The delivery platform developed by Interius BioTherapeutics is an engineered self-inactivating, replication-incompetent lentiviral vector that can target and transduce specific cells types in vivo following a single intravenous administration[81,82]. Interius vectors include proprietary components that enable precise in vivo gene delivery: a high-affinity binder to provide cell-specific targeting of the vector, and a de-targeted fusogen to enable transfer of an integrating lentiviral genome into the target cell.

The first clinical candidate from Interius BioTherapeutics, INT2104, is a CD7-targeted lentiviral vector designed to deliver a CAR20 transgene, encoding an anti-CD20 CAR construct, for treatment of B cell malignancies. To improve cell targeting and vector stability, INT2104 is pseudotyped with Gen 2.1 Fusogen, a modified version of the VSV-G protein engineered with amino acid substitutions that reduce off-target binding and enhance persistence in the bloodstream[82,83]. A single amino acid substitution retargets the fusion protein from its native receptor (low-density lipoprotein receptor (LDL-R)) without disrupting its fusogenic potential. Precise in vivo targeting of INT2104 is achieved by the high-affinity CD7-specific binder on the vector providing tropism to T cells (including both $CD4^+$ and $CD8^+$ subsets) and natural killer cells, resulting in a diverse population of effector anti-CD20 CAR cells after a single intravenous treatment.

In vivo studies with INT2104 were conducted in both NHPs and humanized mouse models to evaluate the safety, specificity of target cell transduction and biological effect in preparation for a first-in-human study. The results of these in vivo studies confirmed the ability of INT2104 to achieve specific transduction of T cells and natural killer cells following intravenous delivery; the generation and persistence of functional anti-CD20 CAR cells; and specific depletion of $CD20^+$ B cells[84]. A good laboratory practice (GLP)-compliant toxicology study carried out in cynomolgus macaques demonstrated that INT2104 was well tolerated with no evidence of toxicity, no CRS and no neurotoxicity following intravenous administration, providing support for the selected dose range and route of administration for the first-in-human clinical study.

In October 2024, the first study participant was dosed in the INVISE study (NCT06539338), a first-in-human phase I clinical trial evaluating the safety of INT2104 intravenous infusion in adults with

refractory/relapsing B cell malignancies[79,85]. Data from the INVISE study are expected to be available in the second half of 2025. Based on the in vitro and in vivo data generated during INT2104 development, Interius anticipates that the clinical data from the INVISE study will support the development of additional products including INT2106, a CAR19 vector comprising an anti-CD19 CAR payload, developed for the treatment of autoimmune diseases. Recently, Kite, a Gilead Company, announced its intention to acquire Interius[86].

VivoVec, the in vivo delivery and CAR-T cell-generating platform developed by Umoja Biopharma, is a surface-engineered, third-generation, self-inactivating, replication-incompetent lentiviral vector particle system engineered to express a multi-domain fusion protein that enables delivery of genetic payloads directly to T cells in vivo[87]. A key differentiating feature of this platform is that VivoVec particles are pseudotyped with the Cocal fusion viral glycoprotein, which is resistant to serum inactivation in humans, which improves in vivo persistence, and enables direct infusion to patients. Additionally, VivoVec products incorporate CD80 and CD58 T cell co-stimulatory ligands combined with an anti-CD3 scFv into a single multi-domain fusion protein, providing high specificity tropism for T cells as well as endowing the particles with the ability to bind, activate and transduce T cells in vivo[88]. In effect, VivoVec particles mimic the antigen-presenting cells which initiate T cell immune responses.

Umoja's first lead programme is UB-VV111, a VivoVec-based drug product that encodes a CD19-targeted CAR along with a rapamycin-activated cytokine receptor (RACR)[88]. UB-VV111 enables direct, in vivo generation of CD19 CAR-T cells that co-express the RACR system, equipping these cells with the capacity for rapamycin-induced expansion and enhanced in vivo survival. Umoja received investigational new drug (IND) clearance from the FDA in July 2024 and has initiated its phase I clinical trial both in the United States and internationally, treating B cell malignancies. AbbVie secured a development and commercialization option for UB-VV111, announced in January 2024 (ref. 89). Evaluation in NHP models has demonstrated that a single infusion of VivoVec drug particles generates peak levels of CAR-T cells comprising up to 65% of circulating T cells following intranodal or intravenous delivery[80,90]. Accompanying rapid and durable B cell aplasia has been demonstrated as well, exceeding what has been observed in benchmark ex vivo NHP CAR models. Interestingly, there was also evidence of secondary CAR-T cell expansion in one of the treated animals, occurring months after treatment and accompanied by B cell decline, ascribable to persistence of viral-transduced CAR-T cells[80]. Inflammatory adverse events in the treated animals were successfully addressed with standard immune suppressive therapy including the anti-IL-6 antibody tocilizumab[80].

Umoja's second lead programme is UB-VV400, a VivoVec-based drug product that encodes a CD22-targeted CAR, also in combination with the RACR system[91]. This product candidate showed preclinical proof of concept in humanized mouse models[92]. Umoja's investigator-initiated trial with Chinese partner IASO Biotherapeutics

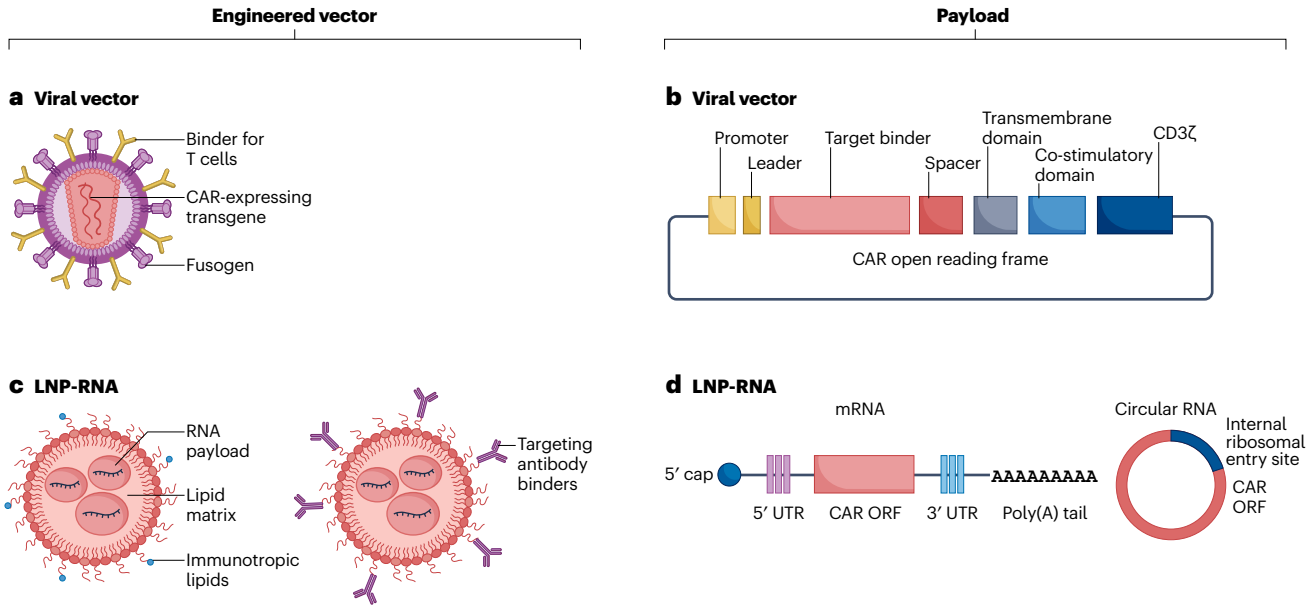

Fig. 2 | Major in vivo CAR platforms with a focus on the engineering vector and payloads. a,**b**, Viral engineering vectors (lentiviral or gamma-retroviral). A retargeted approach in which the viral envelope is modified with T cell-specific binders that enable selective uptake by T cells and fusogenic elements to facilitate cytoplasmic delivery of the payload (chimeric antigen receptor (CAR)-expressing vector) (panel **a**). Structure of the viral payload, which typically includes a CAR-expressing open reading frame (ORF) with regulatory elements similar to those used in conventional ex vivo engineered CAR-T cell products (panel **b**). Companies developing viral-based in vivo CAR-T cell platforms include Interius BioTherapeutics, Umoja Biopharma, Genocury, EsoBiotec, Sana Biotechnology and Kelonia Therapeutics. **c**,**d**, Lipid nanoparticle (LNP)-RNA engineering vectors. LNPs are composed of lipids, including an ionizable lipid that promotes the RNA payload release from endolysosomes into the cytoplasm. Targeting can be achieved using tropic lipids (Myeloid Therapeutics, Carisma Therapeutics, Orna Therapeutics) or antibody-derived binders (Capstan Therapeutics, Sanofi). Two categories of RNA formats are under investigation: linear mRNA optimized for high-level CAR expression through modifications such as improved untranslated regions (UTRs), codon optimization and the use of N_1-methylpseudouridine (panel **c**); and circular RNAs designed to achieve prolonged payload expression by preventing early translation termination (panel **d**). Companies developing this technology include Myeloid Therapeutics, Carisma Therapeutics, Orna Therapeutics, Capstan Therapeutics, Shenzen MagicRNA and Sanofi.

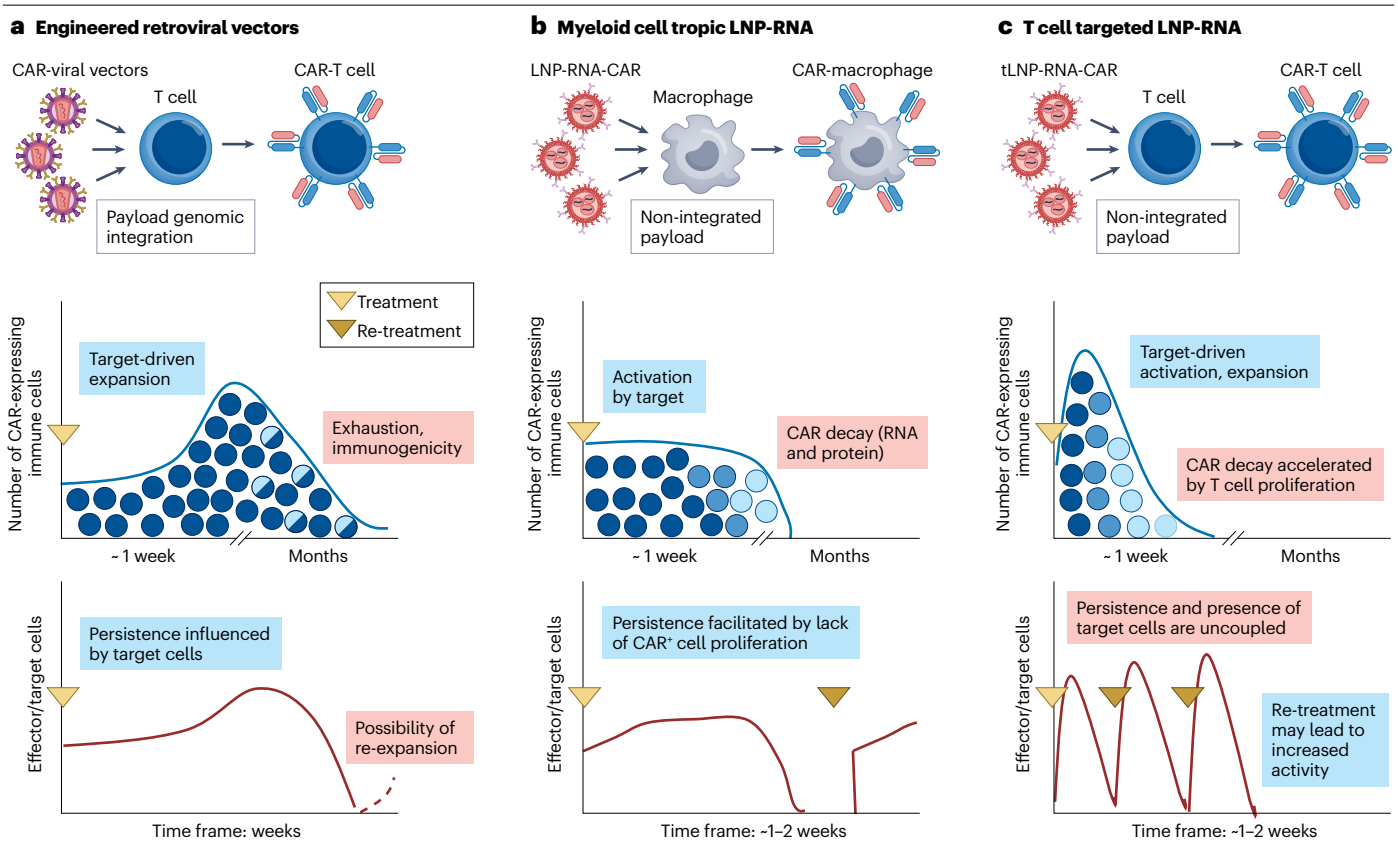

Fig. 3 | Major in vivo CAR platforms in development, and their mechanism of action. a, In vivo platforms using integrating viral vectors (typically lentiviral or gamma-retroviral vectors in general) require targeted delivery to T cells, followed by membrane fusion, transfer of the virion to the cytoplasm or endolysosomal compartment and, eventually, genomic integration of the chimeric antigen receptor (CAR) transgene[66]. Mechanistically, the main features of this approach include target-driven expansion, whereby CAR-expressing T cells proliferate upon encountering their specific antigen on tumour cells. This can lead to persistence of CAR-expressing cells, but also to challenges in preventing amplified activity, undesired persistence or re-expansion leading to delayed or protracted on-target toxicities occurring weeks or months post treatment[81,91]. Companies focused on this technology include Interius BioTherapeutics, Umoja Biopharma, Sana Biotechnology, Genocury, EsoBiotec and Kelonia Therapeutics. **b**, Myeloid cell-tropic lipid nanoparticles (LNPs) are used to deliver CAR mRNA to macrophages, resulting in CAR-expressing macrophages currently tested in solid tumours with promising emerging data[121,127,155]. Prolonged CAR expression in trafficking myeloid cells can be obtained owing to payload optimizations and the slow proliferation of these cells. Level of CAR expression represented through shading of the circles (darker shading corresponds to a higher level). Companies developing this technology include Myeloid Therapeutics and Moderna Inc. with Carisma Therapeutics. **c**, Targeted LNPs (tLNPs) – either expressing antibody binders or comprising immunotropic lipids – can be used to engineer T cells or T cell subsets with CAR in linear[136,137,152] or circular[144–146] RNA formats. CAR expression drives rapid antigen-induced activation and proliferation of engineered cells. However, because the RNA payload is not integrating, CAR expression decays with rapid T cell division. Owing to the immunogenicity profile, LNPs can be re-dosed, achieving increased exposure and even augmenting the pharmacological effect[136]. Companies developing this technology include Shenzen MagicRNA, Orna Therapeutics, Capstan Therapeutics and Sanofi. Filled circles indicate CAR-expressing functional cells; semi-filled circles indicate exhausted CAR-transduced cells.

will commence in 2025, with a focus on B cell malignancies and further investigation in autoimmune conditions[93]. Additional programmes are investigating VivoVec-based therapies in multiple myeloma, solid tumours and autoimmune applications.

As this emerging field is expanding, recently initiated trials yield evidence of clinical activity through case studies. The First Affiliated Hospital of Zhengzhou University and Shenzen Genocury Ltd reported their first patient, with relapsed/refractory diffuse large B cell lymphoma, treated with an anti-CD19 CAR in T cell-targeted, retroviral format[94]. The Varicella zoster virus (VZV)-G protein retroviral vector was engineered to display an anti-CD3 binder and an undisclosed X activation domain, with a CAR payload comprising a murine FMC63 CD19 scFv linked to a CD8 hinge/transmembrane domain, and 41BB and CD3ζ stimulatory domains, respectively. Interestingly and unusually, the treatment protocol comprised apheresis to collect T cells, followed by lymphodepletion conditioning and co-infusion of autologous T cells and viral vector, possibly to also leverage the potential benefit of pre-conditioning. The major side effects were myelotoxicity and grade 1 CRS, without evidence of neurotoxicity or infection. The treatment resulted in CAR-T cell expansion within 3 weeks post infusion, and a partial remission on day 35 documented by clinical imaging and corroborated by reduction of malignant cell counts[95]. This was followed by a more recent update showcasing a complete response 3 months post treatment[96], although it is not clear whether this was a different patient.

Using a comparable approach, based on a third-generation self-inactivating, VSV-G protein targeting-deficient lentivirus vector, EsoBiotec/Shenzen Pregene developed an anti-BCMA CAR product that has some additional features. These include an engineered envelope (ENaBL-T) that is T cell retargeted via a binder specific for TCRαβ, a llama-derived variable domain of heavy-chain only (VHH) nanobody, but immunologically stealthy through major histocompatibility complex (MHC) class I depletion and CD47 expression, and comprising a payload equipped with a T cell-specific promoter[97–99]. This technology achieves effective engineering of both resting and activated T cells, showing preclinical proof of concept in a multiple myeloma model[95]. In addition, the company has initiated clinical activities and recently reported the first treatment of a patient with multiple myeloma treated with their in vivo anti-BCMA product, who achieved an objective response with elimination of disseminated disease in bone marrow[100]. Notably, the response evaluation was performed on day 28 — underscoring the speed of clinical response — thus supporting the paradigm that complete responses can be rapidly achieved by CAR therapy without the need for prolonged CAR exposure. This was complemented by a more recent update describing objective responses in four patients with malignant myeloma, including the one reported earlier[101]. Together with the innocuous adverse events profile, this outcome catalysed the recent acquisition of EsoBiotec by Astra Zeneca heralding the interest of large biopharma in this rapidly evolving field of in vivo CAR therapy[60].

Engineered viral vectors in advanced preclinical research

The in vivo gene placement system (iGPS) developed by Kelonia Therapeutics, an off-the-shelf genetic medicines platform technology, enables in vivo generation of CAR-T cells. iGPS particles are designed to generate potent, persistent CAR-T cells after only a single intravenous dose. This platform, aiming to achieve high specificity when targeting T cells, was developed based on studies geared at identifying antigen-reactive TCR[67]. They initially engineered lentiviral vectors capable of specifically transducing antigen-specific T cells by de-targeting the VSV-G envelope protein to LDL-R, its natural entry receptor, and instead co-expressing a peptide–MHC complex. Kelonia adapted the technology to target all T cells by using CD3 as a viral entry receptor. An anti-CD3 scFv was combined with a de-targeted VSV-G that was selected from numerous variants of mutated VSV-G variants that best inhibited LDL-R binding while maintaining potent T cell transduction. The potential of the iGPS platform has been highlighted in a series of NHP studies in which an anti-CD20 CAR, targeting normal and malignant B cells, was used to transduce T cells in vivo[102]. A single intravenous infusion at dose levels as low as 10^8 viral particles per kilogram of body weight resulted in sufficient anti-CD20 CAR-T cell generation for complete B cell depletion lasting longer than 2 months without any additional treatments. Strikingly, potent CAR-T cell activity occurred without notable toxicities including hepatotoxicity, CRS or neurotoxicity[103].

Kelonia's lead medicine, KLN-1010, encodes a proprietary, fully human anti-BCMA CAR in an iGPS viral particle for treatment of multiple myeloma[103]. In preclinical studies, humanized mouse models of multiple myeloma treated with a single intravenous dose of anti-BCMA iGPS particles resulted in complete tumour clearance, and compared favourably with ex vivo CAR-T cells modelled after the commercial anti-BCMA ex vivo engineered CAR-T cell products idecabtagene vicleucel (ide-cel) or ciltacabtagene autoleucel (cilta-cel). Perhaps related to a higher composition of memory and stem cell-like memory CD4 and CD8 T cells, CAR-T cells generated with KLN-1010 persisted longer than the ex vivo engineered T cells. Most off-target, non-T cell CAR expression was limited to cells capable of phagocytosis, such as macrophages[103]. KLN-1010 is scheduled to enter clinical development for multiple myeloma in 2025 followed by additional internal and partnered programmes in oncology and autoimmunity.

The targeted fusogen platform technology developed by Sana Biotechnology is designed to enable potent and highly specific T cell transduction following systemic administration of a lentiviral vector. This approach uses a third-generation self-inactivating lentiviral vector equipped with a retargetable fusogen derived from paramyxoviruses[63–65]. The fusogen's native receptor binding is disabled ('blinded') and replaced with an engineered domain that binds a selected T cell surface receptor. Upon engagement with this receptor, the fusogen is activated to mediate membrane fusion, enabling vector entry and gene delivery[104,105]. This direct plasma membrane entry mechanism not only improves targeting specificity but also circumvents the non-specific uptake observed with endosomal entry routes — an issue known to affect VSV-G-based vectors in vivo. The receptor-coupled mechanism ensures that transduction occurs only in T cells, minimizing off-target effects caused by unintended interactions with other cell types. Such targeting precision is particularly important because CAR proteins can be incorporated into vector particles during lentiviral production, a known artefact that, when combined with VSV-G fusogens, has been shown to drive off-target transduction[106–108]. Although the fusogen is modular and can target different TCRs such as CD3 and CD4, this proposed therapeutic approach uses a CD8-targeted fusogen. Sana's early work with the CD8-targeted lentiviral vector in mice[109] and NHPs[78] shows specific delivery to $CD8^+$ T cells in the peripheral blood, spleen and other lymphoid organs[109]. Advancement in Sana's suspension-based manufacturing process increased CD8-targeted lentivirus vector (LV) vector titres some 50-fold over earlier processes, while preserving the specificity of the fusogen system[109,110]. This enhanced potency vector reduced the required in vivo dose to achieve robust CAR-T cell production and B cell depletion in mouse models[109] and NHPs. In NHP studies with the enhanced potency vector, highly specific transduction of target $CD8^+$ T cells was observed in the peripheral blood, spleen and other lymphoid organs, with a lack of signal in key off-target organs such as the liver[111].

Sana has used the modular retargetable system to deliver to different T cell subsets, but also hepatocytes and HSCs. In each case, targeted fusosome delivery has been demonstrated to be highly specific for in vivo delivery. Additional proteins can also be added to the vector surface to permit cell activation without changing specificity, for example through the addition of a CD3 ligand for T cell activation. Moreover, the lentiviral vector can be modified for the delivery of gene editing cargos as has been shown for virus-like particles, providing a delivery vehicle with modular cell-specific targeting and diverse payload incorporation. Plans to advance the lead product (in vivo anti-CD19 CAR in an engineered lentiviral vector format targeted to $CD8^+$ T cells) to the clinic are being developed.

Engineered, targeted lentiviruses for CAR therapy are also being developed by other organizations such as Ensoma (multicell lineage engineering through reprogramming HSCs)[112], Vyriad — which announced recently a collaborative agreement with Novartis[113] — and Exuma Biotec (co-engineering of T cells and natural killer cells) with preclinical data available[114] and clinical plans yet to be disclosed (Table 1). Novel technologies comprising pH-sensitive polymer shielding of lentivirus for in vivo CAR therapy are also being explored by Alaya Bio[115,116].

RNA-based platforms

Origins and evolution of RNA delivery systems for in vivo CAR-T cell therapy

RNA-based engineering platforms use either linear mRNA or circular RNA for transient immune cell engineering, delivered via tissue-tropic lipid formulations or via cell-targeted, antibody-functionalized nanoparticles. The rise of this platform was accelerated by the development of mRNA vaccines formulated in LNPs, as a rapid response to the COVID-19 pandemic[5]. Although early work achieved preclinical proof of concept with CD3 and CD8-functionalized polymer-based nanoparticles loaded with anti-CD19 CAR in mRNA format[117], LNPs became a preferred formulation approach owing to mass vaccination experience[5] and innovations with respect to ionizable lipids[118]. Ionizable lipids with high biodegradability and low reactogenicity, relative to those typically used for mRNA-LNP vaccination, are enabling desired target product profiles and the possibility to re-dose to a great extent. Antibody-functionalized LNPs enable effective T cell engineering and cell-type selectivity, whereas immunotropic LNPs identified through high-throughput screening support cell or tissue-specific delivery. These can be used to effectively engineer T cell subsets or entire lymphocyte populations in vivo, whereas another version of this LNP-RNA platform aims to engineer macrophages, leveraging the particle tropism for myeloid cells. This occurs through yet to be identified components of the particle corona interacting with scavenger receptors, probably different to the conventional ApoE/LDL-R-mediated uptake by hepatocytes[119]. Macrophages are well-described sentinel cells of the innate immune system capable of tumour infiltration, phagocytosis, inflammatory cytokine release, T cell recruitment and antigen presentation. Unlike T cells, macrophages[120] lack endogenous antigen specificity; however, they could effectively mobilize multiple arms of immunity.

Below, we describe in more detail the emerging LNP-mRNA in vivo CAR platforms and leading products (Table 2 and Fig. 2). We further categorize RNA-based engineering approaches in technologies that rely on tissue and cell tropism of lipids (Myeloid Therapeutics, Carisma Therapeutics, Orna Therapeutics) or cell-selective targeting antibodies (Capstan Therapeutics, Shenzen MagicRNA, Sanofi, Immorna), respectively.

Non-viral RNA-based systems in clinical development

In vivo myeloid cell programming is pursued by companies such as Myeloid Therapeutics. This in vivo engineering platform specifically targets and activates myeloid cells, redirecting them against tumour cells[121]. This group demonstrated that delivery of LNPs encapsulating CAR in mRNA format resulted in uptake and selective expression by myeloid cells in vivo, leading to tumour elimination in multiple tumour models[121]. The approach leverages the natural tropism of LNPs for the myeloid cell lineage. The CAR design comprised a truncated FcαR (CD89) fusion construct forming a multi-chain complex with the endogenous FcRγ chain, which is required for its stable cell surface expression, thus achieving cell lineage-specific expression and function. The mRNA payloads were also optimized by introducing modifications in the mRNA coding sequence and 3′ untranslated region (UTR) to enhance and prolong the CAR expression. In 2022, Myeloid entered an agreement with Acuitas, an innovator in the field of LNPs, to enable development of novel in vivo CAR therapies for patients with cancer[122]. Initial preclinical proof-of-concept studies in mice used an LNP-mRNA format to deliver CARs targeting glypican 3 (GPC3), tumour-associated calcium signal transducer 2 (TACSTD2; also known as TROP2) and glycoprotein 75 (GP75)[121]. In a mouse lung xenograft model, intravenous administration of TROP2 CAR mRNA-LNP resulted in tumour growth inhibition[123]. Similar to tumour-bearing mice, in cynomolgus monkeys the TROP2 CAR mRNA-LNPs were primarily internalized by myeloid cells.

A similar preclinical dataset was generated for the GPC3 CAR mRNA-LNP product candidate. In vitro transfection of human and cynomolgus whole blood cells resulted in effective LNP uptake by myeloid cells, with the GPC3 CAR selectively expressed in up to 30% of monocytes[124]. Furthermore, dose-dependent expression of the GPC3 CAR was observed in cynomolgus monkeys following infusion. Finally, antitumour activity in a mouse model was associated with increased cytokines, an indicator for CAR engagement with GPC3 on tumour cells. Overall, these findings demonstrate that myeloid cells can be directly engineered with CAR mRNA-LNPs in vivo to eliminate tumour cells and orchestrate an adaptive immune response.

Two product candidates have progressed to phase I trials: MT-302, an in vivo CAR targeting TROP2$^+$ epithelial malignancies, is being evaluated for safety and preliminary efficacy (NCT05969041), with patient dosing initiated on 12 September 2023 (ref. 125); and MT-303, an in vivo CAR targeting primary or metastatic hepatocellular carcinoma overexpressing GPC3, that commenced dosing in a phase I study (NCT06478693) on 31 July 2024 (ref. 126). In TROP2$^+$ cancers, MT-302 resulted in CAR expression in both the peripheral blood and TME[127]. In GPC3$^+$ hepatocellular carcinoma, MT-303 has shown antitumour activity, including lesion reductions in heavily pretreated patients[127]. Across both programmes, tolerability remains acceptable at current dose levels, with no dose-limiting toxicities observed and manageable CRS. These findings demonstrate the mechanism of action and support further dose escalation and expansion into combination studies. Recently, Myeloid Therapeutics extended preclinical proof of concept to CARs against HER2, also applicable to solid tumours[128].

To effectively co-opt T cells, Capstan Therapeutics developed ligand-decorated LNP particles (CellSeeker technology) in support of their in vivo CAR portfolio. This in vivo CAR platform comprises payloads in optimized linear mRNA format delivered by targeted LNPs (tLNPs), with antibody-based binders aimed to selectively engineer immune cell subsets. Compared with LNPs used for mRNA vaccines[129], these tLNPs contain highly biodegradable, less reactogenic ionizable lipids, amenable to repeat intravenous infusion. This platform design was enabled by major progress in the field of mRNA medicines through use of modified nucleotides such as N_1-methylpseudouridine[130], nanomedicines (through the advent of ionizable lipids[131]) and CAR designs enabling potent immune cell function[7]. Foundational science consisted of the generation of anti-fibroblast activated protein (FAP) CAR-T cells in situ, reduction of FAP$^+$ fibroblasts and improvement of organ function in a preclinical cardiac fibrosis model, using an anti-CD5 antibody-decorated tLNP-mRNA format[132]. This was followed by platform and payload optimization, yielding formulations amenable to development, and an anti-CD19 CAR in an anti-CD8 antibody-LNP-mRNA format, recently nominated as a lead clinical development candidate[133].

Similarly to other in vivo CAR technologies that do not rely on integrating vectors, owing to its transient, self-limiting CAR expression, this mRNA-engineering approach may obviate heightened or prolonged on-target toxicities, immunogenicity attributable to viral components and genotoxicity, which can all impact patient access. In addition, owing to the use of targeting antibody binders, this type of technology enables preferential engineering of immune cell subsets without co-opting other cell types such as B cells or CAR-target cells

in general, that may have a detrimental contribution to the outcome. De-risking tLNP immunogenicity and reactogenicity is critically important, especially in disease indications associated with a higher efficacy bar warranting repeat infusions over a prolonged time frame. Finally, mRNA sequence optimization was key to augmenting CAR activity, underscoring the yet to be tapped potential of linear mRNA as a payload type[134].

Non-clinical evidence with Capstan's anti-CD19 CAR lead product candidate comprised comparably efficient in vitro engineering of human T cells from healthy or diseased individuals[135]. Proof of concept in humanized mouse models showed rapid in vivo engineering and profound activity of human $CD8^+$ T cells against primary or malignant B cells[134]. An anti-CD20 CAR in anti-CD8-LNP-mRNA format, a surrogate product applicable to NHPs, showed profound B cell depletion including memory B cells in lymphoid tissues and marrow, followed by repopulation with predominantly naive B cells[136,137], akin to the immune reset achieved with conventional CAR-T cells[23]. A compact treatment cycle of only two infusions 3 days apart sufficed to achieve B cell depletion in lymphoid organs[138]. This supports clinical development of this lead product in autoimmunity, and of other in vivo CAR products and novel treatment concepts for broader indications including genetic disorders[139], pending on demonstrating safety and activity in early-stage clinical trials. A first-in-human trial (NCT06917742) was initiated this year. Capstan intends to develop a broader portfolio of in vivo CAR products applicable to autoimmune disorders, and to other categories of indications (based on targets such as BCMA for plasma cell disorders, and activated fibroblasts for regenerative medicine, autoimmunity and oncology applications). Recently, Abbvie announced the acquisition of Capstan therapeutics, underscoring the broader interest in this rapidly evolving field[61].

Two other organizations have just initiated clinical testing of their in vivo CAR therapies, also using a tLNP-RNA format. Immorna Biotherapeutics started evaluating their tLNP formulation loaded with an anti-CD19 CAR in a phase I trial with patients with non-Hodgkin's lymphoma[140]. This product candidate (JCXH-213) aims to co-reprogramme T cells, natural killer cells and macrophages for an augmented pharmacological effect. Shenzen MagicRNA has also advanced a CD8-tLNP loaded with an anti-CD19 CAR (HN2301) to the clinic[141]. Their Enc-LNP platform includes proprietary antibody fragments and ionizable lipids, to selectively engineer immune cells in vivo. Preclinical evidence showed activity of a surrogate anti-CD19 CAR in a human CD8-transgenic lupus nephritis model, and data indicative of immune reset in NHPs dosed with an anti-CD20 CAR surrogate[142]. This investigator-initiated trial, conducted with corporate participation, is evaluating HN2301 in patients with relapsing and refractory systemic lupus erythematosus, with the objective to evaluate safety and efficacy and broader platform feasibility. At the time of publication of this Review, this approach showed the first evidence of in vivo engineering of anti-CD19 $CD8^+$ CAR-T cells accompanied by evidence of B cell depletion, cytokine production associated to on-target activity and clinically relevant biomarker activity manifested through decreased autoantibody titers in patients with systemic lupus erythematosus[143].

RNA-based platforms in advanced preclinical research

The non-viral platform developed by Orna Therapeutics is distinct in its use of LNPs formulated with immunotropic lipids, which preferentially target immune cells without the need for peptide ligands or any other sort of targeting moiety, which could have considerable advantages from a chemistry, manufacturing and controls (CMC), regulatory and cost perspective. In immune cells, next-generation circular RNA is used to transiently express CARs (panCAR). This enables engineering a precise, transient CAR expression in the patient's immune cells using both LNP design and circular RNA engineering. The Orna platform uses cross-species reactive CAR designs enabling validation of efficacy in both rodent and NHP studies ahead of preclinical toxicology studies. At launch in 2019, based on its foundational MIT technology, Orna had a focus on immune cell engineering using a tissue-specific internal ribosomal entry site within a circular RNA platform[144–146]. Subsequently, it acquired a leading non-viral delivery platform from ReNAgade in 2024 (ref. 147) after ReNAgade demonstrated delivery to immune cells in NHPs including >50% engineering of T cells.

Using anti-CD20 and anti-CD19 CARs in circularized RNA format, this group has demonstrated robust B cell depletion in vitro, in rodent and NHP models after a single dose administration[148]. The anti-CD20 CAR formulation showed significant B cell depletion that manifested as a 75–80% reduction in B cells in the peripheral blood, spleen and bone marrow at 24 h in a humanized mouse model. This effect was sustained for 7 days after dosing in the peripheral blood and bone marrow. Finally, this formulation showed a 95% reduction in B cells in blood, with sustained depletion (82%) for 7 days after a single dose in NHPs[148]. The first clinical programme will use anti-CD19 CAR in circular RNA format in autoimmune diseases across multiple indications. A BCMA programme in multiple myeloma will run shortly after dosing the first patient with the anti-CD19 CAR.

The concept of circular RNA is also pursued by other companies currently in the preclinical development stage — an example is Orbital Therapeutics, initially focused on vaccines[79] — with plans to bring forward a tLNP formulation loaded with a CAR payload against a B cell lineage antigen. In addition, preclinical evidence in vitro or in murine models by Sail Biomedicines[149], Strand Therapeutics[150] and RiboX Therapeutics[151] underscores the tremendous interest in circular RNA platforms as means to further optimize the potential efficacy advantage afforded by the enhanced or prolonged CAR payload expression, with particle functionalization thus augmenting the selectivity and performance of this approach.

Sanofi is also developing a tLNP-based platform, a similar concept to that pursued by Capstan Therapeutics, MagicRNA and Immorna. This is different from typical mRNA-LNP formulations used for vaccines as it is designed to enable selective T cell engineering and control of CAR-T cell exposure. Cell-specific CAR expression is achieved by encapsulating modified mRNA inside the LNP with the addition of antibody-based binders on the surface to target $CD8^+$ cells[152]. The proprietary ionizable lipid used in this formulation is the result of an extensive screen designed to improve the tolerability, stability and activity in T cells. This was achieved by altering both the lipid head and tail groups of the lipid molecules[152]. LNP performance depends heavily on the structure of the ionizable lipid structure, including the hydrophobic chain length, branching, structural isomerism and degree of saturation. Systematic modification of these features led to a 3-fold to 4-fold increase in T cell transfection efficiency compared with LNPs formulated with unoptimized lipids.

The preclinical activity highlights the modularity and flexibility of the tLNP system, supporting potential utility against multiple targets and diseases. Transfection of T cells is achieved by antibody fragments on the surface of the LNP, where they facilitate the interaction and uptake of the targeted nanoparticles[153]. Multiple Fab and nanobody binders were screened using model LNP formulations. Antibody fragments against CD3 enabled rapid uptake and payload

expression but resulted in non-specific activation, which reduced tolerability. Targeting LNPs with fragments against CD8 resulted in rapid payload expression (CAR or fluorescent protein) without non-specific activation and cytokine expression. LNPs combining the functionally screened lipids and T cell binders enabled robust CAR expression in human T cells. CAR expression was limited to $CD8^+$ cells, including both T cells and natural killer cell subsets, and the CAR-expressing cells were active against target cell lines[152]. Cellular transfection was demonstrated in vitro and in a humanized mouse model, in which the platform also demonstrated the ability to control tumour cell growth, systemically and in the spleen[152]. Recently, evidence of anti-CD19 CAR engineering and pharmacological effect was generated in rhesus macaques infused with single or multiple doses of CD8-tLNPs containing CD19 CAR mRNA. Rapid B cell depletion was documented in both blood (>95% relative to baseline) and bone marrow (90% relative to control animals), with B cells returning to pre-infusion levels by day 29 in marrow and day 50 in blood[154]. Plans to initiate clinical development have yet to be disclosed.

Although Myeloid Therapeutics entered clinical development as described above, the prospect of reprogramming myeloid cells ex vivo or in vivo has also been pursued by others. Carisma Therapeutics developed human CAR macrophages and monocytes (CAR-M), demonstrating that CARs can redirect macrophage effector function against tumour-associated antigens[155]. Pierini et al. demonstrated that CAR-M remodel the solid tumour TME, recruit T cells, drive epitope spreading and sensitize solid tumours to checkpoint inhibition[156]. Subsequently, the concept of CAR-M was expanded by directly introducing CARs to $CD14^+$ classical monocytes, which differentiated into CAR-M in situ while enabling enhanced trafficking and persistence[157,158]. The first CAR-M, CT-0508 (autologous, anti-HER2), was evaluated without lymphodepletion chemotherapy in 14 patients with advanced/metastatic HER2-overexpressing solid tumours, and demonstrated feasibility, safety, TME remodelling and induction of adaptive immunity[159]. Antitumour activity was demonstrated in patients with $HER2^+$ solid tumours based on deep but transient reductions in circulating tumour DNA[158]. A key limitation of the ex vivo CAR-M approach is the maximum potential dose; monocytes and macrophages are terminally differentiated and do not expand, which directly caps the dose by apheresis output. In addition, allogeneic CAR-M approaches have limited utility in the oncology context as MHC mismatch limits antigen presentation. Thus, direct in vivo delivery of CARs to myeloid cells was an appealing alternative, in line with the potential advantages discussed earlier. In 2022, Carisma Therapeutics and Moderna initiated a collaboration to develop in vivo CAR-M using mRNA-LNP technology[160]. The platform was developed using an LNP with robust capability to transfect myeloid cells in vivo and a novel CAR comprising optimized structural and signalling domains for myeloid cell antitumour activity. In vitro data demonstrated that the CAR was expressed for >7 days and induced antigen-dependent cell death, cytokine release, serial killing (the ability to kill multiple target cells in sequence) and polarized macrophages towards an inflammatory phenotype post antigen stimulation[161]. In vivo, systemic dosing of mRNA-LNP led to robust antitumour activity in humanized mice engrafted with metastatic solid tumours in both HER2 and GPC3-positive tumour models[161,162]. Myeloid cells were the primary CAR-expressing immune cells after intravenous mRNA-LNP administration[161,162]. Carisma and Moderna announced that an anti-GPC3 in vivo CAR-M development candidate was nominated in 2024 for further clinical development in hepatocellular carcinoma. Meanwhile, the companies continued to develop in vivo CAR-M for additional undisclosed oncology targets and expanded the collaboration in 2024 to include two targets in autoimmune disease[163]. Carisma and Moderna have since discontinued the anti-HER2 programme but continued development of CAR-M targeting GPC3 (ref. 164).

Other companies are exploring improvements to the tLNP design and manufacturing process applicable to in vivo CAR products, as pursued, for example, by Acuitas Therapeutics[165], Mote Therapeutics[166], Nitto Denko Corporation[167], Aera Therapeutics[168] and Grit Biotechnology[169]. A particular interest is represented by non-viral, all-RNA payload approaches, to permanently insert a CAR construct gene in T cells, in vivo, in specific loci. Such alternate knock-in technologies pioneered by Tessera Therapeutics, obviating the use of viral vectors[170,171], may be greatly enabled by the advent of RNA writing, highly compatible with the LNP platform. Preclinical mouse modelling, supported by NHP evidence, yielded proof of concept for CD19, CD20 and BCMA-directed CARs, utilizing this CAR knock-in approach[171–173]. A similar path is also pursued by other organizations, such as Stylus Medicine[174] and Integra Therapeutics[175], as an alternative to LV-based in vivo CAR platforms, in the quest to achieve a permanent yet more controlled CAR payload integration and expression profile. Finally, to this aim, NanoCell is developing a hybrid payload approach, comprising CAR in a DNA format and transposase delivered as mRNA[176].

In vivo CAR-T cells in clinical development

As the field enters the clinical stage, first with engineered lentiviral vectors and myeloid cell-tropic LNP-mRNA formulations explored in malignant disorders, clinical case studies point to favourable safety, tolerability and biological response or clinical efficacy. These first-in-human trials are either investigator-initiated studies with corporate participation or company-sponsored trials and are taking place worldwide, including in China, the United States, Australia and the European Union, illustrating the global nature of this emerging ecosystem. Objective responses afforded by anti-BCMA[60,97–99,101] and anti-CD19 (refs. 95,96) CARs in T cell-targeted lentivirus format were described in multiple myeloma and non-Hodgkin's lymphoma, respectively. Recently, four patients with multiple myeloma with deep objective responses treated with an in vivo lentiviral anti-BCMA CAR therapy have been reported[101]. One of the patients showed stringent CR by day 28 and another minimal residual disease clearance in marrow by day 28, underscoring the possibility to achieve rapid and profound responses in cancer, provided that the CAR exposure is sufficiently high. The CRS profile appeared biphasic[101], with the first peak within hours likely co-contributed by an acute infusion reaction to T cell-activating virions plus activity mediated by CAR^+ effector cells, and the second typical of viral CARs, possibly mediated by de novo differentiated effector cells about 7–14 days post treatment. Although the field is still in its infancy, the magnitude and speed of CAR-T cell expansion followed by a clinical response, measurable within weeks post treatment, points to the scenario that a clinically meaningful outcome can be afforded during a short period of CAR exposure. This may also bode well for CAR products with transient expression by design when CAR exposure can be calibrated by repeating infusions or adjusting the dose. Although emerging data with myeloid cell-tropic LNP-RNA formulations comprising CAR constructs against solid tumour targets (TROP2 and GPC3)[127] support this observation, additional evidence is needed.

Although all these trials are designed to carefully monitor the safety profile, owing to the novelty of these platform technologies and risks emerging from integrating components with distinct and potentially synergistic toxicities, no serious adverse events or

dose-limiting toxicities have been reported to date. In addition to the slow dose escalation with low starting dose, these studies may include sentinel patients, in-hospital treatment and post-infusion follow-up, prolonged observation intervals between cohorts, strict stopping rules and definition of dose-limiting toxicities, as well as extensive safety biomarker evaluation pertaining to infusion reactions, possible on-target toxicities and product immunogenicity. These efforts are also aimed at supporting selection of dose regimens for subsequent trials. Based on the number of companies in the preclinical stage and the expanding interest in this field, we anticipate that the number of companies entering clinical development will continue to rapidly increase beyond 30, a tremendous double-digit expansion during the last 2 years (Tables 1 and 2).

In vivo immune engineering: key challenges

As the first in vivo CAR therapies enter clinical evaluation (Tables 1 and 2 and Fig. 4), several key risks and unanswered questions remain (Box 3). To address these, early-phase trials are incorporating a broad array of safety and pharmacological response biomarkers to guide further development. Most notably, in terms of safety, this approach represents uncharted territory, combining distinct technologies, each with its own risk profile.

Technical risks and current limitations

To anticipate risks associated with in vivo CAR therapy, NHP modelling could be highly informative. In addition, physicians are becoming more experienced with commercial CAR-T cell products; however, there is only very limited clinical experience with direct in vivo CAR-T cell therapy with lentiviral vectors and LNP-RNA products[177]. Hence, it is key to deploy a risk-based monitoring approach in early clinical development, factoring in the specifics of the platform technology (viral or LNP-RNA-based) and the experience to date, in the clinic, with conventional CAR-T cell products.

Owing to the integrating nature of the payload, lentiviral-based CAR products present inherent challenges, mainly stemming from limited achievable control with respect to the CAR activation, expansion and persistence. Self-adjustment of CAR-T cell expansion, in proportion to the target cell burden, can enhance treatment efficacy. However, uncontrolled pharmacological responses, amplified by predisposing factors, could trigger serious inflammatory adverse events such as CRS, ICANS and IEC-HS. These require aggressive management and hospitalization. A key challenge is that this category of adverse events may occur after a lag interval post treatment, and are therefore difficult to predict, thus warranting prolonged monitoring of patients. Current efforts with conventional ex vivo engineered CAR-T cells in autoimmune diseases should continue to be very informative[178]: whereas the prevalence and severity of inflammatory adverse events seems to be lower than in oncology settings for reasons that are being elucidated[179], protracted CAR-T cell persistence and even re-expansion accompanied by severe toxicities such as high-grade IEC-HS[180], months after CAR-T cell infusion, have been recently reported. Recently, a novel toxicity has been described in patients with autoimmune disease dosed with anti-CD19 CAR-T cells: local immune effector cell-associated toxicity syndrome (LICATS)[181]. However, this seems to be a disease flare up, or bystander CAR-mediated activity transiently affecting the organs involved in the autoimmune process; 30% of the treated patients were managed with low-dose corticosteroids and 8% required hospitalization. Half of the treated patients developed LICATS at least 2 weeks post treatment, underscoring again the importance of long-term monitoring of patients with autoimmune disease treated with viral-engineered CAR-T cells. Finally, prolonged persistence of CAR-T cells may lead to protracted on-target toxicities manifested through B cell aplasia and hypogammaglobulinaemia — requiring prophylaxis of infections in the case of targeting the B cell lineage — or Parkinsonism[182], which has been reported with ex vivo anti-BCMA CAR-T cell products. In addition to long-term monitoring, to enhance the safety profile and controllability of in vivo CARs relying on genomic payload integration, one may need to integrate features that ensure spatial-temporal control of CAR expression such as cell lineage-specific promoters and kill switches[183].

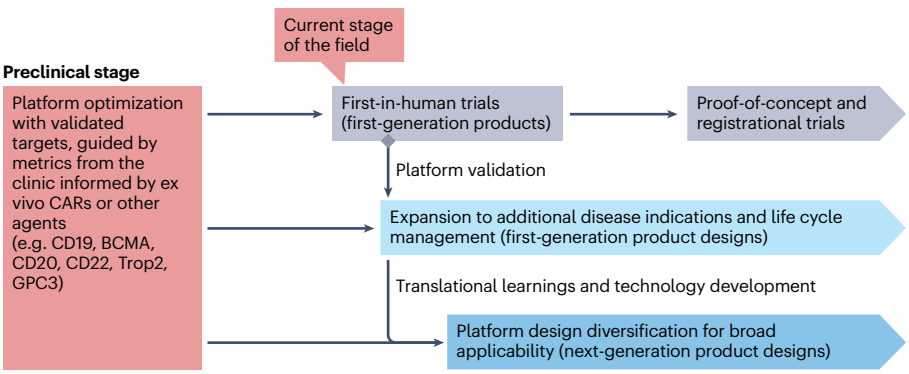

Fig. 4 | Top-line perspective of development of in vivo CAR therapies. At this stage, most efforts are focused on platform and product design and optimization using validated targets, guided by metrics from clinical experience with other treatment modalities. These targets are B cell lineage antigens (CD19, B cell maturation antigen (BCMA), CD20, CD22) with dual applicability to oncology and autoimmunity, and solid tumour targets (TROP2, glypican 3 (GPC3)). Several product candidates are currently being translated to the clinic, including both engineered viruses and lipid nanoparticle (LNP)-RNAs. Upon demonstrating safety, tolerability and clinically relevant biological activity with the first-wave product candidates, we envisage a rapid progression towards proof of concept and registrational trial, respectively, accompanied by an expansion of clinical activities across diverse clinical indications (oncology and autoimmunity). Owing to the novelty of these platforms, and the yet to be defined therapeutic indexes, it is likely that most product candidates will be clinically tested first in oncology indications, followed by autoimmune disorders or regenerative medicine; except for product candidates deemed to have a safety profile more amenable to non-oncologic indications such as those based on RNA . Pending on continuous technology development, we envisage a diversification of product concepts, payload architectures and targeted delivery vehicles, leading to increasingly innovative and disruptive in vivo therapies for diseases that are hard to treat with conventional technologies. CAR, chimeric antigen receptor.

Box 3 | In vivo CAR therapy: risks and questions to be answered

Viral vector-based in vivo CAR therapies

Owing to permanent genomic integration of the chimeric antigen receptor (CAR) sequence, there is a likelihood of amplified CAR-T cell activation, expansion, re-expansion and/or persistence in a subset of patients, leading to cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), immune effector cell-associated haemophagocytic lymphohistiocytosis-like syndrome (IEC-HS) and prolonged B cell aplasia, and hypogammaglobulinaemia in the case of B cell-directed products. These may require monitoring or intensive management utilizing tocilizumab, corticosteroids, anakinra, intravenous immunoglobulin and other supportive therapies. There is potential genotoxicity and a low probability of clonal events, requiring long-term monitoring and influencing the utilization of this technology in indications with a high safety bar. Immunogenicity against certain viral components may also influence utilization and re-dosing with these products. Finally, insertional mutagenesis that may lead to clonal events warrants long-term monitoring of patients treated with retroviral-based in vivo CAR products.

Lipid nanoparticle (LNP)-RNA-based in vivo CAR therapies

Owing to the transient engineering nature, this approach may need re-dosing to achieve sufficient exposure, hence product reactogenicity or immunogenicity may be limiting, or this modality may be more suitable for indications with a lower efficacy bar. Depending on the formulation specifics and due to the tropism for liver and myeloid cells, there is a possibility for acute infusion reactions, liver toxicities and product immunogenicity leading to hypersensitivity reactions. In the case of suboptimal cell selectivity, co-engineering of non-target cells may limit efficacy or facilitate organ immunopathology. Although this technology is tunable with respect to dosing, one cannot rule out the possibility of aggravated pharmacology (CRS, ICANS, IEC-HS) that may require adequate management. Finally, the lack of clinical experience with this type of product that brings together the safety profile of nanomedicines, RNA molecules and CAR products, respectively, warrants comprehensive risk-based monitoring during early clinical development.

Key questions

- Can in vivo CARs be curative, on a par with currently available ex vivo CAR products?
- Is this concept scalable and broadly applicable to both non-oncology and oncology indications, the outpatient setting and early lines of treatment?
- What are the novel dose-limiting toxicities warranting adequate prophylaxis and management?
- What future optimizations are needed to maximize the footprint of in vivo CAR therapies?

A particularly notable aspect related to retroviral vectors is the insertional oncogenesis due to the relatively stochastic nature of genomic integration. Some recent reports showed that patients treated with conventional viral-engineered CAR-T cell therapy developed CAR^+ secondary T cell malignancies[184]. Nevertheless, establishing a direct causal link between CAR insertion and tumour development is challenging, given the low numbers of cases, the underlying cancer susceptibility of this patient population and the inherent difficulty of transforming differentiated T cells[184–186]. Clearly, additional investigation into this matter — specifically, elucidating the relative contribution of the CAR vector insertion and identity of co-factors such as locus integration and interaction with oncogenes[187] — is necessary. Regulatory guidance for industry is available to guide product developers with respect to long-term monitoring after administration of human gene products[188], although these may need to be further adjusted to enable development of in vivo retroviral-based CAR therapies.

In turn, RNA-based CARs relying on LNP formulations may present safety risks resulting from the interaction of the three major components of the technology: nanoformulations that can mediate infusion reactions or acute phase response[189]; RNA payloads with intrinsic innate immune activating effect[190]; and CAR constructs with profound immune pharmacologic activity that may result, as discussed above, in inflammatory adverse events or uncontrolled on-target pharmacology. Owing to the transient nature of CAR expression off an RNA template, especially in the face of rapid T cell division, repeat dosing or other optimizations may be necessary to achieve sufficient CAR exposure during a critical interval, required for clinical effectiveness. This raises several key challenges, including de-targeting the liver to pre-empt organ-specific toxicities due to the inherent liver-tropism of such formulations[191]. Secondly, repeat administration of antibody-tLNPs or untargeted LNPs may lead to induction of antibody and T cell responses against the LNP components, targeting moiety and/or expressed payload. These could, in principle, limit the effectiveness of these therapies but could also elicit immune-mediated adverse events[192] including type I, III and IV hypersensitivity reactions.

Altogether, these potential limitations, combined with the lack of clinical experience with in vivo CARs in LNP-RNA format, underscore the importance of de-risking such technologies in multiple ways in diverse preclinical settings including NHPs. Previously approved RNA-nanomedicines such as ONPATTRO[177] may provide key building blocks for a roadmap to develop in vivo LNP-RNA CAR products, but they need to be integrated with CAR treatment-related considerations[193] in future guidelines. Meanwhile, comprehensive risk-based monitoring[194] of all potential toxicities during early clinical development remains key to a successful translation to the clinic of this treatment modality. This needs to include product immunogenicity, acute phase reactions, organ (liver) toxicities and on-target inflammatory or other categories of adverse events. It remains to be seen whether in vivo CAR technologies comprising transiently expressed payloads may obviate relatively delayed or chronic on-target or off-target toxicities seen with viral CARs, such as LICATS, IEC-HS associated with secondary CAR-T cell expansion, movement disorders, delayed cytopenia[195] or prolonged B cell aplasia.

Below, we propose a product development roadmap and potential initial applicability (Figs. 4 and 5) for the main categories of in vivo CAR therapies based on their target product profiles. Finally, we provide a longer-term perspective on the concept of programming the immune system in vivo, with a potential transcending those of conventional treatment modalities.

Product development strategies

Most sponsors have prioritized well-validated targets (CD19, CD20, BCMA, CD22, TROP2, GPC3) to guide the optimization of their platform technology, thus enabling clinical translation and development of commercial products. The overarching aim is to expand the clinical applicability of CAR therapy, by displacing or outcompeting less scalable ex vivo engineered cell-based products or underperforming biologics. The first wave of human trials with in vivo CARs, in addition to safety and evidence of clinical activity, will enable dose-regimen selection for subsequent proof of concept and registrational trials (Fig. 6). These initial trials in B cell malignancies and autoimmune diseases involving B cells will investigate risks including infusion reactions, product immunogenicity, acute, subacute and chronic on-target toxicities, and genotoxicity. Therefore, a comprehensive translational analysis is critical to build an adequate safety monitoring and management plan, to inform optimizations prior to phase II trials and to select adequate dose regimens for registrational trials.

In haemato-oncology, owing to the strong rationale of advancing immunotherapy to earlier lines[196,197] and the advent of minimal residual disease testing[198], a major opportunity will be treating high-risk patients in a front-line setting, for curative intent. In autoimmunity, a key objective will be to advance scalable therapies that afford 'immune reset', consisting of deep immune cell lineage depletion and rapid repopulation with a normal immune cell repertoire, both leading to durable, drug-free responses[23] without any tissue or genomic sequelae. In regenerative medicine, fibrosis[21] and senescence[199], a major opportunity will be to eliminate pathogenic cells and restore the normal functioning tissue architecture, through in vivo CAR engineering of immune cells leading to the ablation of pathogenic cells in tissues. Based on the predicted target product profiles for the viral and mRNA based in vivo CAR-T cell treatment modalities, we envisage different commercial entry points, although first targeting solid tumours with CAR mRNA-engineered macrophages may represent an exception (Fig. 5). Iterative product and platform optimizations based on deep reverse translation will likely yield improvements in product performance and target product profiles, leading to broader clinical applicability across all these platforms.

Towards in vivo reprogramming of the immune system

Clinical validation of these treatment platforms enabled by the first waves of in vivo CAR-T cell products may pave the way to re-thinking immunotherapy altogether in the future. The differentiating characteristics for in vivo immune engineering from other treatment modalities are numerous, and the therapeutic potential is vast (Box 4). For example, in oncology one could envisage multi-receptor CARs or TCR engineering of multiple categories of immune cells that work in concert with each other, direct reprogramming of the immune microenvironment, effective co-opting of endogenous immunity, modulating the transcriptome and signalling pathways of immune cells, and combating immune checkpoints — all through in vivo engineering, as detailed below.

In autoimmunity, transplant rejection management, allergy and other immune conditions, in vivo CAR-T cell products could reset or modulate the immune system in various ways, through co-deploying counter-regulatory mechanisms or by inducing operational tolerance[200], thereby achieving very durable drug-free responses. Other promising directions include expanding CAR payload designs to target a broader array of molecules[60,201], selectively directing CARs to antigen-specific pathogenic cells[202,203] or using dual-targeted CAR constructs to broaden activity and enhance clinical safety and efficacy in certain settings[204]. A decade after the initial demonstrations of antibody-guided LNP-mediated in vivo T cell engineering[205], current efforts continue to focus on improving precision delivery to defined immune cell subsets, with the use of highly selective antibody fragments and emerging classes of binders such as nanobodies[152].

Novel platforms including implantable devices may greatly enable this wholistic immune system engineering approach[206]. Similar to

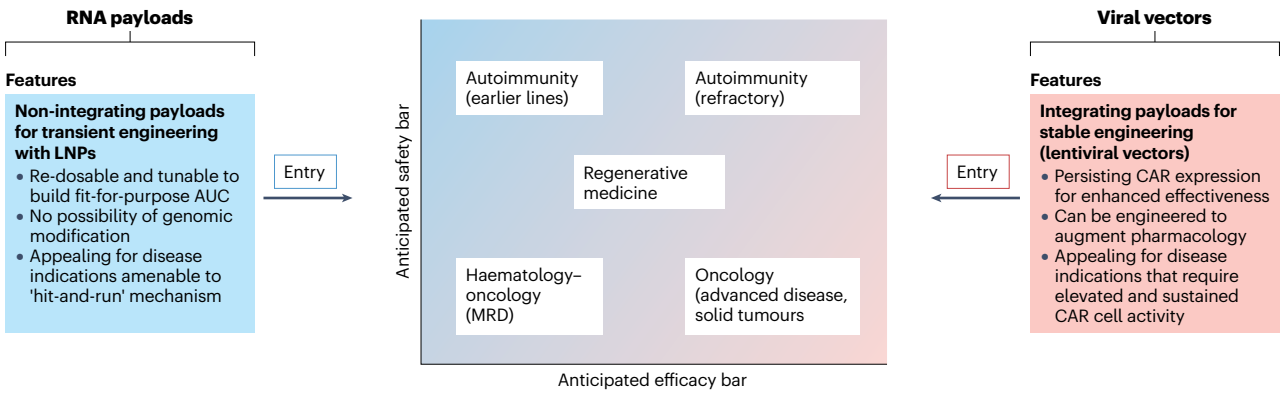

Fig. 5 | In vivo engineering platform features inform on target product profile characteristics and therapeutic applicability. The in vivo chimeric antigen receptor (CAR) product candidates that contain non-integrating payloads for transient engineering with lipid nanoparticle (LNP)-RNAs are, in principle, re-dosable and tunable, thus enabling modulation of the CAR exposure function of therapeutic efficacy and safety bars. In addition, considering the lack of genomic integration of the payload, these are appealing for disease indications with high safety bars amenable to 'hit-and-run' mechanisms (such as induction of 'immune reset' in autoimmunity or elimination of residual disease in cancer). Nevertheless, macrophage-tropic LNP formulations delivering CAR mRNA, which enable repeat administration and co-opt endogenous immune mechanisms, are currently being tested in solid tumours with promising emerging results[127]. The in vivo CAR products based on integrating payloads for stable T cell engineering using viral (lentiviral or gamma-retroviral) vectors carry the mechanistic advantage of self-calibrated CAR-T cell expansion and persistence as a function of the target antigen burden. This is appealing for disease indications that require elevated and sustained CAR cell activity. Nevertheless, the genomic integration aspect may pose specific challenges related to possible CAR^+ cell re-expansion, exaggerated on-target pharmacology and requirement to monitor genotoxicity. Hence, in principle, such products could be more easily translated first for disease indications with higher efficacy and a lower safety bar, such as blood cancers in an advanced stage or solid tumours. AUC, area under the curve; MRD, minimal residual disease.

injectables, implantable devices are promising new technologies to genetically manipulate patient T cells in vivo. For example, biomaterial implants can recruit host T cells in situ, genetically equipping them with tumour-specific receptors, and rapidly expanding and dispersing the cells into the tumour milieu[207]. The device is a functionalized porous collagen scaffold, with a potent T cell chemoattractant, a CAR-encoding lentiviral vector and stimulatory anti-CD3/CD28 antibodies. In a mouse model of breast cancer, the implants quickly amassed and programmed cancer-specific host lymphocytes and drove long-term tumour regression. A potential advantage of this localized implant technology is that it reduces the required vector dose considerably, saving costs and minimizing systemic toxicity risk. The devices could be placed near a solid tumour during or post primary debulking surgery, or be positioned strategically near lymph nodes to facilitate rapid dissemination of in situ programmed CAR-T cells throughout the body. Furthermore, these 'off-the-shelf' scaffolds can work with the same lentiviral vectors that are used for any of the current FDA-approved CAR-T cell therapies.

With six medicinal products on the market for in vivo gene therapy, adeno-associated viral (AAV) vectors may become a versatile vector of choice for in vivo delivery. Owing to intrinsic limitations of AAVs such as immunogenicity, their episomal location and, thus, transient activity in proliferating cells, the use of AAV vectors for CAR-T cell generation has been very limited to date. Yet combining AAV-based CAR delivery with endonuclease-mediated genomic targeting enabled stable CAR integration[208,209]. Another potential combination is with the recently described T cell-targeted AAVs based on a capsid design strategy such as the engineering of lentivirus envelope, which may soon yield an alternative vector system for transient or permanent precision engineering of CAR-T cells in vivo[209–211]. Optimizations may include bispecific AAVs recognizing combinations of target receptors with logic 'AND' gating[210]. An alternative approach to directly reprogramming T cells is HSC engineering to generate CAR-T cells, CAR natural killer cells and CAR-M, by using virus-like particles based on helper-dependent adenoviruses with a sleeping beauty integration payload along with a CAR construct[212].

Alternative knock-in technologies that obviate the use of viral vectors[169,170] may also enable the next generation of in vivo CAR-T cell therapies. The advent of RNA gene writing presents a tremendous opportunity to develop integrating payload technologies with increased control of CAR gene insertion; and late breaking preclinical data generated from in vitro and mouse models yielded proof of concept for CD19, CD20 and BCMA-directed CARs in this format, delivered as an all-RNA payload through tLNPs[170,213].

Novel technologies to further optimize the safety and efficacy profile will be critical to enabling the clinical translation of such in vivo engineering platforms, especially in disease indications with high safety and efficacy bars. The academic research continues to drive this field, with several additional concepts that may greatly enable next-generation product design as described recently[214]. With respect to obviating toxicities and enhancing targeting selectivity, major directions include spatial-temporal control of payload expression[215] and combinatorial antigen recognition[216]. Other avenues include leveraging tissue and cell lineage-specific promoters for genomic integrating technologies, and in case of RNA-based CARs and other payloads, utilization of tissue-specific microRNA binding sites that restrict undesired translation in the liver[217] or other organs. Payloads with enhanced biological

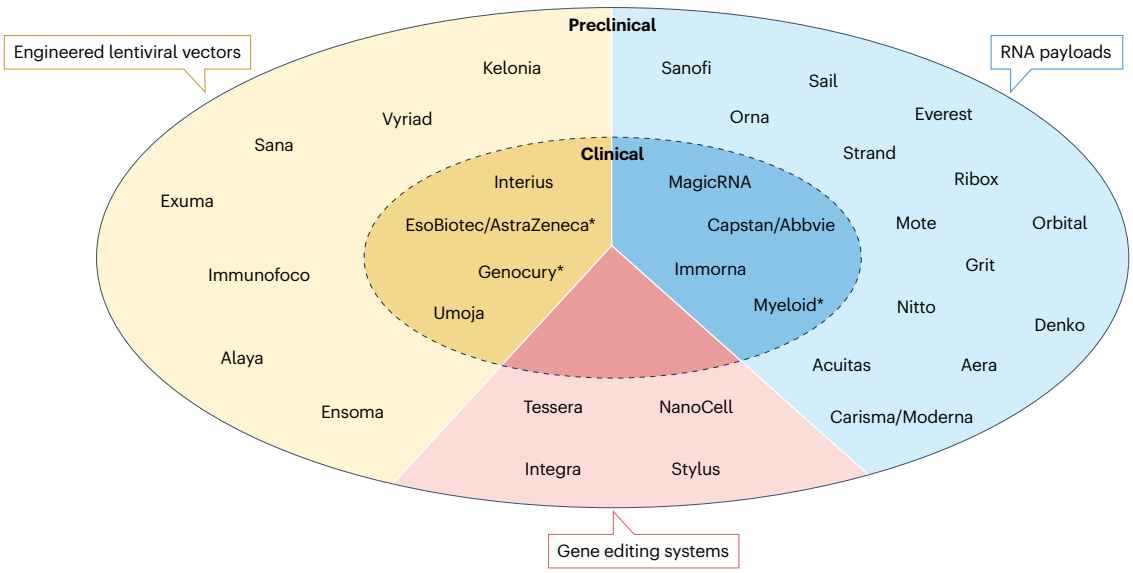

Fig. 6 | The rapidly evolving, in vivo CAR therapy ecosystem. The main technologies entering clinical testing or in a preclinical optimization phase divided according to the payload technology (viral or non-viral) and whether it relies on chimeric antigen receptor (CAR) construct integration into the genome. The leading technologies with emerging proof of concept are based on integrating engineered lentiviral vectors and mRNA formulated in myeloid cell-tropic lipid nanoparticles (LNPs), evaluated in B cell malignancies and solid tumours, respectively. These are followed by other engineered lentiviral vectors and targeted LNP (tLNP)-RNA technologies entering the clinical stage or in advanced preclinical development (Table 1). Additional technologies comprising hybrid or non-viral gene editing systems are in early research or the preclinical development stage as described in the text. The inner circle shows companies in the clinical testing stage and the outer circle shows companies in the preclinical stage. Companies developing engineered lentiviral vector formats are shown in the orange sections, whereas companies developing RNA-based approaches are shown in the blue section. Finally, the red sections shows companies pursuing in vivo engineering, based on target delivery of gene editing systems. *Companies with emerging proof of concept based on reported case studies: Esobiotec in multiple myeloma, Genocury in non-Hodgkin's lymphoma and Myeloid Therapeutics in solid tumours.

Box 4 | Major differentiating features and potential advantages of in vivo immune engineering over conventional immunotherapies

Relative to antibodies and immune cell engagers

- Augmented activity: owing to immune cell trafficking and chimeric antigen receptor (CAR) design comprising multiple co-stimulatory domains
- Higher versatility in terms of engineering-specific immune cell subsets
- Capability to simultaneously co-opt multiple immune cell types that work in concert with each other
- More diverse cell engineering modalities, including modifying the cell functionality from within through reprogramming the genome, transcriptome, cell signalling networks, receptors or other aspects of the immune cell biology
- Co-delivery of payloads: by expressing biological response modifiers, checkpoint modulators or other biomolecules for local activity, within the microenvironment, thereby enabling a broader range of mechanisms with an augmented safety profile

Relative to ex vivo engineered cell products

- Obviates cell manipulation, supporting broader scalability and access, especially as compared with autologous products
- Avoids the use of chemotherapy-based lymphodepletion conditioning that may limit access and narrow applicability to indications with a low safety bar
- Possibility to co-engineer in vivo multiple categories of immune cells that may work in concert with each other
- Build on pre-existing immunity through activating rather than lymphodepleting conditioning approaches
- Increased tunability, depending on the nature of the payload, through dose adjustment and re-dosing, both key to attaining the desired exposure fitting a broader range of indications with their specific performance metrics

activity warrant better performing and tissue or cell-selective nanomedicines, facilitated by high-throughput screening augmented by bar coding[218–220], and designs guided by artificial intelligence[221]. Another approach to enhance the safety of LNPs as a vehicle for in vivo CAR therapy is co-formulation of immunomodulating factors such as short hairpin RNA to knock down the production of IL-6 (refs. 222,223) or of other pro-inflammatory molecules. A highly specific tissue and cell delivery vehicle becomes even more critical as the payloads diversify to include gene editing systems[219]. In parallel, there is continuous focus on exploring diverse formulation and payload chemistry, functionalization and manufacturing optimization[224–227]. To meet a higher efficacy bar presented by disease indications such as solid tumours, diversification of payloads including biological response modifiers for immune cell armouring[228], immune cell engagers co-opting additional effector mechanisms[229] and immune checkpoint modulators[230] are all being considered.

Glossary

Circular RNA
A single-stranded RNA molecule that forms a continuous loop. Although naturally a type of non-coding RNA that is found in many species, circular RNA can be adapted for cell engineering.

Designed ankyrin repeat protein
(DARPin). A genetically engineered antibody mimetic protein typically exhibiting highly specific and high-affinity target protein binding, derived from natural ankyrin repeat proteins.

Fusogen
A protein on the surface of a virus that helps the virus enter a host cell by fusing the viral membrane with the host cell membrane. This process releases the genetic material of the virus into the host cell's cytoplasm.

Hypogammaglobulinaemia
A condition characterized by abnormally low levels of immunoglobulins in the blood, making individuals more susceptible to infections.

Internal ribosomal entry site
A specific RNA sequence that allows ribosomes to bind to mRNA and initiate protein synthesis at an internal location, bypassing the typical cap-dependent initiation process.

Ionizable lipids
Lipid molecules that can change their charge depending on the pH level of their environment. They are a key component of lipid nanoparticles (LNPs), which are used to deliver RNA therapeutics.

Lymphodepletion conditioning
A treatment that prepares the body for chimeric antigen receptor (CAR)-T cell therapy or adoptive cell transfer in general. It involves using chemotherapy drugs (most frequently cyclophosphamide and fludarabine) to deplete endogenous immune cells.

Minimal residual disease
A term used for a small number of cancer cells that remain in the body after treatment. Minimal residual disease can occur in blood cancers such as leukaemia and lymphoma, as well as solid tumours.

N_1-Methylpseudouridine
A chemical compound found in tRNA and mRNA vaccines. Although a natural component of archaea, it is presently utilized in biochemistry and molecular biology.

Nipah virus (NiV) glycoproteins
NiV uses two key glycoproteins on its surface: the attachment glycoprotein (G) and the fusion glycoprotein (F), which are crucial for viral entry into host cells. The G protein facilitates attachment to the host cell, whereas the F protein triggers membrane fusion, allowing the virus to enter the cell.

PiggyBac transposon system
A genetic engineering tool used to introduce and integrate DNA sequences into a genome, often in a stable and reproducible manner. It utilizes a transposase enzyme to 'cut and paste' a transposon (a DNA sequence) into a new location in the genome.

Pseudotype
A virus particle that has been engineered to display a foreign viral envelope protein on its surface.

Outlook

Thirty-five years after the first evaluation of in vivo gene-based immunization with a simple antigen in a plasmid DNA format[5,231], 25 years after the first intravenous infusion of a retroviral vector for therapeutic application in clinic[232] and 8 years after the approval of the first CAR-T cell products[11,12], we envisage a future comprising precise and complex in vivo programming of the immune system. These technologies (Fig. 6), presently entering the clinical stage, will eventually afford spatial-temporal control and tunability of payload expression, and accommodate diverse mechanisms working in concert with each other. They will thus transcend the capabilities of current treatment modalities, leading to augmented clinical performance across a broader range of diseases. More specifically, the conceptual shift from ex vivo to in vivo CAR-T cell therapy redefines the scalability and accessibility of immune therapies, facilitated by massive reduction of the cost of goods, with profound socio-economic implications leading to democratization of life-saving therapies.

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References

1. Leavy, O. The birth of monoclonal antibodies. *Nat. Immunol.* **17**, 13 (2016).
2. Dumontet, C., Reichert, J. M., Senter, P. D., Lambert, J. M. & Beck, A. Antibody–drug conjugates come of age in oncology. *Nat. Rev. Drug. Discov.* **22**, 641–661 (2023).
3. Rosenberg, S. A. IL-2: the first effective immunotherapy for human cancer. *J. Immunol.* **192**, 5451–5458 (2014).
4. Baeuerle, P. A. & Reinhardt, C. Bispecific T-cell engaging antibodies for cancer therapy. *Cancer Res.* **69**, 4941–4944 (2009).
5. Wolff, J. A. et al. Direct gene transfer into mouse muscle in vivo. *Science* **247**, 1465–1468 (1990).
6. Pardi, N., Hogan, M. J., Porter, F. W. & Weissman, D. mRNA vaccines—a new era in vaccinology. *Nat. Rev. Drug. Discov.* **17**, 261–279 (2018).
7. June, C. & Sadelain, M. Chimeric antigen receptor therapy. *N. Engl. J. Med.* **379**, 64–73 (2018).
8. Hinrichs, C. & Rosenberg, S. A. Exploiting the curative potential of adoptive T-cell therapy for cancer. *Immunol. Rev.* **257**, 56–71 (2014).
9. Cappell, K. M. & Kochenderfer, J. N. Long-term outcomes following CAR T cell therapy: what we know so far. *Nat. Rev. Clin. Oncol.* **20**, 359–371 (2023).
10. Melenhorst, J. J. et al. Decade-long leukaemia remissions with persistence of CD4$^+$ CAR T cells. *Nature* **602**, 503–509 (2022).
11. US Food and Drug Administration. KYMRIAH. *FDA* https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/kymriah (2017).
This label corresponds to the first CAR-T cell product approved.
12. US Food and Drug Administration. YESCARTA. *FDA* https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/yescarta (2017).
13. US Food and Drug Administration. TECARTUS. *FDA* https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/tecartus (2020).
14. US Food and Drug Administration. BREYANZI. *FDA* https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/breyanzi-lisocabtagene-maraleucel (2021).
15. US Food and Drug Administration. ABECMA. *FDA* https://www.fda.gov/vaccines-blood-biologics/abecma-idecabtagene-vicleucel (2021).
16. US Food and Drug Administration. CARVYKTI. *FDA* https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/carvykti (2022).
17. US Food and Drug Administration. AUCATZYL. *FDA* https://www.fda.gov/vaccines-blood-biologics/aucatzyl (2024).
18. Joy, R., Phair, K., O'Hara, R. & Brady, D. Recent advances and current challenges in CAR-T cell therapy. *Biotechnol. Lett.* **46**, 115–126 (2024).
19. Lonez, C. & Breman, E. Allogeneic CAR-T therapy technologies: has the promise been met? *Cells* **13**, 146 (2024).
20. Wang, X. et al. Allogeneic CD19-targeted CAR-T therapy in patients with severe myositis and systemic sclerosis. *Cell* **187**, 4890–4904.e9 (2024).
21. Mougakiakos, D. Allogeneic CAR T cells for autoimmune diseases: a glimpse into the future. *Signal. Transduct. Target. Ther.* **9**, 276 (2024).
22. Aghajanian, H. et al. Targeting cardiac fibrosis with engineered T cells. *Nature* **573**, 430–433 (2019).
This work is the first preclinical proof of concept with CAR-T cells in a fibrotic disease model.
23. Kansal, R. et al. Sustained B cell depletion by CD19-targeted CAR T cells is a highly effective treatment for murine lupus. *Sci. Transl. Med.* **11**, eaav1648 (2019).
This work is the first preclinical proof of concept with CAR-T cells in an autoimmune disease model.
24. Müller, F. et al. CD19 CAR T-cell therapy in autoimmune disease—a case series with follow up. *N. Engl. J. Med.* **390**, 687–700 (2024).
25. Mougiakakos, D. et al. CD19-targeted CAR T cells in refractory systemic lupus erythematosus. *N. Engl. J. Med.* **385**, 567–569 (2021).
This work is the first clinical proof of concept with CAR-T cells in patients with autoimmune disease.
26. Müller, F. et al. CD19-targeted CAR T cells in refractory antisynthetase syndrome. *Lancet* **401**, 815–818 (2023).
27. Haghikia, A. et al. Anti-CD19 CAR T cells for refractory myasthenia gravis. *Lancet Neurol.* **22**, 1104–1105 (2023).
28. Pecher, A. C. et al. CD19-targeting CAR T cells for myositis and interstitial lung disease associated with antisynthetase syndrome. *JAMA* **329**, 2154–2162 (2023).
29. Zhang, W. et al. Treatment of systemic lupus erythematosus using BCMA-CD19 compound CAR. *Stem Cell Rev. Rep.* **17**, 2120–2123 (2021).
30. Granit, V. et al. Safety and clinical activity of autologous RNA chimeric antigen receptor T-cell therapy in myasthenia gravis (MG-001): a prospective, multicentre, open-label, non-randomised phase 1b/2a study. *Lancet Neurol.* **22**, 578–590 (2023).
31. Fischbach, F. et al. CD19-targeted chimeric antigen receptor T cell therapy in two patients with multiple sclerosis. *Med* **5**, 550–558.e2 (2024).
32. Oliver-Caldes, A. First report of CART treatment in AL amyloidosis and relapsed/refractory multiple myeloma. *J. Immunother. Cancer* **9**, e003783 (2021).
33. Korell, F. et al. First third-generation CAR T cell application targeting CD19 for the treatment of systemic IgM AL amyloidosis with underlying marginal zone lymphoma. *Biomark. Res.* **14**, 11–91 (2023).
34. Qin, C. et al. Anti-BCMA CAR T-cell therapy CT103A in relapsed or refractory AQP4-IgG seropositive neuromyelitis optica spectrum disorders: phase 1 trial interim results. *Signal. Transduct. Target. Ther.* **8**, 5 (2023).
35. Tobias, A. & Grego, A. Hematopoietic stem cell transplantation and cellular therapies for autoimmune diseases: overview and future considerations from the Autoimmune Diseases Working Party (ADWP) of the European Society for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant.* **57**, 1055–1062 (2022).
36. Muraro, P. A. et al. Autologous haematopoietic stem cell transplantation for treatment of multiple sclerosis. *Nat. Rev. Neurol.* **13**, 391–405 (2017).
This review discusses clinical evidence of immune reset by a radical procedure — high-dose chemotherapy followed by rescue with autologous stem cell transplantation — thus supporting similar approaches with the aim to elicit durable, drug-free responses in autoimmunity.
37. Brudno, J. N. & Kochenderfer, J. N. Recent advances in CAR T-cell toxicity: mechanisms, manifestations and management. *Blood Rev. Mar.* **34**, 45–55 (2018).
38. Lee, D. W. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol. Blood Marrow Transplant.* **25**, 625–638 (2019).
39. Santomasso, B. D. et al. Management of immune-related adverse events in patients treated with chimeric antigen receptor T-cell therapy: ASCO guideline. *J. Clin. Oncol.* **39**, 3978–3992 (2021).
40. Hines, M. R. et al. Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome. *Transplant. Cell. Ther.* **29**, 438.e1–438.e16 (2023).
41. Mikhael, J., Fowler, J. & Shah, N. Chimeric antigen receptor T-cell therapies: barriers and solutions to access. *JCO Oncol. Pract.* **18**, 800–807 (2022).
This review describes major hurdles to successfully deploying ex vivo engineered CAR-T cells.
42. Sterner, R. C. & Sterner, R. M. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J.* **11**, 69 (2021).
43. Borgert, R. Improving outcomes and mitigating costs associated with CAR T-cell therapy. *Am. J. Manag. Care* **27**, S253–S261 (2021).
44. Lickefett, B. Lymphodepletion—an essential but undervalued part of the chimeric antigen receptor T-cell therapy cycle. *Front. Immunol.* **14**, 1303935 (2023).
45. Bot, A. et al. Cyclophosphamide and fludarabine conditioning chemotherapy induces a key homeostatic cytokine profile in patients prior to CAR T cell therapy. *Blood* **126**, 4426 (2015).
46. Warkentin, P. I. Voluntary accreditation of cellular therapies: foundation for the accreditation of cellular therapy (FACT). *Cytotherapy* **5**, 299–305 (2003).
47. Locke, F. L. et al. Tumor burden, inflammation, and product attributes determine outcomes of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv.* **4**, 4898–4911 (2020).
48. Scholler, N. et al. Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. *Nat. Med.* **28**, 1872–1882 (2022).
49. Locke, F. L. et al. Impact of tumor microenvironment on efficacy of anti-CD19 CAR T cell therapy or chemotherapy and transplant in large B cell lymphoma. *Nat. Med.* **30**, 507–518 (2024).
50. Sotillo, E. et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. *Cancer Discov.* **5**, 1282–1295 (2015).
51. Plaks, V. et al. CD19 target evasion as a mechanism of relapse in large B-cell lymphoma treated with axicabtagene ciloleucel. *Blood* **138**, 1081–1085 (2021).
52. Da Via, M. C. et al. Homozygous BCMA gene deletion in response to anti-BCMA CAR T cells in a patient with multiple myeloma. *Nat. Med.* **27**, 616–619 (2021).
53. Smith, T. T. et al. In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriers. *Nat. Nanotechnol.* **12**, 813–820 (2017).
This work is the first preclinical report of in vivo cell engineering with non-viral payload delivered via a nanoformulation.

54. Pfeiffer, A. et al. In vivo generation of human CD19-CAR T cells results in B-cell depletion and signs of cytokine release syndrome. *EMBO Mol. Med.* **10**, e9158 (2018).
This work is the first preclinical report of in vivo CAR-T cell engineering utilizing a viral vector-based approach.
55. Zhou, Q. et al. T-cell receptor gene transfer exclusively to human CD8$^+$ cells enhances tumor cell killing. *Blood* **120**, 4334–4342 (2012).
56. Mullin, R. Gilead to acquire Kite Pharma for $11.9 billion. *Chem. Eng. N.* **95**, 15 (2017).
57. Themeli, M., Rivière, I. & Sadelain, M. New cell sources for T cell engineering and adoptive immunotherapy. *Cell Stem Cell* **16**, 357–366 (2015).
58. Jaeger, A. J. CAR-T in the courts. *Genet. Eng. Biotechnol. N.* **37**, 31–33 (2017).
59. Sanofi. Sanofi R&D Day. *sanofi.com* https://www.sanofi.com/assets/dotcom/content-app/events/investor-presentation/2023/r-and-d-day-2023/Presentation.pdf (2023).
60. AstraZeneca to acquire EsoBiotec to advance cell therapy ambition. *Astra Zeneca* (17 March 2025); https://www.astrazeneca.com/media-centre/press-releases/2025/astrazeneca-to-acquire-esobiotec.html.
61. Mullard, A. In vivo CAR T cells gain traction, with AbbVie's US$2.1 billion acquisition of Capstan therapeutics. *Nature* https://www.nature.com/articles/d41573-025-00131-w (2025).
62. Klebanoff, K. A., Khong, H. T., Antony, P. A., Palmer, D. C. & Restifo, N. P. Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. *Trends Immunol.* **26**, 111–117 (2005).
63. Anliker, B. et al. Specific gene transfer to neurons, endothelial cells and hematopoietic progenitors with lentiviral vectors. *Nat. Methods* **7**, 929–935 (2010).
64. Funke, S. et al. Targeted cell entry of lentiviral vectors. *Mol. Ther.* **16**, 1427–1436 (2008).
65. Bender, R. R. et al. Receptor-targeted Nipah virus glycoproteins improve cell-type selective gene delivery and reveal a preference for membrane-proximal cell attachment. *PLoS Pathog.* **12**, e1005641 (2016).
66. Charitidis, F. T. et al. CAR gene delivery by T-cell targeted lentiviral vectors is enhanced by rapamycin induced reduction of antiviral mechanisms. *Adv. Sci.* **10**, e2302992 (2023).
67. Dobson, C. S. et al. Antigen identification and high-throughput interaction mapping by reprogramming viral entry. *Nat. Methods* **19**, 449–460 (2022).
68. Strebinger, D. et al. Cell type-specific delivery by modular envelope design. *Nat. Commun.* **14**, 5141 (2023).
69. Frank, A. M. et al. CD8-specific designed ankyrin repeat proteins improve selective gene delivery into human and primate T lymphocytes. *Hum. Gene Ther.* **31**, 679–691 (2020).
70. Agarwal, S., Weidner, T., Thalheimer, F. B. & Buchholz, C. J. In vivo generated human CAR T cells eradicate tumor cells. *Oncoimmunology* **8**, e1671761 (2019).
71. Agarwal, S. et al. In vivo generation of CAR T cells selectively in human CD4$^+$ lymphocytes. *Mol. Ther.* **28**, 1783–1794 (2020).
72. Frank, A. M. et al. Combining T-cell-specific activation and in vivo gene delivery through CD3-targeted lentiviral vectors. *Blood Adv.* **4**, 5702–5715 (2020).
73. Huckaby, J. T. et al. Bispecific binder redirected lentiviral vector enables in vivo engineering of CAR-T cells. *J. Immunother. Cancer* **9**, e002737 (2021).
74. Braun, A. H., Frank, A. M., Ho, N. & Buchholz, C. J. Dasatinib is a potent enhancer for CAR T cell generation by CD3-targeted lentiviral vectors. *Mol. Ther. Methods Clin. Dev.* **28**, 90–98 (2022).
75. Kapitza, L. et al. CD62L as target receptor for specific gene delivery into less differentiated human T lymphocytes. *Front. Immunol.* **14**, 1183698 (2023).
76. Ho, N. et al. In vivo generation of CAR T cells in the presence of human myeloid cells. *Mol. Ther. Methods Clin. Dev.* **26**, 144–156 (2022).
77. Krug, A. et al. In vivo CAR T cell therapy against angioimmunoblastic T cell lymphoma. *J. Exp. Clin. Cancer Res.* **43**, 262 (2024).
78. Cunningham, J. et al. In vivo delivery of a CD20 CAR using a CD8-targeted fusosome in southern pig-tail macaques (*M. nemestrina*) results in B cell depletion. *Blood* **138**, 2769 (2021).
79. Mullard, A. In vivo CAR T cells move into clinical trials. *Nat. Rev. Drug. Discov.* **23**, 727–730 (2024).
This news feature reports the translation of in vivo CAR technology to the clinic.
80. Nicolai, C. J. et al. In vivo CAR T-cell generation in nonhuman primates using lentiviral vectors displaying a multidomain fusion ligand. *Blood* **144**, 977–987 (2024).
This work is the first peer-reviewed publication describing preclinical evidence of activity of in vivo CAR therapy in NHPs.
81. Andorko, J. I. et al. Targeted in vivo generation of CAR T and NK cells utilizing an engineered lentiviral vector platform. *Blood* **142**, 763 (2023).
82. Andorko, J. I. et al. Targeted in vivo delivery of genetic medicines utilizing an engineered lentiviral vector platform results in CAR T and NK cell generation. *Molecular Therapy* https://doi.org/10.1016/j.ymthe.2025.06.036 (2025).
83. Russell, R. M. et al. Rational design of a detargeted vesiculovirus fusogen to enable targeted in vivo gene delivery. In: *27th American Society of Gene & Cell Therapy (ASGCT) Annual Meeting* https://doi.org/10.1016/j.ymthe.2024.04.020 (2024).
84. Mullen, K. F. et al. In vivo generation of both CAR T cells and CAR NK cells using a CD7 targeted lentiviral vector. In: *27th American Society of Gene & Cell Therapy (ASGCT) Annual Meeting* https://doi.org/10.1016/j.ymthe.2024.04.020 (2024).
85. Grinstein, J. D. First patient dosed with in vivo gene therapy for CAR T. *Inside Precision Medicine* https://www.insideprecisionmedicine.com/topics/precision-medicine/first-patient-dosed-with-in-vivo-gene-therapy-for-car-t/ (2024).
86. Kite to acquire Interius BioTherapeutics to advance in vivo platform [press release]. *Kite* (21 August 2025); https://www.kitepharma.com/news/press-releases/2025/8/kite-to-acquire-interius-biotherapeutics-to-advance-in-vivo-platform.
87. Michels, K. R. et al. UB-VV100, a novel platform for in vivo engineering of therapeutic anti-CD19 CAR T cells, shows effective T cell transduction, B cell depletion, and tumor control in a humanized murine model. *Umoja Biopharma* https://www.umoja-biopharma.com/wp-content/uploads/2021/05/Umoja-Biopharma-ASGCT-FINAL.pdf (2021).
88. Nicolai, C. et al. VivoVec: a novel lentiviral-based in vivo CAR T cell generation platform with viral particle surface engineering incorporating T cell activating and co-stimulatory ligands. *Umoja Biopharma* https://www.umoja-biopharma.com/wp-content/uploads/2022/11/CN-ASGCT-Poster-Umoja1.pdf (2022).
89. Michels, K. R. et al. Preclinical proof of concept for VivoVec, a lentiviral-based platform for in vivo CAR T-cell engineering. *J. Immunother. Cancer 2023* **11**, e006292 (2023).
90. AbbVie and Umoja Biopharma announce strategic collaboration to develop novel in-situ CAR-T cell therapies. *Umoja Biopharma* https://www.umoja-biopharma.com/news/abbvie-and-umoja-biopharma-announce-strategic-collaboration-to-develop-novel-in-situ-car-t-cell-therapies/ (2024).
91. Beitz, L. et al. In vivo generation of B cell depleting CAR T cell therapies for treatment of autoimmune diseases. In: *ACR Convergence Annual Meeting* https://acrabstracts.org/abstract/in-vivo-generation-of-b-cell-depleting-car-t-cell-therapies-for-treatment-of-autoimmune-diseases/ (2024).
92. Teoh, J. et al. Development of a surface engineered lentiviral vector for in vivo generation of CD22-directed CAR T cells. In: *SITC Annual Meeting* https://jitc.bmj.com/content/12/Suppl_2/A1267.info (2024).
93. Friesen, T. et al. Preclinical development of UB-VV400, a surface engineered lentiviral vector for the in vivo generation of CD22-directed CAR T cells. In: *ASGCT Annual Meeting* Abstract 795 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
94. IASO BIO announces new development partnership with umoja biopharma to develop ex vivo and in vivo cell and gene therapies. *Umoja Biopharma* https://www.umoja-biopharma.com/news/iaso-bio-announces-new-development-partnership-with-umoja-biopharma-to-develop-ex-vivo-and-in-vivo-cell-and-gene-therapies/ (2024).
95. Chen, X.-F. et al. In-vivo CAR-T cells produced by specific virus in refractory B cell lymphoma. In: *SITC Annual Meeting* https://doi.org/10.1136/jitc-2024-SITC2024.1482 (2024).
96. Chen, X. et al. Lentiviral particle-mediated generation of in-vivo CAR-T cells for treating refractory B-cell lymphoma. In: *ISCT Annual Meeting* https://www.isct-cytotherapy.org/article/S1465-3249%2825%2900518-3/abstract (2025).
97. Galvis, L. et al. ENaBL, a novel lentiviral vector platform for in vivo engineering of therapeutic anti-BCMA CAR T cells, shows effective T cell transduction and tumor control in a humanized murine model. In: *ASGCT Annual Meeting* Abstract 793 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
98. Weerens, L. et al. Development of novel T cell-specific synthetic promoters for safer and more effective in vivo CAR-T cell therapies. In: *ASGCT Annual Meeting* Abstract 868 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
99. Doix, B. et al. Engineering T cell-specific lentiviral vectors for safe and effective in vivo CAR-T therapy. In: *ASGCT Annual Meeting* Abstract 879 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
100. EsoBiotec doses first patient in investigator initiated trial of in vivo BCMA CAR-T candidate ESO-T01 for multiple myeloma [press release]. *EsoBiotec* https://www.esobiotec.com/esobiotec-doses-first-patient-in-investigator-initiated-trial-of-in-vivo-bcma-car-t-candidate-eso-t01-for-multiple-myeloma (2025).
101. Xu, J. et al. In-vivo B-cell maturation antigen CAR T-cell therapy for relapsed or refractory multiple myeloma. *Lancet* **406**, 228–231 (2025).
This is the first clinical proof of concept published in a peer-reviewed journal describing the efficacy and safety of an in vivo CAR technology.
102. Contrastano, S. G. et al. Potent in vivo transduction by iGPS particles generates CAR T cells with durable anti-tumor activity. *Kelonia* https://keloniatx.com/wp-content/uploads/2023/05/2023-ASGCT-Kelonia-Presentation.vFINAL.pdf (2023).
103. Beura, E. T. et al. T cell-specific in vivo transduction with preclinical candidate KLN-1010 generates BCMA directed CAR T cells with potent anti-multiple myeloma activity. *Cancer Res.* https://doi.org/10.1158/1538-7445.AM2024-48 (2024).
104. Bender, R. R. et al. Receptor-targeted Nipah virus glycoproteins improve cell-type selective gene delivery and reveal a preference for membrane-proximal cell attachment. *PLoS Pathog.* **12**, 1–28 (2016).
105. Nakamura, T. et al. Rescue and propagation of fully retargeted oncolytic measles viruses. *Nat. Biotech.* **23**, 209–214 (2005).
106. Cordes, N. et al. Anti-CD19 CARs displayed at the surface of lentiviral vector particles promote transduction of target-expressing cells. *Mol. Ther. Methods Clin. Dev.* **21**, 42–53 (2021).
107. Ruella, M. et al. Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. *Nat. Med.* **24**, 1499–1503 (2018).
108. Amatya, S. et al. Enabling potent T cell delivery and mitigating key off-target concerns of in vivo CAR T lentiviral vectors with the targeted paramyxovirus fusogen system. *Blood* **144**, 2047 (2024).
109. Cruite, P. et al. Retargeted "fusosomes" for in vivo delivery to T cells. *Mol. Ther.* **30**, 1081 (2022).
110. Chavan, H. et al. In vitro and in vivo specificity and biodistribution of a novel CD8-targeted fusosome. *Blood* **142**, 3630 (2023).
111. Trudeau, K. et al. Advancements in manufacturing of CD8- targeted fusosomes enhance transduction of resting T cells in vitro and in vivo. *Mol. Ther.* **31**, 760 (2023).

112. Chokshi, C. et al. In vivo engineering of hematopoietic stem cells with virus-like particles to generate multi-lineage CAR immune cell therapy for cancer. In: *ASGCT annual meeting* Abstract 1783 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
113. Vyriad announces strategic collaboration with Novartis to develop in vivo CAR-T cell therapies [press release]. *PR Newswire* https://www.prnewswire.com/news-releases/vyriad-announces-strategic-collaboration-with-novartis-to-develop-in-vivo-car-t-cell-therapies-302310717.html (2024).
114. In vivo delivery of CD3-directed CD19-CAR lentivectors leads to the generation of CAR-T and NK-like (CAR-TaNK) cells capable of complete ablation of B cells in the blood, bone marrow, and tissue in NSG-SGM3 CD34$^+$ humanized mice. In: *AACR Annual Meeting* https://exumabio.com/wp-content/uploads/2023/04/AACR-Poster-2023-Final-2.pdf (2023).
115. Coillard, A. et al. A novel polymeric gene delivery platform to precisely target and reprogram cells in vivo for haematological malignancies & beyond. In: *ASGCT Annual Meeting* Abstract 1213 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
116. Jaudoin, C. et al. Lentiviral vector engineering with polymer surface modification for car-T cell therapy. In: *ASGCT Annual Meeting* Abstract 1214 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
117. Parayath, N. N., Stephan, S. B., Koehne, A. L., Nelson, P. S. & Stephan, M. T. In vitro-transcribed antigen receptor mRNA nanocarriers for transient expression in circulating T cells in vivo. *Nat. Commun.* **11**, 6080 (2020).
This seminal preclinical study demonstrates in vivo CAR engineering using mRNA formulated in nanocarriers.
118. Cullis, P. R. & Hope, M. G. Lipid nanoparticle systems for enabling gene therapies. *Mol. Ther.* **25**, 1467–1475 (2017).
This review describes the discovery and development of current-generation LNPs comprising ionizable lipids and facilitating the design of first-in-class RNA nanomedicines.
119. Kularatne, R. N. The future of tissue-targeted lipid nanoparticle-mediated nucleic acid delivery. *Pharmaceuticals* **15**, 897 (2022).
120. Park, M. D., Silvin, A., Ginhoux, F. & Merad, M. Macrophages in health and disease. *Cell* **185**, 4259–4279 (2022).
121. Wang, Y. et al. In vivo programming of myeloid cells by mRNA mediated delivery of novel FcA fusion receptor activates anti-tumor immunity. *J. Immunother. Cancer* https://doi.org/10.1136/jitc-2022-SITC2022.1237 (2022).
122. Myeloid Therapeutics enters into agreement with Acuitas Therapeutics for lipid nanoparticle (LNP) system, enabling the first-ever delivery of mRNA-encoded CARs directly to humans [press release]. *Myeloid* https://myeloidtx.com/myeloid-therapeutics-enters-into-agreement-with-acuitas-therapeutics-for-lipid-nanoparticle-system/ (2022).
123. Argueta, S. et al. In vivo programmed myeloid cells expressing novel chimeric antigen receptors show potent anti-tumor activity in preclinical solid tumor models. *Front. Immunol.* https://doi.org/10.3389/fimmu.2024.1501365 (2024).
124. Argueta, S. et al. Preclinical development of MT-303, a novel LNP-formulated GPC3-specific CAR mRNA, for in vivo programming of monocytes to treat hepatocellular carcinoma. *J. Immunother. Cancer* https://doi.org/10.1136/jitc-2024-SITC2024.1125 (2024).
125. Myeloid Therapeutics initiates patient dosing with MT-302, a novel TROP2-targeting RNA CAR, in phase 1 study for advanced or metastatic epithelial tumors [press release]. *Myeloid* https://myeloidtx.com/myeloid-therapeutics-initiates-patient-dosing-with-mt-302-a-novel-trop2-targeting-rna-car-in-phase-1-study-for-advanced-or-metastatic-epithelial-tumors/ (2023).
126. Myeloid Therapeutics initiates patient dosing with MT-303, a novel GPC3 targeting RNA CAR, in phase 1 study for advanced hepatocellular carcinoma (HCC) [press release]. *Myeloid* https://myeloidtx.com/myeloid-therapeutics-initiates-patient-dosing-with-mt-303-a-novel-gpc3-targeting-rna-car-in-phase-1-study-for-advanced-hepatocellular-carcinoma-hcc/ (2024).
127. Myeloid therapeutics to present at 43rd Annual JP Morgan Healthcare Conference [press release]. *Myeloid* https://myeloidtx.com/myeloid-therapeutics-to-present-at-43rd-annual-jp-morgan-healthcare-conference/ (2025).
128. Wang, Y. et al. Novel innate immune receptor-based chimeric antigen receptors for in vivo programming of myeloid cells show potent anti-tumor activity in preclinical solid tumor models. In: *ASGCT Annual Meeting* Abstract 1727 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
129. Parhiz, H., Atochina-Vasserman, E. N. & Weissman, D. mRNA-based therapeutics: looking beyond COVID-19 vaccines. *Lancet* **403**, 1192–1204 (2024).
130. Karikó, K. et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Molecular Therapy* **16**, 1833–1840 (2008).
This report describes the seminal discovery that incorporation of synthetic nucleotides enables the utilization of RNA.
131. Cullis, P. R. & Felgner, P. L. The 60-year evolution of lipid nanoparticles for nucleic acid delivery. *Nat. Rev. Drug. Discov.* **23**, 709–722 (2024).
132. Rurik, J. G. CAR T cells produced in vivo to treat cardiac injury. *Science* **375**, 91–96 (2022).
This is the first preclinical report of in vivo CAR-T cell therapy in a non-oncologic indication.
133. Hunter et al. A novel product candidate (CPTX2309) for in vivo mRNA engineering of anti-CD19 CAR T cells utilizing novel CD8-targeted lipid nanoparticles. In: *American College of Rheumatology Annual Meeting* (2024).
134. Adams, G. et al. In vivo engineering of CAR T cells using a novel targeted LNP-mRNA technology. *J. Immunother. Cancer* https://doi.org/10.1136/jitc-2023-SITC2023.1202 (2023).
135. Zhang, Y. et al. Effective engineering of CD8$^+$ T cells from autoimmune disease patients utilizing a CD8-targeted lipid nanoparticle encoding an anti-CD19 CAR mRNA(CPTX2309). In: *American College of Rheumatology Annual Meeting* (2024).
136. Franz, A. et al. Profound B Cell depletion and repopulation with predominantly naïve B cells in non-human primates achieved through a novel in vivo CD8-targeted lipid nanoparticle mRNA CAR. In: *American College of Rheumatology Annual Meeting* (2024).
137. Hunter, T. et al. In vivo CAR T cell generation for cancer and autoimmune disease. *Science* **388**, 1311–1317 (2025).
This work is the first preclinical report in NHPs demonstrating proof of concept with respect to in vivo CAR-T cell generation utilizing a non-viral platform.
138. Aghajanian, H. et al. A two-infusion regimen with a novel in vivo non-viral chimeric antigen receptor (CAR) achieves up to 90% CD8$^+$ T cell engineering and tissue depletion of target cells in non-human primates (NHPs). In: *ASGCT Annual Meeting* Abstract 258 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
139. Breda, L. et al. In vivo hematopoietic stem cell modification by mRNA delivery. *Science* **381**, 436–443 (2023).
140. Immorna Biotherapeutics announces first clinical administration of JCXH-213, a breakthrough mRNA-based in vivo CAR therapy [press release]. *Pipeline Review* https://pipelinereview.com/immorna-biotherapeutics-announces-first-clinical-administration-of-jcxh-213-a-breakthrough-mrna-based-in-vivo-car-therapy/ (2025).
141. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/study/NCT06801119 (2025).
142. Xiao, T. et al. An vivo CAR T producer (HN2301) for autoimmune disease enables B cell depletion in NHP tissues and performs therapeutic effect in an SLE mouse model. In: *ASGCT Annual Meeting* Abstract 794 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
143. Wang, Q. et al. In vivo CD19 CAR T-cell therapy for refractory systemic lupus erythematosus. *N. Engl. J. Med.* https://doi.org/10.1056/NEJMc2509522 (2025).
144. Wesselhoeft, R. A., Kowalski, P. S. & Anderson, D. G. Engineering circular RNA for potent and stable translation in eukaryotic cells. *Nat. Commun.* **9**, 2629 (2018).
145. Wesselhoeft, R. A. et al. RNA circularization diminishes immunogenicity and can extend translation duration in vivo. *Mol. Cell* **74**, 508–520.e4 (2019).
146. Becker, A. M. et al. In situ CAR therapy using oRNATM lipid nanoparticles regresses tumors in mice. In: *SITC Annual Meeting* https://www.ornatx.com/wp-content/uploads/2022/11/Orna_SITC-2022.pdf (2022).
147. Orna Therapeutics announces strategic acquisition of ReNAgade therapeutics to solidify leadership in circular RNA therapies. *Orna* https://www.ornatx.com/orna-therapeutics-announces-strategic-acquisition-of-renagade-therapeutics-to-solidify-leadership-in-circular-rna-therapies/ (2024).
148. Crabtree, J. et al. In vivo panCAR-mediated depletion of B cells in non-human primates using a circular (oRNA®) anti-CD20 CAR. In: *ASH Annual Meeting* https://ash.confex.com/ash/2024/webprogram/Paper210256.html (2024).
149. Beaulieu, A. et al. In vivo transient programming of T cells with Sail's targeted nanoparticles encapsulating eRNATM-encoded hCD19 CAR achieves deep depletion of B cells in blood and lymphoid tissues in a B-cell repopulating humanized mouse model. In: *ASGCT Annual Meeting* Abstract 774 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
150. Sharma, K. et al. Long-acting circular RNA CAR drives potent and durable tumor elimination invivo. In: *ASGCT Annual Meeting* Abstract 781 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
151. Pan, X. et al. In vivo generation of anti-CD19 CAR T cells utilizing circular RNA encapsulated in targeted lipid nanoparticles. In: *ASGCT Annual Meeting* Abstract 1762 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
152. Lemgart, V. et al. In vitro and in vivo activity of a lipid nanoparticle system for the in vivo generation of CAR T cells. In: *ASH Annual Meeting* https://doi.org/10.1182/blood-2024-207566 (2024).
153. Kamoun, W. S. et al. Antitumour activity and tolerability of an EphA2-targeted nanotherapeutic in multiple mouse models. *Nat. Biomed. Eng.* **3**, 264–280 (2019).
154. Lemgart, V. et al. CD19 CAR T cells generated in vivo by T cell targeted lipid nanoparticles demonstrate robust and durable B cell depletion in non-human primates. In: *ASGCT Annual Meeting* Abstract 260 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
155. Klichinsky, M. et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat. Biotechnol.* **38**, 947–953 (2020).
156. Pierini, S. et al. Chimeric antigen receptor macrophages (CAR-M) sensitize solid tumors to anti-PD1 immunotherapy. *J. Immunother. Cancer* https://doi.org/10.1136/jitc-2022-SITC2022.0371 (2022).
157. Gabitova, L. et al. Pre-clinical development of a CAR monocyte platform for cancer immunotherapy. *J. Immunother. Cancer* https://doi.org/10.1136/jitc-2022-SITC2022.0318 (2022).
158. Carisma Therapeutics corporate presentation November [corporate presentation]. *Carisma* https://ir.carismatx.com/static-files/42a456e9-9007-4574-8aaa-e7c8db3ac852 (2024).
159. Abdou, Y. et al. PO2-04-08 translational insights from a phase 1, first-in-human (FIH) clinical trial of the anti-HER2 CAR macrophage CT-0508 in participants with HER2 positive metastatic breast cancer and other HER2 overexpressing solid tumors. In: *SABCS* (2023).

160. Moderna and Carisma establish collaboration to develop in vivo engineered chimeric antigen receptor monocytes (CAR-M) for oncology [press release]. *Mpderna* https://investors.modernatx.com/news/news-details/2022/Moderna-and-Carisma-Establish-Collaboration-to-Develop-in-vivo-Engineered-Chimeric-Antigen-Receptor-Monocytes-CAR-M-for-Oncology/default.aspx (2022).
161. Varghese, B. et al. In vivo CAR-M: redirecting endogenous myeloid cells with mRNA for cancer immunotherapy. *J. Immunother. Cancer* https://doi.org/10.1136/jitc-2023-SITC2023.1514 (2023).
162. Bagashev, A. et al. Pre-clinical efficacy of a novel anti-GPC3 in vivo CAR-M for hepatocellular carcinoma. *J. Immunother. Cancer* https://doi.org/10.1136/jitc-2024-SITC2024.0329 (2024).
163. Carisma and Moderna expand collaboration to develop two in vivo CAR-M therapies for autoimmune diseases [press release]. *Carisma* https://ir.carismatx.com/news-releases/news-release-details/carisma-and-moderna-expand-collaboration-develop-two-vivo-car-m (2024).
164. Carisma Therapeutics provides corporate updates [press release]. *Carisma* https://ir.carismatx.com/news-releases/news-release-details/carisma-therapeutics-provides-corporate-updates#:~:Text=The%20Company%20will%20assess%20a,value%20of%20all%20our%20assets (2025).
165. Yan, F. et al. Improved T cell delivery and expression of mRNA using CD8-targeted lipid nanoparticles with Athebody® designed ankyrin repeat proteins (DARPins). In: *ASGCT Annual Meeting* Abstract 667 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
166. Fang, J. et al. Mobilize LNPs: a novel platform for in vivo targeted delivery of RNA therapeutics to T cells. In: *ASGCT Annual Meeting* Abstract 483 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
167. Hashiba, K. et al. Optimizing VHH ligand density in T cell-targeted LNPs: an inverted U-shaped relationship for effective in vivo CAR T cell generation. In: *ASGCT Annual Meeting* Abstract 665 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
168. Khairallah, C. et al. In: *ASGCT Annual Meeting* Abstract 770 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
169. Sun, J. et al. In vivo CAR-T therapy powered by novel CLAMP technology for T cell targeted mRNA delivery. In: *ASGCT Annual Meeting* Abstract 1282 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
170. Magee, M. S. et al. Deploying an RNA-based gene writer system and lipid nanoparticle (LNP) delivery to generate functional chimeric antigen receptor (CAR) T cells with in vitro and in vivo anti-tumor activity. *Blood* **142**, 2073 (2023).
171. Rodriguez, J. et al. Targeted lipid nanoparticle delivery of an RNA gene writer in vivo enables generation of CAR-T cells in a humanized mouse model. *Blood* **144**, 4799 (2024).
172. De Iaco, A. et al. In: *ASGCT Annual Meeting* Abstract 153 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
173. Monte, M. et al. In vivo RNA delivery to T cells and hematopoietic stem cells in humanized mice and non-human primates using targeting lipid nanoparticles. In: *ASGCT Annual Meeting* Abstract 668 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
174. Wang, Z. et al. Efficient and effective in vivo CAR T generation using recombinase-based, precise, high-capacity, off-the-shelf, LNP-based in vivo genomic engineering. In: *ASGCT Annual Meeting* Abstract 797 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
175. Petazzi, P. et al. Precise gene writing system for CAR-T cell therapy generation. In: *ASGCT Annual Meeting* Abstract 1140 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
176. Bimbo, J. et al. Novel non-viral DNA-based gene therapy vector for CAR T engineering in vivo. In: *ASGCT Annual Meeting* Abstract 213 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
177. Urits, I. et al. A review of patisiran (ONPATTRO®) for the treatment of polyneuropathy in people with hereditary transthyretin amyloidosis. *Neurol. Ther.* **9**, 301–315 (2020).
178. Patil, H. et al. CAR-T cell therapy in rheumatic diseases: a review article. *Clin Rheumatol*. https://doi.org/10.1007/s10067-025-07451-7 (2025).
179. Mueller, F. et al. Comparison of the safety profiles of CD19-targeting CAR T-cell therapy in patients with SLE and B-cell lymphoma. *Blood* https://doi.org/10.1182/blood.2025028375 (2025).
180. Pecher, A. C. et al. CD19-targeting CAR T cell therapy in five patients with systemic sclerosis unsuitable for autologous stem cell transplantation. *EULAR Rheumatology* https://doi.org/10.1016/j.ero.2025.03.005 (2025).
181. Hagen, M. et al. Local immune effector cell-associated toxicity syndrome in CAR T-cell treated patients with autoimmune disease: an observational study. *Lancet Rheumatol*. https://doi.org/10.1016/S2665-9913(25)00091-8 (2025).
182. Gust, J. BCMA-CAR T-cell treatment-associated parkinsonism. *Blood* **142,** 1181–1183 (2023).
183. Di Stasi, A. et al. Inducible apoptosis as a safety switch for adoptive cell therapy kills switches. *N. Engl. J. Med.* **365**, 1673–1683 (2011).
184. Hu, J. & Dunbar, C. E. T-cell lymphomas in recipients of CAR-T cells: assessing risks and causalities. *Blood* **144**, 2473–2481 (2024).
185. Hamilton, M. P. et al. Risk of second tumors and T-cell lymphoma after CAR T-cell therapy. *N. Engl. J. Med.* **390**, 2047–2060 (2024).
186. Ruella, M. & June, C. H. CAR T-cell resistance to oncogenic transformation. *Blood Cancer Discov.* **5**, 229–233 (2024).
187. Fraietta, J. A. et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T-cells. *Nature* **558**, 307–312 (2018).
188. US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry: long term follow-up after administration of human gene therapy products. *FDA* https://www.fda.gov/media/113768/download (2020).
189. Szebeni, J., Simberg, D., González-Fernández, A., Barenholz, Y. & Dobrovolskaia, M. A. Roadmap and strategy for overcoming infusion reactions to nanomedicines. *Nat. Nanotechnol.* **13**, 1100–1108 (2018).
190. Uehata, T. & Takeuchi, O. RNA recognition and immunity—innate immune sensing and its posttranscriptional regulation mechanisms. *Cells* **9**, 1701 (2020).
191. Hosseini-Kharat, M., Bremmell, K. E. & Prestidge, C. A. Why do lipid nanoparticles target the liver? Understanding of biodistribution and liver-specific tropism. *Mol. Ther. Methods Clin. Dev.* https://doi.org/10.1016/j.omtm.2025.101436 (2025).
192. Szebeni, J. et al. Applying lessons learned from nanomedicines to understand rare hypersensitivity reactions to mRNA-based SARS-CoV-2 vaccines. *Nat. Nanotechnol.* **17**, 337–346 (2022).
193. FDA Center for Biologics Evaluation and Research (CBER) Office of Therapeutic Products (OTP). Guidance document: considerations for the development of chimeric antigen receptor (CAR) T cell products. *FDA* https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considerations-development-chimeric-antigen-receptor-car-t-cell-products (2024).
194. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Center for Devices and Radiological Health (CDRH), Office of Good Clinical Practice (OGCP), Office of Regulatory Affairs (ORA). Guidance for industry: oversight of clinical investigations—a risk-based approach to monitoring. *FDA* https://www.fda.gov/media/116754/download (2013).
195. Rejeski, K. et al. Immune effector cell-associated haematotoxicity after CAR T-cell therapy: from mechanism to management. *Lancet Haematol.* **11**, e459–e470 (2024).
196. Neelapu, S. S. et al. Axicabtagene ciloleucel as first-line therapy in high-risk large B-cell lymphoma: the phase 2 ZUMA-12 trial. *Nat. Med.* **28**, 735–742 (2022).
197. Swan, D., Madduri, D. & Hocking, J. CAR-T cell therapy in multiple myeloma: current status and future challenges. *Blood Cancer J.* **14**, 206 (2024).
198. Costa, L. J. MRD accelerating myeloma drug development. *Blood* **144**, 345–347 (2024).
199. Amor, C. et al. Senolytic CAR T cells reverse senescence-associated pathologies. *Nature* **583**, 127–132 (2020).
200. Fuchs, E. J. Transplantation tolerance: from theory to clinic. *Immunol. Rev.* **258**, 64–79 (2014).
201. Su, Y., Yuan, C. & Shi, M. Editorial: screening and verification of new targets for CAR-T immunotherapy in cancer. *Front. Immunol.* **14**, 1189773 (2023).
202. Lee, J. et al. Antigen-specific B cell depletion for precision therapy of mucosal pemphigus vulgaris. *J. Clin. Invest.* **130**, 6317–6324 (2020).
203. Reincke, S. M. et al. Chimeric autoantibody receptor T cells deplete NMDA receptor-specific B cells. *Cell* **186**, 5084–5097.e18 (2023).
204. Yang, J. et al. Dual-targeted CAR T-cell immunotherapies for hematological malignancies: latest updates from the 2023 ASH annual meeting. *Exp. Hematol. Oncol.* **13**, 25 (2024).
205. Ramishetti, S. et al. Systemic gene silencing in primary T lymphocytes using targeted lipid nanoparticles. *ACS Nano* **9**, 6706–6716 (2015).
This article provides evidence for the first time of in vivo delivery to a T cell subset using targeted nanomedicine.
206. Inamdar, V. V., Hao, S., Stephan, S. B. & Stephan, M. T. Biomaterial-based scaffolds for direct in situ programming of tumor-infiltrating T lymphocytes. *J. Control. Rel.* **370**, 310–317 (2024).
207. Nyberg, W. A. et al. An evolved AAV variant enables efficient genetic engineering of murine T cells. *Cell* **186**, 446–460.e19 (2023).
208. Hale, M. et al. Homology-directed recombination for enhanced engineering of chimeric antigen receptor T cells. *Mol. Ther. Methods Clin. Dev.* **4**, 192–203 (2017).
209. Demircan, M. B. et al. T-cell specific in vivo gene delivery with DART-AAVs targeted to CD8. *Mol. Ther.* **32**, 3470–3484 (2024).
210. Theuerkauf, S. A. et al. AAV vectors displaying bispecific DARPins enable dual-control targeted gene delivery. *Biomaterials* **303**, 122399 (2023).
211. Chen, H. et al. In vivo CAR-T cell therapy using modified AAV capsids targeting human T cells. In: *ASGCT Annual Meeting* Abstract 1739 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
212. Decker, C. et al. In vivo VLP-based engineering generates multi-lineage CAR-immune cells that mediate potent and durable anti-tumor activity. In: *SITC Annual Meeting* https://ensoma.com/wp-content/uploads/SITC-2024-HER2-poster-final.pdf (2024).
213. Tessera Therapeutics to present new data across preclinical programs for its gene writing™ and delivery platforms at the American Ssociety of Gene and Cell Therapy 28th Annual Meeting [press release]. *Tessera* https://www.tesseratherapeutics.com/news/tessera-therapeutics-to-present-new-data-across-preclinical-programs-for-its-gene-writing-and-delivery-platforms-at-the-american-society-of-gene-and-cell-therapy-28th-annual-meeting (2025).
214. Li, Y.-R., Zhu, Y., Halladay, T. & Yang, L. In vivo CAR engineering for immunotherapy. *Nat. Rev. Immunol.* https://doi.org/10.1038/s41577-025-01174-1 (2025).
215. Lu, L., Xie, M., Yang, B., Zhao, W. B. & Cao, J. Enhancing the safety of CAR-T cell therapy: synthetic genetic switch for spatiotemporal control. *Sci. Adv.* **10**, eadj6251 (2024).

216. Dannenfelser, R. et al. Discriminatory power of combinatorial antigen recognition in cancer T cell therapies. *Cell Syst.* **11**, 215–228.e5 (2020).
217. Parrett, B. J. et al. Reducing off-target expression of mRNA therapeutics and vaccines in the liver with microRNA binding sites. *Mol. Ther. Methods Clin. Dev.* **33**, 1101402 (2025).
218. Xue, L. et al. High-throughput barcoding of nanoparticles identifies cationic, degradable lipid-like materials for mRNA delivery to the lungs in female preclinical models. *Nat. Commun.* **15**, 1884 (2024).
219. Rhym, L. H. et al. Peptide-encoding mRNA barcodes for the high-throughput in vivo screening of libraries of lipid nanoparticles for mRNA delivery. *Nat. Biomed. Eng.* **7**, 901–910 (2023).
220. Da Silva Sanchez, A. J. et al. Universal barcoding predicts in vivo ApoE-independent lipid nanoparticle delivery. *Nano Lett.* **22**, 4822–4830 (2022).
221. Das, K. P. & Chandra, J. Nanoparticles and convergence of artificial intelligence for targeted drug delivery for cancer therapy: current progress and challenges. *Front. Med. Technol.* **4**, 1067144 (2023).
222. Zhou, J. et al. Lipid nanoparticles produce chimeric antigen receptor T cells with interleukin-6 knockdown in vivo. *J. Controlled Rel.* **350**, 298–307 (2022).
223. Kath, J. et al. PASTA: CRISPR knock-in paired with integrase for simple, precise, and efficient large transgene insertion in T cells. In: *ASGCT Annual Meeting* Abstract 649 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
224. Amabile, A. et al. Bispecific antibody targeting of lipid nanoparticles. Preprint at *bioRxiv* https://doi.org/10.1101/2024.12.20.629467 (2024).
225. Kim, M. et al. Development of in vivo CAR T cell therapy using nanoparticle and RNA-based gene delivery systems. In: *ASGCT Annual Meeting* Abstract 796 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
226. Divita, G. et al. In vivo mRNA CAR-T cell engineering utilizing a novel CD3/CD5 peptide based targeted nanoparticles. In: *ASGCT Annual Meeting* Abstract 1283 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
227. Lee, C. et al. In vivo CAR delivery as supercoiled DNA with lymphatic tropism polyasparagine nanoparticles. In: *ASGCT Annual Meeting* Abstract 699 https://www.sciencedirect.com/journal/molecular-therapy/vol/33/issue/4/suppl/S1 (2025).
228. Lian, H. et al. Cytokine therapy combined with nanomaterials participates in cancer immunotherapy. *Pharmaceutics* **14**, 2606 (2022).
229. Huang, C. et al. Lipid nanoparticle delivery system for mRNA encoding B7H3-redirected bispecific antibody displays potent antitumor effects on malignant tumors. *Adv. Sci.* https://doi.org/10.1002/advs.202205532 (2022).
230. Hamilton, A. G. et al. Ionizable lipid nanoparticles with integrated immune checkpoint inhibition for mRNA CAR T cell engineering. *Adv. Healthc. Mater.* **12**, e2301515 (2023).
231. Danko, I. & Wolff, J. A. Direct gene transfer into muscle. *Vaccine* **12**, 1499–1502 (1994).
232. University Of Pittsburgh Medical Center. University of Pittsburgh Medical Center performs first in vivo gene therapy for hemophilia A. *Science Daily* https://www.sciencedaily.com/releases/1999/06/990604081001.htm (1999).
233. Kuwana, Y. et al. Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived c regions. *Biochem. Biophys. Res. Commun.* **149**, 960–968 (1987).
This work is the first report of design and in vitro activity of a construct comparable with current CARs.
234. Gross, G., Waks, T. & Eshhar, Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc. Natl Acad. Sci. USA* **86**, 10024–10028 (1989).
235. Eshhar, Z., Waks, T., Gross, G. & Schindler, D. G. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the γ or ζ subunits of the immunoglobulin and T-cell receptors. *Proc. Natl Acad. Sci. USA* **90**, 720–724 (1993).
236. Finney, H. M., Lawson, A. D., Bebbington, C. R. & Weir, A. N. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *J. Immunol.* **161**, 2791–2797 (1998).
237. Maher, J., Brentjens, R. J., Gunset, G., Rivière, I. & Sadelain, M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ/CD28 receptor. *Nat. Biotechnol.* **20**, 70–75 (2002).
238. Kochenderfer, J. N. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood* **116**, 4099–4102 (2010).
This article reports the first evidence of objective clinical response afforded by CAR-T cell intervention in a patient with B cell lymphoma.
239. Porter, D. L., Levine, B. L., Kalos, M., Bagg, A. & June, C. J. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N. Engl. J. Med.* **365**, 725–733 (2011).
240. Umoja Biopharma announces FDA clearance of IND application for UB-VV111, a CD19 directed in situ CAR T for hematologic malignancies press release. *Umoja Biopharma* https://www.umoja-biopharma.com/news/umoja-biopharma-announces-fda-clearance-of-ind-application-for-ub-vv111-a-cd19-directed-in-situ-car-t-for-hematologic-malignancies/ (2024).
241. Papahadjopoulos, D., Vail, W. J., Jacobson, K. & Poste, G. Cochleate lipid cylinders: formation by fusion of unilamellar lipid vesicles. *Biochim. Biophys. Acta* **394**, 483–491 (1975).
242. Dimitriadis, G. J. Translation of rabbit globin mRNA introduced by liposomes into mouse lymphocytes. *Nature* **274**, 923–924 (1978).
243. Malone, R. W., Felgner, P. L. & Verma, I. M. Cationic liposome-mediated RNA transfection. *Proc. Natl Acad. Sci. USA* **86**, 6077–6081 (1989).
This seminal report describes the use of mRNA to directly transfect human, rat, mouse, *Xenopus* and *Drosophila* cells.
244. Conry, R. M. et al. Characterization of a messenger RNA polynucleotide vaccine vector. *Cancer Res.* **55**, 1397–1400 (1995).
245. Hafez, I. M., Ansell, S. & Cullis, P. R. Tunable pH-sensitive liposomes composed of mixtures of cationic and anionic lipids. *Biophysical J.* **79**, 1438–1446 (2000).
246. Karikó, K., Buckstein, M., Ni, H. & Weissman, D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* **23**, 165–175 (2005).
247. Barrett, D. M. et al. Treatment of advanced leukemia in mice with mRNA engineered T cells. *Hum. Gene Ther.* **22**, 1575–1586 (2011).
248. US Food and Drug Administration. COMIRNATY FDA approval. *FDA* https://www.fda.gov/media/151710/download?attachment (2021).
249. US Food and Drug Administration. SPIKEVAX FDA approval. *FDA* https://www.fda.gov/media/155815/download?attachment (2022).
250. Uslu, U. & June, C. H. Beyond the blood: expanding CAR T cell therapy to solid tumors. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-024-02446-2 (2024).
251. Aghajanian, H., Rurik, J. G. & Epstein, J. A. CAR-based therapies: opportunities for immuno-medicine beyond cancer. *Nat. Metab.* **4**, 163–169 (2022).
252. Wat, J. & Barmettler, S. Hypogammaglobulinemia after chimeric antigen receptor (CAR) T-cell therapy: characteristics, management, and future directions. *J. Allergy Clin. Immunol. Pract.* **10**, 460–466 (2022).

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Author contributions

J.I.A., A.B., A.S., K.F., L.G., R.H., M.K., F.N., J.V.S., A.J.S. and K.T. generated the sections 'Viral vectors' and 'RNA-based platforms'. A.B. conceived the article layout. M.T.S., C.J.B., D.W. and C.H.J. contributed to the Abstract, Introduction and Outlook. All authors reviewed and edited the manuscript before submission. C.J.B.'s contribution to the manuscript represents his own perspective and not the official view of the Paul-Erlich Institute.

Competing interests

J.I.A. is a paid employee of Interius BioTherapeutics, Inc., holds equity in the company, and is an inventor on patents issued and/or pending related to this work. A.B. is a shareholder and employee of Capstan Therapeutics, a company developing in vivo chimeric antigen receptor (CAR) therapies, and inventor or co-inventor on multiple relevant patents in the CAR and related fields. C.J.B. is a co-inventor on patents covering T cell-targeted lentiviral vectors. K.F. is a shareholder and member of the Board of Directors of Kelonia Therapeutics, and is also an inventor on patent applications assigned to Kelonia. L.G. is a shareholder and employee of Moderna, and a shareholder of Tessera. A.S. is a shareholder and employee of Umoja Therapeutics, a company developing in vivo CAR therapies. R.H. is a shareholder and employee of Myeloid Therapeutics, a company developing in vivo CAR therapies. C.H.J. is an inventor of multiple CAR patents and shareholder of several companies developing CAR products. M.K. is a founder, employee and shareholder of Carisma Therapeutics; a founder, shareholder and board director of Chymal Therapeutics; and an inventor of multiple patents related to CAR macrophages and monocytes (CAR-M) that have been licensed by Carisma Therapeutics. F.N. is a shareholder and employee of Orna Therapeutics, a company developing in vivo CAR therapies. A.J.S. is a shareholder and current employee of Sanofi. J.V.S. is an employee and shareholder in Mirai Bio, and a consultant and shareholder in Sana Biotechnology. M.T.S. is co-founder of and has received stock options from Persistence Therapeutics (Jupiter Bioventures); and has IP Licensing with Sanofi, Juno Therapeutics (now Bristol Myers Squibb) and Jupiter Bioventures. K.T. is a shareholder and employee of Sana Therapeutics, a company developing in vivo CAR and cell gene therapies. D.W. has filed patent applications based on some aspects of this work; those interests were fully disclosed to the University of Pennsylvania, with an approved plan in place for managing any potential conflicts arising from licensing of these patents.

Additional information

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