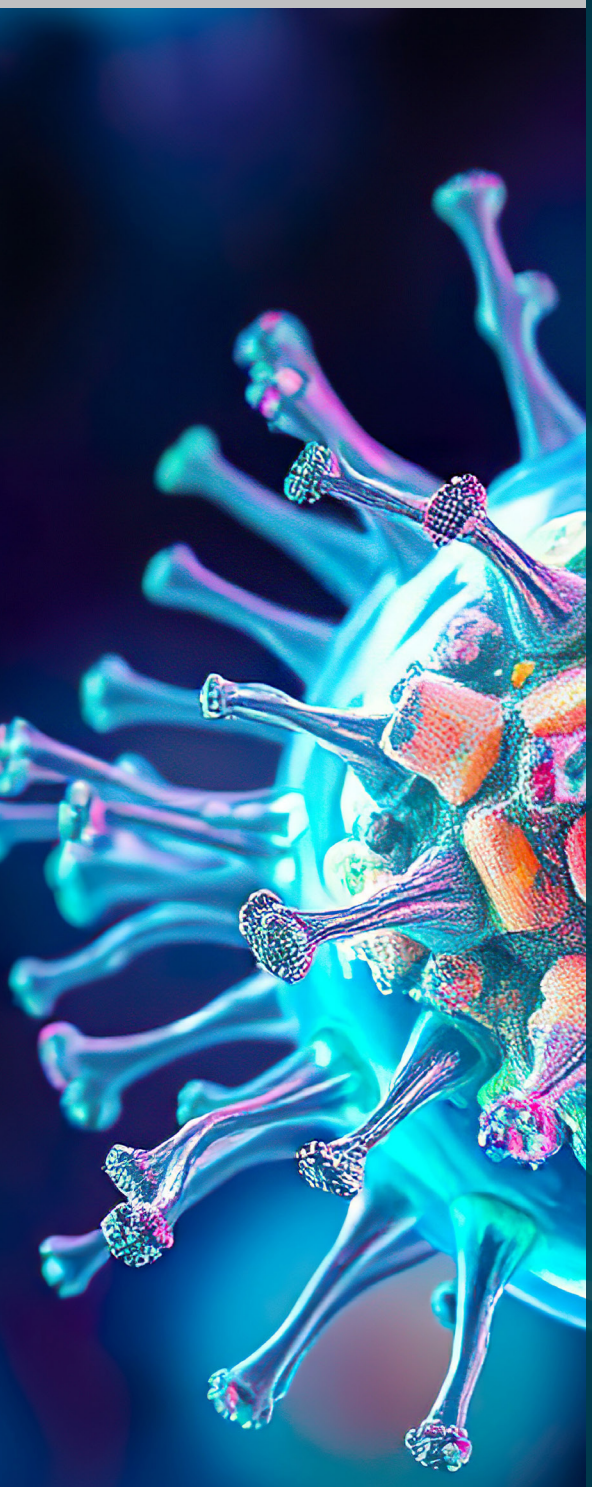
NOVOTECH™
Biotech's Partner at Every Phase



MAY 29, 2025

IN-VIVO CAR THERAPIES – GLOBAL RESEARCH AND DEVELOPMENT LANDSCAPE (2025)

novotech-cro.com

IN-VIVO CAR THERAPIES

GLOBAL CLINICAL TRIAL LANDSCAPE (2025)

#DYK Did you know?

- In-vivo CAR **assets surged post-2022**, crossed 35 in 2024
- **Early 2025:** 70+ disclosed assets; most in preclinical stages (75%); 5 therapies have entered clinical trials
- Assets projected to **exceed 100** by 2025
- **Broad Focus:** oncology (leukemia, lymphoma, solid tumors)
- **Autoimmune Interest Rising:** lupus, multiple sclerosis; 10+ assets in pipeline

2028
$2.5B
(CAR MARKET)

2032
2M
(PATIENTS)

Overcomes ex-vivo CAR-T limitations like **manufacturing delays** and **centralized treatment**

Enables **earlier intervention** with reduced toxicity potential

Expands patient access to community care

Simplifies administration, easing provider burden

Aligns with payer models via **cost-efficiency**

Fits within **existing supply chains** for distribution

In-vivo CARs **target TROP-2, GPC3, CD19, and CD20**

Delivered via **viral (lentivirus, AAV) and non-viral (LNPs, tLNPs, poly-mers)** systems

LNPs **lead among non-viral vectors,** building on mRNA vaccine tech

Simplified, scalable production may reduce cost and expand access vs. ex vivo CAR-T

CONTENTS

1. INTRODUCTION
2. CARS – TECHNOLOGICAL FEATURES AND EVOLUTION
3. GLOBAL RESEARCH AND DEVELOPMENT LANDSCAPE
4. EMERGING INNOVATIONS IN DELIVERY SYSTEMS FOR IN-VIVO CAR-T THERAPY
5. COMMERCIAL VIABILITY OF IN-VIVO CAR-T
6. CHALLENGES AND STRATEGIC FOCUS AREAS IN IN-VIVO CAR-T THERAPY
7. REGULATORY CONSIDERATIONS FOR ADVANCING IN-VIVO CAR-T THERAPY
8. BEYOND CAR-T AND BEYOND ONCOLOGY
9. FUTURE DIRECTIONS AND OUTLOOK
10. FEEDBACK FROM KEY OPINION LEADERS
11. CURRENT DISCUSSIONS AND OPINION ON IN-VIVO CAR-T

INTRODUCTION

Chimeric Antigen Receptor (CAR) T-cell therapy has transformed cancer treatment, offering remarkable efficacy for hematologic malignancies. However, conventional autologous and allogeneic CAR-T therapies face significant challenges, including high manufacturing costs, logistical complexities, and patient accessibility issues. The emergence of in-vivo CAR-T therapy aims to overcome these barriers by enabling the direct engineering of T-cells within the patient's body, eliminating the need for complex ex-vivo manufacturing.

Autologous CAR-T therapies, while highly personalized, require cell extraction, gene modification, and reinfusion—a time-consuming and costly process that limits patient access. Allogeneic, or "off-the-shelf," CAR-T therapies promise greater scalability but introduce risks such as graft-versus-host disease (GvHD), immune rejection, and T-cell exhaustion—as larger expansions (10^{11}–10^{12} cells) increase the risk of functional decline. Meanwhile, point-of-care (POC) manufacturing has attempted to decentralize production but remains constrained by infrastructure and regulatory hurdles.

In-vivo CAR-T therapy seeks to combine the durability of autologous therapy with the scalability of allogeneic approaches, potentially making CAR-T treatments safer, more affordable, and accessible beyond specialized centers. By using viral vectors, lipid nanoparticles (LNPs), and bioinstructive scaffolds, researchers are developing innovative methods to reprogram immune cells directly within the body, reducing manufacturing time and associated costs.

Several biotech companies are driving in-vivo CAR-T innovations with novel delivery platforms. For example, EXUMA Biotech utilizes GCAR lentiviral vectors, while Umoja Biopharma develops VivoVec viral particles for in-body CAR-T generation. Others like Vector BioPharma focuses on SHREAD (Shielded, Retargeted Adenovirus) adenoviral vectors, Ensoma on non-viral Engenious vectors, Capstan Therapeutics is exploring mRNA-based CAR-T therapy, with Interius BioTherapeutics advancing in-vivo CAR-T for blood cancers.

Adding to these developments, the year 2024 emerged as a landmark year for cell therapy, highlighted by the first-ever approvals of TIL and TCR cell therapies for treating solid tumors in the US. Additionally, CAR-T therapies have advanced to earlier lines of treatment, becoming approved as second- and third-line options for multiple myeloma in both the US and EU—signifying a major shift toward broader and earlier clinical adoption.

With multiple biotech companies and pharmaceutical giants now investing in-vivo CAR platforms, the field is poised for significant advancements. As clinical trials progress, this revolutionary approach could redefine cell therapy, expanding its reach beyond oncology into broader therapeutic areas. By addressing existing limitations, in-vivo CAR therapy holds the promise of transforming patient outcomes while making cellular immunotherapy more efficient, cost-effective, and widely accessible. [1,11]

This report delves into the evolving in-vivo CAR therapy landscape, examining key technological innovations, major industry players, recent clinical progress, and regulatory developments. Additionally, it explores the expanding role of in-vivo CAR beyond oncology, with potential applications in autoimmune diseases, fibrosis, and genetic disorders. By assessing emerging research and market trends, this paper provides valuable insights into how in-vivo CAR therapies could redefine the future of cell-based immunotherapy.

CARS – TECHNOLOGICAL FEATURES AND EVOLUTION

Overview of CAR Structure and Evolution

CARs are modular synthetic receptors composed of four key regions: extracellular domain, hinge region, transmembrane domain, and intracellular domain, each playing a crucial role in CAR-T cell function.

Extracellular Domai	Hinge Region & Transmembrane Domain	Intracellular Domain
Responsible for recognizing and binding cancer cells, typically derived from monoclonal antibodies as single-chain variable fragments (scFvs). It can also include modified ligands or peptides to enhance specificity and minimize off-target effects.	These provide flexibility and stability, ensuring proper receptor function and integration into T-cell membranes.	Triggers T-cell activation and proliferation, incorporating T-cell receptors such as CD3ζ, CD28, 4-1BB, or IL-2 signaling for enhanced cytotoxicity and persistence.

Figure 1: Structure of CAR-T cell

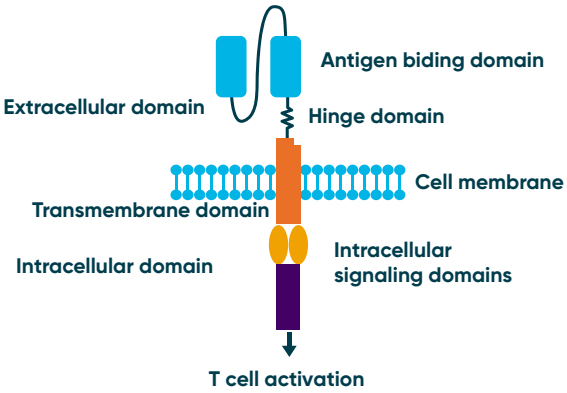


Image source: DOI:10.34172/ia.2022.06

Evolution of CAR-T Cell Therapy: Key Milestones from First TIL Treatment to Latest Applications

CAR-T therapy remains a groundbreaking immunotherapy, particularly for cancers. The representation below and Figure 2 summarize the key milestones of CAR-T development. These advancements have paved the way for the progressive evolution of CAR-T cell generations, each incorporating new features to enhance efficacy, persistence, and safety. [6] The section that follows further explores the key innovations and improvements across different CAR-T generations.

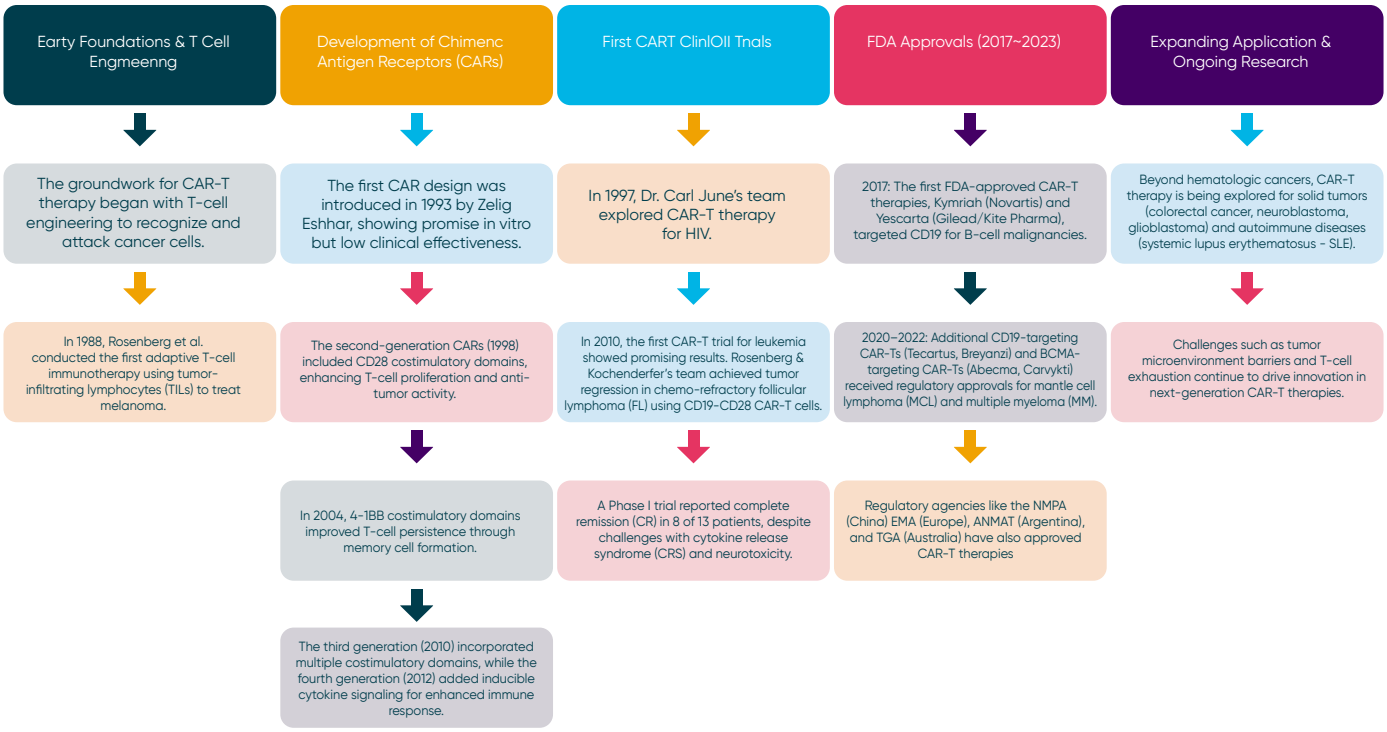
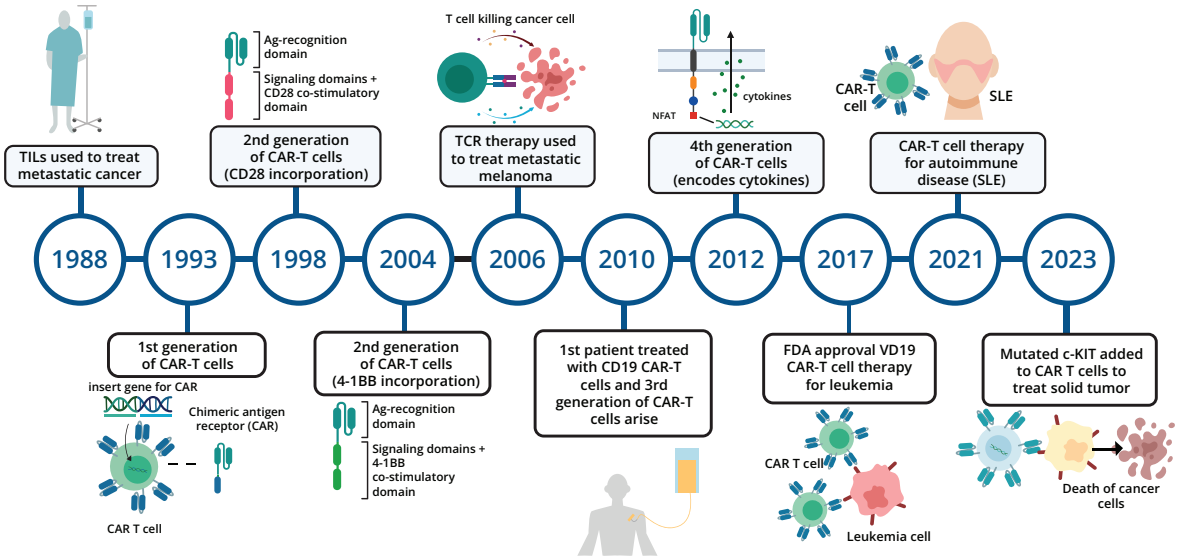


Figure 2: Key Milestones of CAR-T Cells



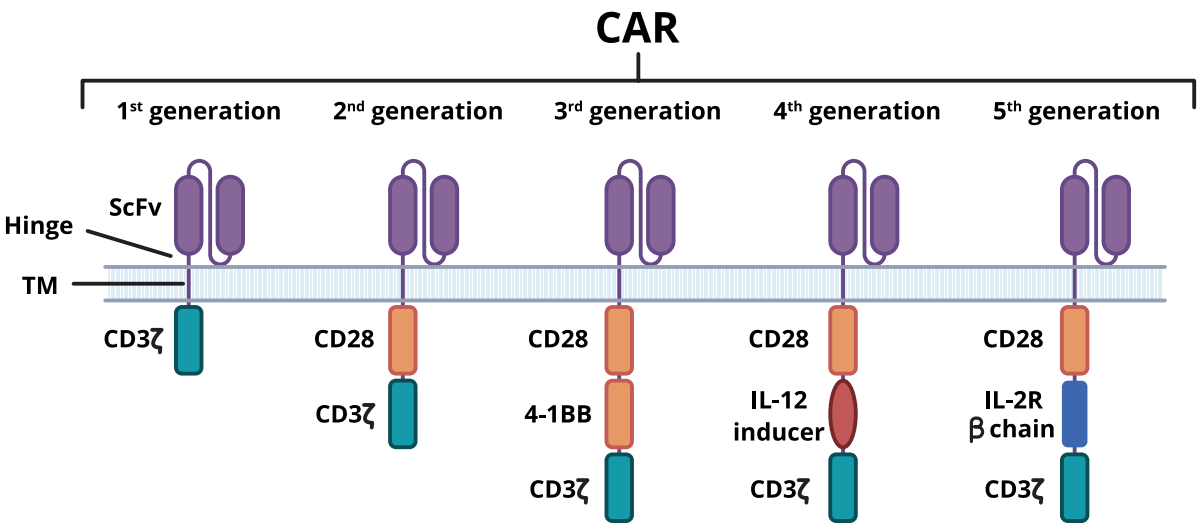
Source: Int. J. Mol. Sci. 2023, 24(21), 15688; https://doi.org/10.3390/ijms242115688

Evolution of CAR Generations

CARs have evolved through five generations (Figure 3.1), each with distinct anti-tumor capabilities driven by intracellular domain structures. CAR constructs across all five generations share the above-mentioned four key domains (extracellular (ScFv) for tumor antigen recognition, hinge, transmembrane (TM), and intracellular for signaling). Within the intracellular domains, the CD3ζ domain drives T-cell activation, proliferation, and cytotoxicity, while CD28 and 4-1BB enhance persistence and function. The IL-12 inducer promotes cytokine release in the tumor microenvironment, strengthening the immune response against cancer. The IL-2Rβ-chain mimics IL-2 signaling, enhancing CAR-T cell survival, proliferation, and long-term persistence, allowing for more controlled and sustained therapeutic effects.

- First Generation: Contains CD3ζ as the primary intracellular signaling domain, responsible for T-cell activation but with low persistence and efficacy.
- Second Generation: Introduces the CD28 co-stimulatory domain alongside CD3ζ, improving T-cell proliferation and persistence in CARs (Examples such as Kymriah and Yescarta). CAARs on the other hand substitute a recombinant autoantigen in place of the scFv. Figure 3.2 illustrates therapeutic examples across different disease areas, including anti-FAP CARs for fibrotic disorders, anti-CD19 CARs, CAARs (chimeric autoantibody receptor) targeting autoantigens for autoimmune diseases, and anti-uPAR CARs for conditions linked to cellular senescence.
- Third Generation: Builds on the second generation by adding 4-1BB (another co-stimulatory domain), enhancing T-cell activation, longevity, and antitumor efficacy.
- Fourth Generation: Incorporates an IL-12 inducer along with CD28 and CD3ζ, enabling cytokine release in the tumor microenvironment to enhance immune response and tumor destruction.
- Fifth Generation: Retains the CD28 and CD3ζ domains but includes an IL-2Rβ-chain, allowing drug-dependent regulation of CAR-T cell function, offering better control, persistence, and safety

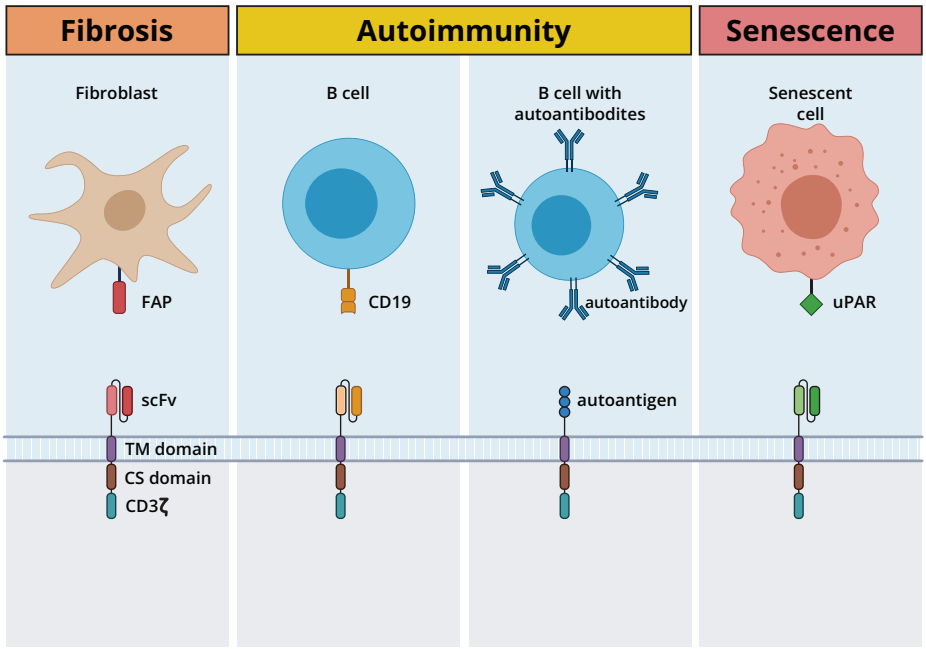
Figure 3.1: Evolution of CAR Constructs



Source: Bui, Thuy Anh et al.eBioMedicine, Volume 106, 105266

All currently approved CAR-T cell therapies—are second-generation products featuring a single co-stimulatory domain (CD28 or 4-1BB) alongside CD3ζ, while third, fourth, and fifth-generation CAR-Ts remain investigational and are not yet approved for clinical use.

Figure 3.2: Second generation CARs - Expanding CAR and CAAR T Cell Therapies: Applications Beyond Oncology



Source: Aghajanian H et al. Nat Metab. 2022 Feb 28;4(2):163–169. doi: 10.1038/s42255-022-00537-5

The structural evolution of CARs has significantly improved their therapeutic potential, from basic T-cell activation to enhanced targeting, persistence, and cytokine modulation. While fifth-generation CARs introduce more control over therapy, challenges like off-target effects and tumor specificity remain areas of ongoing research. [3] As these advancements in CAR architecture continue to unfold, it is equally important to examine the global research and development landscape driving these innovations forward.

TECHNOLOGY ENABLERS FOR CAR-T CELL GENERATION

Gene editing platforms

Various gene-editing systems facilitate stable CAR expression in primary T cells, including transposons, zinc finger nucleases (ZFNs), TALENs, and CRISPR-Cas9. Among these, transposons, ZFNs, and CRISPR support both in-vivo and ex-vivo CAR-T generation, offering the potential for next-gen cancer therapies. Table 1 summarizes the benefits, challenges, and safety concerns linked to various CAR-T-cell engineering approaches.

• Transposon Systems (Sleeping Beauty & PiggyBac) enable stable transgene insertion with low genotoxicity and cost-effectiveness but pose challenges such as stability issues and off-tar-get effects.

• ZFNs allow precise gene modifications and have been used in HIV-resistant CD4+ T cells and cancer models but require complex protein optimization.

• TALENs are effective for blood cancers like AML, B-ALL, and multiple myeloma but have limitations, including size constraints and extensive nuclease modifications.

• CRISPR-Cas9, the most advanced tool, enables precise T-cell modifications and is being ex-plored in early clinical trials for enhanced CAR-T efficacy. [3,18]

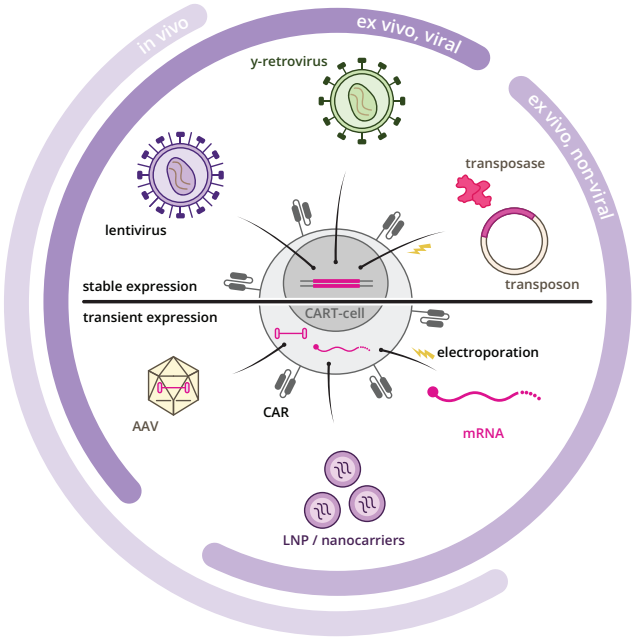
Table 1: Benefits, challenges, and safety concerns linked to various CAR-T-cell engineering approaches.

Benefits, Challenges & Safety concerns	Gene editing technologies				
	ZFN, TALEN	CRISPR/Cas9	Base editing	Prime editing	PASTE
Applicability CART cell engineering	Complex	Complex	Complex	Complex	Extremely complex
Clinically validated	No	Yes	No	No	No
Clinically evaluated for CAR-T-cell generation	Yes	Yes	Yes	No	No
Multiplexing	Yes difficult	Yes difficult	Yes difficult	Yes difficult	N/A
Efficiency in CAR-T-cells	Low	Good but decreases withthe number targets	Low	Low	Low
Risk of genotoxic effects	High increases with the number targets	High increases with the number targets	Moderate	Moderate	Moderate
Risk of off-target effects	Moderate	Moderate	High	High	Low

Transgene delivery systems

Multiple strategies are currently under investigation for transgene delivery, including both vector-based methods (such as y-retroviruses and lentiviruses) and non-vector-based techniques (such as transposons, nanovectors, and mRNA), as outlined in Figure 4 and Table 2 below. The methodologies are categorized into in-vivo, ex-vivo viral, and ex-vivo non-viral approaches. Techniques involving lentiviruses, retroviruses, and transposons integrate the CAR construct into the genome, resulting in stable expression (upper half). Conversely, AAV, LNP/nanocarriers, and mRNA-based methods yield transient CAR expression (lower half).[18]

Figure 4: Viral and Non-viral Transgene Delivery Systems



Source: Rossi M and Breman E (2024) doi: 10.3389/fimmu.2024.1411393

Table 2: Benefits, challenges and safety risks associated with the different transgene delivery systems

Benefits, challenges and safety risks	Viral delivery systems		Non-viral delivery systems			In-vivo delivery systems		
	γ-retroviruses	Lenti viruses	Transposons	Nanovectors	mRNA	Lentiviruses	AAVs	LNPs, NCs
Transfer method to the target cells	Transduction (ex-vivo)	Transduction (ex-vivo)	Electroporation	Electroporation	Electroporation, cationic lipids or polymers	Transduction (in-vivo)	Transduction (in-vivo)	Endocytosis
Efficiency	High	High	Moderate to low	Moderate	High	High	High	High
Cargo size	Limited (<10 kb)	Limited (<10 kb)	Large (~14 kb, >100 kb with BACs)	N/A	N/A	Limited (<10 kb)	Limited (<4 kb)	N/A
Integration	Semi-random	Semi-random	Random (SB), semi-random (PB)	No	No	Semi-random	No	No
Stability of gene expression	High	High	High	Transient	Transient	High	Transient	Transient
Immunogenicity	N/A	N/A	N/A	N/A	N/A	Low	High	Very low
Manufacturing complexity	High	High	Moderate	Low	Low	High	High	Low
Manufacturing costs	High	High	Moderate	Low	Low	High	High	Low
Clinically evaluated | for CAR-T-cell generation	Yes	Yes	Yes	No	Yes	No[1]	No[1]	No
Theoretical risk of genotoxic effects	Yes	Yes	Yes	Extremely low	No	Yes	Extremely low	Extremely low
Reported genotoxic effects in the clinics	Yes	Yes	Yes	N/A	No	Yes	No	N/A

1 Clinically evaluated for gene therapy applications.

AAVs – adeno-associated viruses; LNPs – lipid nanoparticles; NCs – nanocarriers; BACs – bacterial artificial chromosomes; SB – Sleeping Beauty; PB – Piggy Bac

Source: https://doi.org/10.3389/fimmu.2024.1411393

GLOBAL RESEARCH AND DEVELOPMENT LANDSCAPE

Explosive Growth in In-Vivo CAR Assets Since 2021

The clinical and commercial interest in in-vivo CAR platforms has accelerated in recent years, with over $2 billion in funding and several key partnerships formed. As of early 2025, more than five in-vivo CAR programs have entered clinical trials, and the number of disclosed assets is expected to surpass 100 within the year.

The in-vivo CAR-Therapy landscape has witnessed remarkable momentum over the past three years. While the first in-vivo CAR construct—targeting CD19 via a DNA nanoparticle polymer—surfaced in 2018 and was formally disclosed in a 2019 patent by Rongze Biotech, the broader pipeline expansion is a recent phenomenon.

By 2024, the number of disclosed in-vivo CAR assets crossed 35 in a single year, contributing to more than 70 cumulative assets. (Figure 5) This surge has been propelled by increasing scientific visibility, with multiple assets presented at leading oncology and gene therapy conferences including AACR, ASGCT, SITC, and ASH.

Notably, only a small fraction of these assets (approximately 18%) remains inactive. This indicates that the majority of these therapies are still active in development pipelines or have been recently introduced, demonstrating sustained innovation and ongoing interest from biotech developers.

Table 2: Benefits, challenges and safety risks associated with the different transgene delivery systems

Current Development Landscape
The in-vivo CAR therapy landscape has expanded significantly, particularly from 2022 onward. Around 75% of disclosed assets are still preclinical , while 7% have entered early clinical trials.

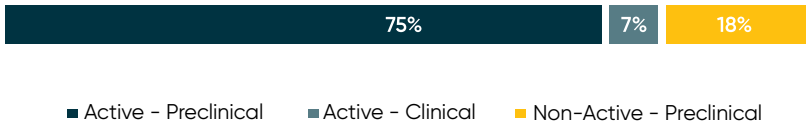
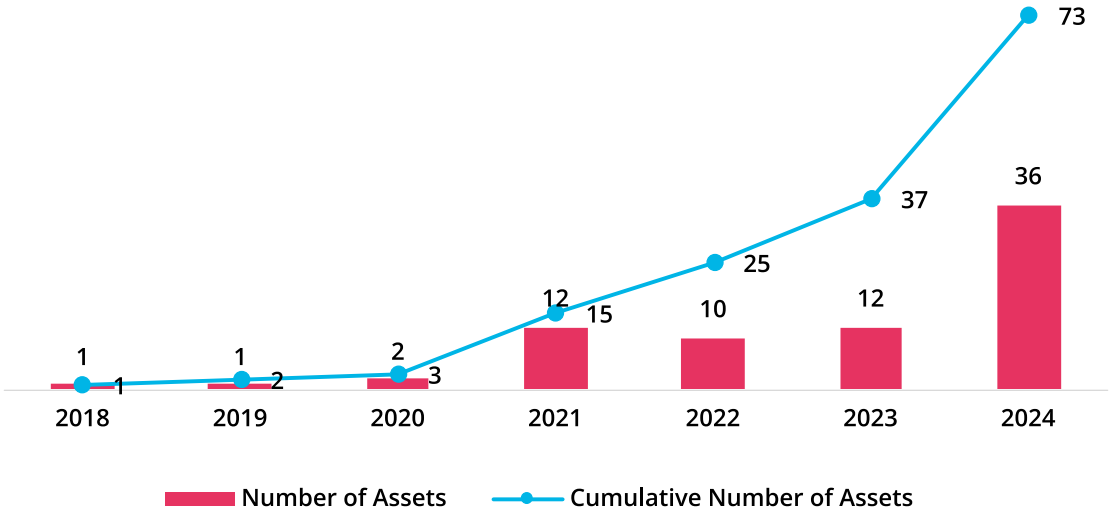


Figure 5: Growth of In-Vivo CAR-Therapies by Year



Source: Beacon Intelligence

The rapid rise in disclosed in-vivo CAR assets reflects increasing scientific and clinical interest in this novel modality. As of now, these therapies are in the early stages of clinical development, with only a limited number of trials initiated. For instance, Umoja Biopharma's Phase 1 clinical trial, INVICTA-1, is among the first in-vivo CAR-T cell therapy trials in the United States, with clinical updates expected in 2025.

In contrast, ex-vivo CAR-T therapies—where T cells are extracted, modified outside the body, and then reinfused—have been more extensively studied. A systematic review indicates that China and the USA are leading regions in clinical studies involving CAR-T therapies.

Given the nascent stage of in-vivo CAR-T therapies, they constitute a small fraction of the overall CAR-T clinical trial landscape. However, with ongoing research and development, the number of in-vivo CAR-T trials is expected to increase in the coming years.

Several in-vivo CAR-Therapies are in clinical development with five listed below in Table 1: Myeloid Therapeutics' MT-302 and MT-303, Umoja Biopharma's UB-VV111, Interius Bioscience's INT-2104, and Genocury's unnamed trial. These programs target TROP-2, GPC3, CD19, and CD20 using both viral and non-viral systems.

Table 3: In-vivo CARs in clinical development

Therapy	Developer	Cell Type	Target	Trial Start Date
MT-302	Myeloid Therapeutics	Myeloid Cells	TROP-2	2nd August 2023
MT-303	Myeloid Therapeutics	Myeloid Cells	GPC3	1st July 2024
In-vivo CAR-T	Genocury Biotech	T Cells	Undisclosed	18th August 2024
INT-2104	Interius Bioscience	T Cells	CD20	31st October 2024
UB-VV111	Umoja Biophama	T Cells	CD19	30th November 2024

Umoja's VivoVec platform in particular, is designed to revolutionize CAR-T cell therapy by shifting the entire manufacturing process into the patient's body. Using a lentiviral delivery system, the platform enables rapid in-vivo generation of CAR-T cells—potentially within hours—eliminating the need for complex external production and lymphodepleting chemotherapy. Unlike traditional ex-vivo approaches, VivoVec offers a single-dose, off-the-shelf treatment with the potential for long-lasting immune responses. This reduces cost, simplifies logistics, and improves patient experience. Umoja's Phase 1 trial, INVICTA-1, recently cleared by the FDA, is set to be the first in-vivo CAR-T-cell therapy tested in humans in the U.S. Preclinical primate studies have shown durable, sustained CAR-T cell responses, supporting the platform's clinical promise.

Similarly, Myeloid Therapeutics is advancing two in-vivo mRNA CAR programs—MT-302 (the first intravenously delivered mRNA-based CAR therapy) and MT-303—delivered via lipid nanoparticles (LNPs) to reprogram myeloid cells directly in the body. MT-302, targeting TROP2, is in a Phase 1 trial for epithelial cancers, including breast and lung. Myeloid recently presented first-in-human (FIH) data for MT-302 at ASCO 2025, representing a significant milestone in RNA-based immuno-oncology. Pre-clinical models showed potent tumor suppression without the need for lymphodepletion. MT-303, directed at GPC3 in hepatocellular carcinoma (HCC), began clinical evaluation in July 2024. At SITC 2024, Myeloid presented early data highlighting MT-303's ability to activate CAR-expressing myeloid cells in-vivo, with encouraging tumor control in preclinical HCC models. Both programs support Myeloid's goal to develop off-the-shelf, durable, and simplified cell therapies that eliminate the need for ex-vivo manufacturing. [19,20,28]

Key development milestones from a selection of In-vivo CAR developers

As in-vivo CAR therapy gains traction as a potential alternative to expensive ex-vivo CAR-T treatments, 2025 has already seen significant developments. Several biotech companies are advancing their in-vivo CAR programs, with key clinical and regulatory milestones shaping the evolving landscape. (Table 4)

Table 4: Research and development milestones of In-vivo CAR developers

Company	Therapy/Platform	Indication	Development Phase	Recent Activities
Myeloid Therapeutics	MT-302 (TROP2) & MT-303 (GPC3) using LNPs	TROP2+ Epithelial Cancers, GPC3+ Hepatocellular Carcinoma	Phase 1 Trials	• ASCO 2025 FIH data unveiled on MT-302 • SITC 2024 oral presentation on MT-303 • Demonstrated tumor control in preclinical HCC models • Both programs are actively recruiting.
EsoBiotec	ESO-T01 (anti-BCMA)	Multiple Myeloma	Phase 1 Clinical Trial	• Dosed its first multiple myeloma patient with the ESO-T01 (anti-BCMA) therapy, developed in partnership with Pregene. • AstraZeneca to acquire EsoBiotec for up to $1B.
Interius BioTherapeutics	INT-2104 (anti-CD20)	CD20+ B-cell Malignancies	Phase 1 (Australia, Germany)	• Received first-in-human trial clearance • The Invise trial dosed its first patient in Australia (with plans to dose up to 30 patients) • Expanded into Germany via Paul Ehrlich Institute - as the first in-vivo CAR-T therapy to be evaluated in Europe • Includes both CAR-Ts and CAR-NKs.
Umoja Biopharma	UB-VV111 (CD19) & UB-VV400 (CD22) using VivoVec™	B-cell Malignancies	Phase 1 (CD19, CD22)	• Launched UB-VV111 trial (Nov 2024) • UB-VV400 also advanced to phase I trials (Dec 2024) • AbbVie holds opt-in rights to UB-VV111.
Carisma Therapeutics	CAR-M (Macrophage) targeting GPC3	Hepatocellular Carcinoma	Preclinical	• Partnered with Moderna • Presented promising preclinical data on CAR-M therapy.
Capstan Therapeutics	CPTX2309 (mRNA-LNP based)	CD19+ Autoimmune Diseases	Preclinical	• $175M Series B raised. • Demonstrated in-vivo CD8+ CAR-T engineering and B-cell depletion in NHPs.
Kelonia Therapeutics	KLN-1010	BCMA	Preclinical	• Discovery deal with Astellas • Preclinical data presented at AACR 2024.
Orna Therapeutics	ORN-101	CD19	Preclinical	• Circular RNA with LNP delivery • Expected to enter clinic by 2026.
Exuma Biotech	GCAR™ Platform (CD3-targeted lentiviral vector)	Solid Tumors	Preclinical	• Presented early clinical and translational findings at AACR 2023 • Plans to complete non-human primate studies in 1H25 to support first in-vivo CAR-T product.
Vyriad / Novartis	Undisclosed	Undisclosed	Discovery	• Discovery deal with Novartis signed in Nov 2024; Lentiviral vector, possibly delivered via G proteins.
Sanofi	3 programmes	Undisclosed	Early Discovery	• 3 CD8+ T cell-based in-vivo CAR programs were revealed at R&D Day in Dec 2023.
Tessera	Undisclosed	Undisclosed	Research	• Presented technical poster at ASGCT 2024 outlining platform approach.
Orbital Therapeutics	Undisclosed	Undisclosed	Research	• Early-stage company; program details yet to be disclosed.

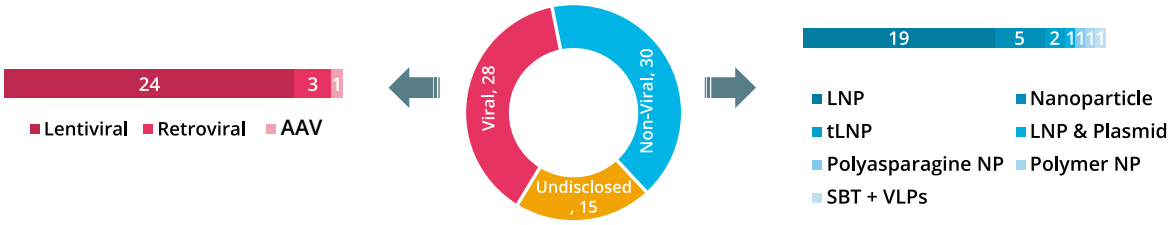
Source: Hung Trinh, Vertex Biopharm Consulting, February 2025; https://www.asgct.orgt

The expansion of in-vivo CAR-Trials into Europe (Germany) and Australia reflects a growing global interest in this approach. Interius's Invise trial reaching European evaluation marks a significant regulatory milestone. Additionally, big pharma involvement, as seen with AbbVie's partnership with Umoja, signals confidence in the potential of in-vivo CAR therapies to reshape treatment paradigms by addressing cost and scalability challenges. [2,9,10] The sections below discuss in detail the various CAR delivery systems, indications being investigated, strategic partnerships, and funding landscape.

Delivery I Systems : Viral vs Non-Viral

In-vivo CAR constructs are delivered using both viral (lentiviral, retroviral, AAV) and non-viral (LNPs, tLNPs, biodegradable polymers) platforms. LNPs currently lead among non-viral vectors, leveraging mRNA delivery technology validated during the COVID-19 vaccine era.

Figure 6.1: In-vivo CAR therapies by delivery systems

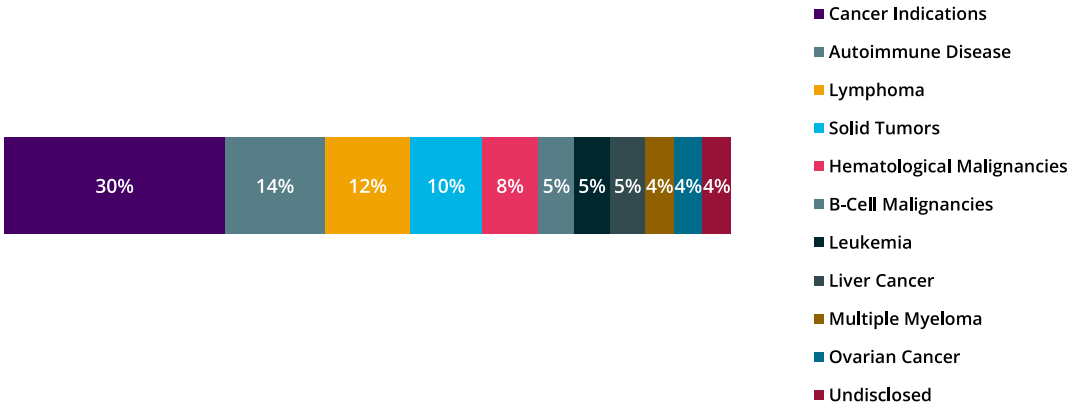


Source: Beacon Intelligence

IndIcatIons Under InvestIgatIon

- Most programs are directed at broad cancer indications, but specific assets also target lymphoma, leukemia, and autoimmune diseases such as lupus and multiple sclerosis. Solid tumors and liver cancer are also under active investigation.
- A significant portion of in-vivo CAR assets- 30%- remain broadly categorized under general "cancer indications," with no specific disease target disclosed.
- Meanwhile, autoimmune disorders are gaining interest, with 14 assets under investigation. Notably, this includes programs emerging from the Moderna-Carisma collaboration, as well as a CD19-directed program from Orna Therapeutics, which expanded its RNA capabilities through the acquisition of ReNAgade Therapeutics in 2024.
- The interest in autoimmune indications is driven by the growing evidence of CAR-T efficacy in diseases like lupus and multiple sclerosis.
- In-vivo CAR platforms, with their simplified and scalable production, may help address the cost and complexity barriers seen with ex vivo CAR-T manufacturing, unlocking broader patient access.

Figure 6.2: In-vivo CAR therapies - top indications



Collaborations and Financial Momentum

- Notable deals include AbbVie-Umoja (2024), Alaya.bio-Memorial Sloan Kettering (2023) and Moderna-Carisma (2021). More than $2B has been raised by in-vivo CAR developers s ince 2020.
- Investment momentum for in-vivo CAR therapy developers has been sustained over recent years, with particular emphasison RNA-focused companies leveraging adaptable, proprietary technologies to enable CAR-based applications. These financing rounds reflect growing confidence in the potential of in-vivo platforms to transform the cell therapy space.

2021

• Mustang Bio initiated a research collaboration with the Mayo Clinic to investigate novel CART cell platforms.

• Moderna and Carisrna Therapeutics entered a strategic agreement to codevelop in vivo-engineered CAR monocyte therapies targeting oncological indications.

• Abintus Bio established a technology agreement to optimize in vivo CAR cell programing with a focus on enhancing antitumor potency and persistence in hematologic and solid tumor malignancies.

2022

• CellOrigin Biotech and Xinhe Biotech formalized a technical cooperation agreement to advance mRNA-based genome editing technolog es for modifying immune cells in vivo.

2023

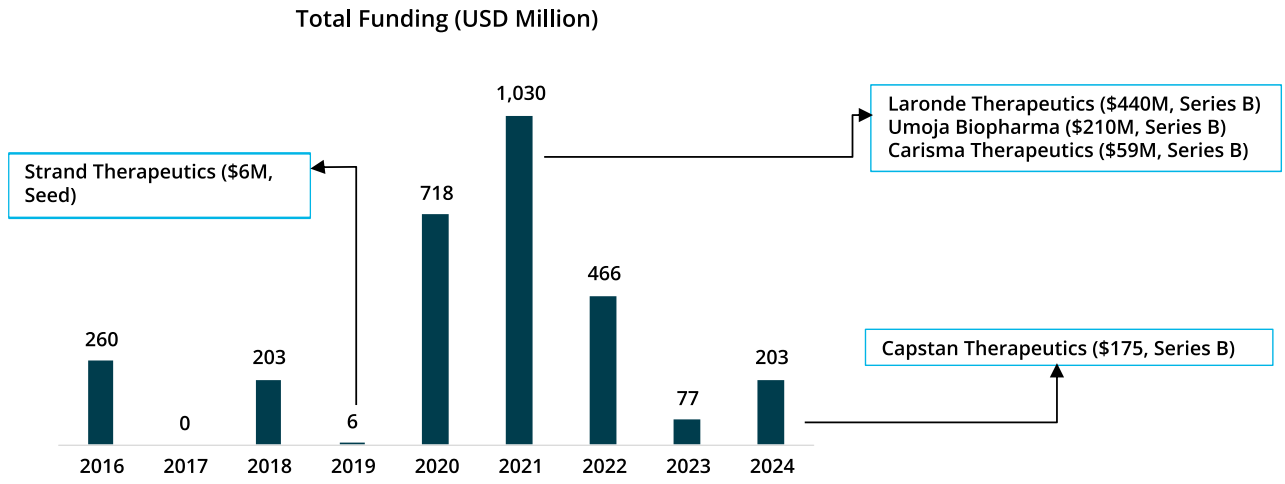
• Simnova and Orna Therapeutics executed a collaboration agreement aimed at advancing circular RNA-based in vivo engineering technolog es within the Chinese biotech market.

2024

• Alaya.bio announced a translational research collaboration with Memorial Sloan Kettering Cancer Center to advance in situ CAR-T therapeutic modalities.

• AbbVie and Umoja Biopharma entered a multiprogram strategic alliance encompassing exclusive option and license agreements to develop in vivo-generated CAK-T cell therapies.

Figure 7: Key Financing Milestones in In-vivo CAR Therapy

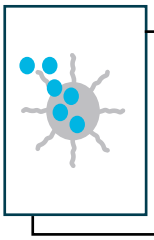


Source: Beacon Intelligence

EMERGING INNOVATIONS IN DELIVERY SYSTEMS FOR IN-VIVO CAR-T THERAPY

Academic and Industrial research advancements in targeted CAR-T Delivery

The rapid evolution of in-vivo CAR-T therapy is being driven by both academic research and industrial advancements, aiming to overcome the complexity, cost, and accessibility limitations of traditional ex-vivo CAR-T treatments. Academic research focuses on optimizing delivery strategies, including viral vectors, lipid nanoparticles (LNPs), and hybrid nanocarriers, to enhance targeted gene transfer, safety, and therapeutic efficacy. Recent advancements in vector design and targeting have enabled successful in-vivo CAR-T generation in mouse models, marking a breakthrough in cancer immuno-therapy. These findings underscore the crucial role of vector engineering in advancing gene therapy applications.



Optimized Viral and Nanocarrier Delivery:

- Lentiviral vectors (LVs) and CD3-modified bispecific LV platforms enable direct T-cell targeting and in vivo CAR transgene delivery.
- CD3-targeted lipid nanocarriers (AntiCD3-LNP/CAR19 + shIL6) enhance CAR expression, anti-tumor effects, and reduce cytokine release syndrome.
- References: Pfeiffer et al., 2022; Michels et al., 2023; Zhou et al., 2023; Smith et al., 2023.

Hybrid and Targeted Delivery Systems:

- CD3-targeted polymer nanoparticles and biodegradable polymers enhance CAR-T gene transfer in leukemia and lymphoma models.
- Hybrid approaches using exosome-engineered lipid nanoparticles improve in vivo CAR-T expansion and long-term gene expression.
- References: Fan et al., 2023; Jones et al., 2024.

Safety & Toxicity Mitigation:

- Polymer-based nanocarriers require engineering to address biodegradability and instability issues affecting CAR production.
- Lipid-polymer hybrid modifications emerge as safer alternatives to mitigate toxicity and enhance therapeutic effects.
- References: Jones et al., 2024; Smith et al., 2023.

Advancements in CAR-T Targeting & Expansion:

- Advanced ligand engineering in lentiviral and retroviral vectors improves CD3+ T-cell targeting.
- Capsid-modified AAV enhances T-cell-specific gene delivery.
- Hybrid nanocarrier systems combining lipid nanoparticles, polymer nanocarriers, and exosomes enhance in vivo CAR-T generation while reducing immune activation risks.
- References: Michels et al., 2023; Jones et al., 2024; Smith et al., 2023; Fan et al., 2023.

Currently, viral vectors and nanocarriers are the most widely explored delivery platforms for in-vivo CAR-T generation (Michels et al., 2023). This section discusses the academic research developments in viral vector and nanoparticle-based delivery systems for CAR-Therapy

Viral Vector-Based Delivery

Viral vectors such as lentivirus (LV), retrovirus, and adeno-associated virus (AAV) have been optimized for in-vivo CAR-T therapy. These vectors are engineered to target specific T-cell populations, ensuring efficient gene delivery and CAR expression (Agarwalla et al., 2023).

1. Lentiviral and Retroviral Vectors

Lentivirus (LVs) targeting CD8+ T cells: Early studies demonstrated that LV-based CAR constructs can transduce CD8+ T cells in-vivo, leading to effective expansion and tumor cell elimination (Pfeiffer et al., 2022).

Retroviral scaffolds: A novel implantable scaffold was developed to co-deliver patient-derived T cells and CD19-encoding retroviral particles, condensing the CAR-T manufacturing process into a single day while improving T-cell activation and expansion (Agarwalla et al., 2023).

2. Adeno-Associated Virus (AAV) for CAR-T Targeting

AAV-based CAR-T targeting CD4+ T cells: AAV vectors engineered to deliver CD4 CAR constructs have been tested for treating T-cell leukemia, achieving regression in mouse models (Smith et al., 2023).

Capsid-modified AAV for improved T-cell targeting: To enhance specificity, engineered ankyrin repeat proteins (DARPins) have been inserted into AAV2 capsids, increasing CAR gene delivery efficiency by 20-fold in CD8+ T cells (Jones et al., 2024).

3. Safety Considerations for Viral Vectors: While viral vectors have demonstrated high efficiency, challenges such as immune activation, inflammation, and off-target effects remain. Researchers are developing strategies to modify viral capsids and optimize transduction efficiency for safer clinical applications (Michels et al., 2023).

Nanocarrier-Based Delivery

Nanocarriers offer an alternative to viral vectors, reducing immunogenicity and enabling scalable production (Fan et al., 2023). These systems include lipid nanoparticles (LNPs), polymer nanoparticles, and exosomes, each designed for specific CAR-T gene delivery applications.

1. Lipid Nanoparticles (LNPs) for CAR-T Targeting

LNPs for CD19 CAR delivery: Microfluidics-based LNP synthesis has been developed to encapsulate mRNA encoding CD19 CAR constructs, enabling efficient in-vivo T-cell reprogramming (Smith et al., 2023).

LNP-modified exosomes for dual targeting: Hybrid LNP-exosome platforms engineered to target both endogenous T cells and tumor cells have improved CAR-T expansion and therapeutic efficacy (Fan et al., 2023).

2. Polymer Nanoparticles for CAR-Mediated Therapy

Cationic polymer nanoparticles for CD8+ T-cell targeting: These nanoparticles form stable electrostatic complexes with CAR constructs, enhancing T-cell uptake and persistence (Jones et al., 2024).

Safety concerns and optimization: High toxicity remains a challenge, and lipid-polymer hybrids are being explored to improve biocompatibility and minimize side effects (Michels et al., 2023).

3. Exosome-Based Approaches for CAR-T Therapy

Tumor-antigen-stimulated exosomes for CD3+ CAR delivery: Engineered dendritic cell-derived exosomes (tDC-Exo) have been designed to deliver CAR constructs targeting T cells, improving antigen specificity and therapeutic outcomes (Fan et al., 2023).

Challenges with gene packaging: Due to their small size, exosomes face limitations in delivering large gene-editing tools like CRISPR/Cas9. Exosome engineering and hybrid delivery systems are being explored to enhance cargo loading efficiency (Smith et al., 2023).

The industrial development of in-vivo CAR-T therapy is also advancing with innovations in viral vectors and nanoparticle-based delivery systems. The biopharmaceutical industry is accelerating clinical translation, with companies like EXUMA Biotech, Umoja Biopharma, and Capstan Therapeutics developing scalable, off-the-shelf in-vivo CAR-T platforms that can revolutionize patient care.

Viral Vector Innovations:

EXUMA Biotech has developed a lentiviral vector encoding a CD19 CAR, which successfully targets and activates CD3+ T cells in preclinical NSG-SGM3 mouse models. Their therapy also extends to CCT303-406, a Phase I investigational treatment for HER2-positive metastatic solid tumors.

Umoja Biopharma's VivoVec™ utilizes surface-engineered lentiviral particles carrying a CD19-targeted CAR-Transgene. In non-human primates, a single dose induced potent CAR-T cell generation and persistent B-cell depletion for up to 76 days with no toxicity.

Nanoparticle-Based Advancements:

Capstan Therapeutics employs mRNA-lipid nanoparticle (LNP) technology for in-vivo CD5-specific CAR-T generation, achieving 20% CAR-T cell formation within 48 hours of LNP injection in mouse heart disease models. The resulting CAR-T cells consist of 87% CD4+ and 9–10% CD8+ T cells, demonstrating high therapeutic efficacy.

These advancements suggest a potential shift toward off-the-shelf in-vivo CAR-T therapies, addressing the limitations of ex-vivo CAR-T treatments while improving efficacy, safety, and scalability.

The convergence of academic innovation and industrial expertise is paving the way for next-generation in-vivo CAR-T therapies, offering more accessible, cost-effective, and scalable solutions. By integrating advanced viral vector engineering, nanoparticle-based gene delivery, and immune-targeting technologies, these approaches are expanding applications beyond hematologic malignancies to solid tumors and non-oncologic diseases.

Current advancements leverage viral and non-viral systems for efficient gene transfer and T-cell reprogramming. While viral vectors offer high transduction efficiency, nanocarriers provide scalability and reduced immunogenicity. Ongoing research into hybrid approaches, immune shielding, and precision targeting is crucial to optimizing safety, stability, and clinical effectiveness, bringing in-vivo CAR-T therapy closer to broad clinical adoption.[3]

COMMERCIAL VIABILITY OF IN-VIVO CAR-T

In-vivo CAR-T therapy is revolutionizing immunotherapy by addressing the accessibility, cost, and logistical limitations of conventional ex-vivo CAR-T treatments. By directly modifying T cells within the patient's body, this approach eliminates the need for complex cell collection and processing, making it a ready-to-use (RTU) treatment that integrates seamlessly into existing healthcare infrastructures while ensuring broad patient access and durable therapeutic outcomes. To better appreciate the advantages of in-vivo CAR-T, it is important to first understand the persistent challenges associated with ex-vivo CAR-T approaches—and how in-vivo strategies are uniquely positioned to overcome them.

Limitations of Ex-vivo CAR-T Therapy and How In-vivo CAR-T Addresses Them

Despite its therapeutic potential, ex-vivo CAR-T therapy remains inaccessible to many eligible patients. In 2022, only ~3,500 first-time CAR-T infusions were administered in the U.S., with less than 20% of eligible diffuse large B-cell lymphoma (DLBCL) patients receiving treatment.

Key barriers include patient eligibility, complex manufacturing, high costs, and lengthy processing times. The therapy requires specialized facilities, cell harvesting, transportation, and weeks or months-long production, delaying treatment for critically ill patients.

In-vivo CAR-T Therapy: A Targeted, Scalable Alternative

To address the limitations of ex-vivo CAR-T production, in-vivo CAR-T therapies are being developed to bypass external manufacturing and enable direct T-cell engineering within the patient. This strategy reduces production complexity, lowers costs, shortens turnaround times, and significantly improves patient accessibility.

In this approach, gene-editing constructs—delivered via viral vectors or nanoparticles—are administered systemically to program T cells in-vivo. These modified T cells then express CARs, enabling them to identify, expand, and destroy cancer cells within the bloodstream or tumor microenvironment, offering a more efficient and scalable therapeutic approach. Figure 8 below summarizes the key limitations of ex-vivo CAR-T therapy and illustrates how the emerging in-vivo CAR-T technology addresses these gaps. Figure 9 provides an overview of key factors driving the commercial adoption of in-vivo CAR. [4,8]

Figure 8: Ex-vivo CAR Vs In-vivo CAR

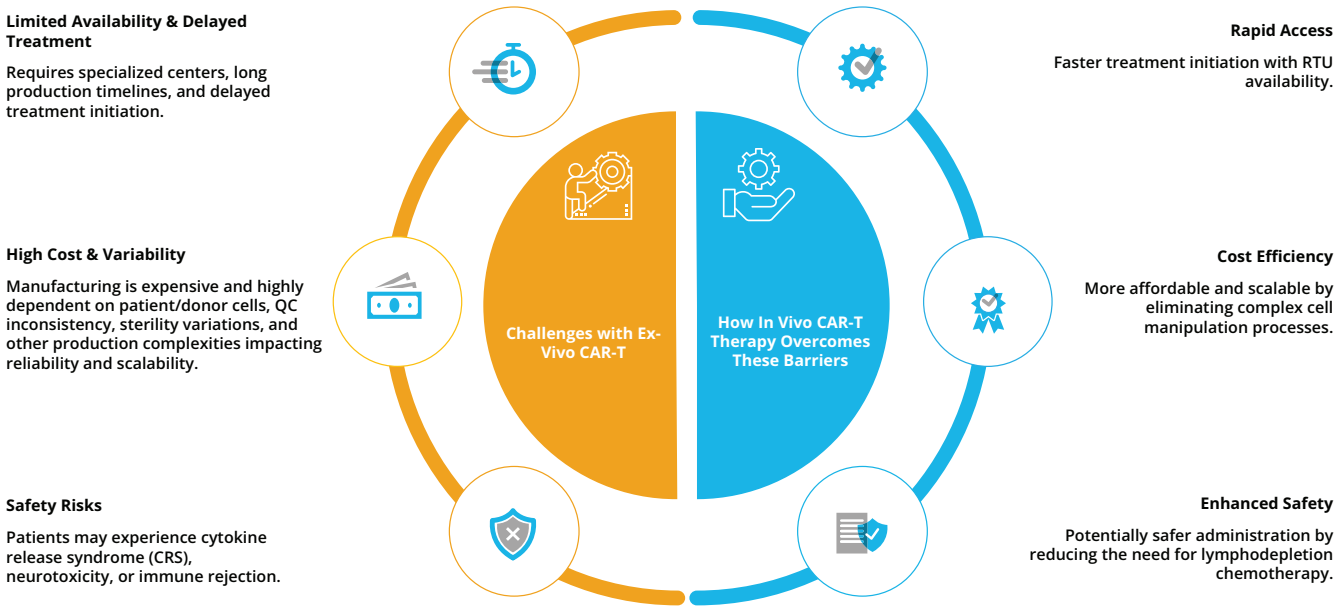
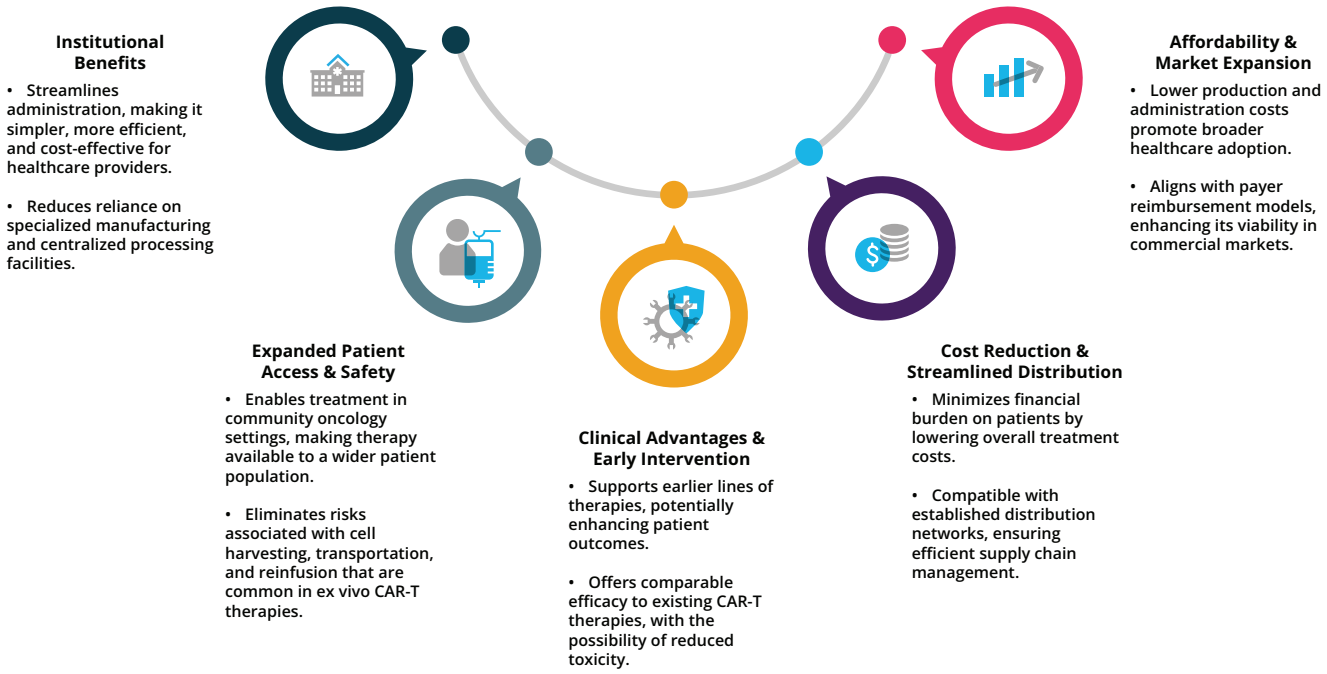


Figure 8: Ex-vivo CAR Vs In-vivo CAR



Positioning for Success: A Balanced Approach

A commercially viable In-vivo CAR-T therapy must blend widespread patient accessibility with strong clinical efficacy. As illustrated below in the patient access versus treatment outcomes framework (Figure 10), therapies in this category (i.e., In-vivo CAR-T) offer greater scalability and accessibility as well as therapeutic impact than autologous CAR-T therapies, which remain effective but limited in accessibility.

The manufacturing process for ex-vivo CAR-T therapy is complex, costly, and time-consuming, often requiring weeks for production and quality checks. Personalized "one patient, one batch" models limit scalability and delay treatment. While allogeneic CAR-T platforms address some of these challenges using gene editing tools like CRISPR/Cas9, they introduce added costs and regulatory hurdles.

In contrast, in-vivo CAR-T therapies offer off-the-shelf availability by programming T cells directly inside the body. Moreover, unlike antibody drugs such as blinatumomab (a CD3/CD19-directed bispecific T-cell engager), which require repeated dosing and pose infection risks, in-vivo CAR-T cells self-expand and sustain tumor-killing activity.

Manufacturing is streamlined through GMP-grade nanoparticle platforms, which allow for high-yield, stable, and cost-effective production. These therapies also eliminate the need for lymphodepletion chemotherapy, reducing toxicity and infection risk—making in vivo CAR-T a promising, scalable solution for broader clinical use.

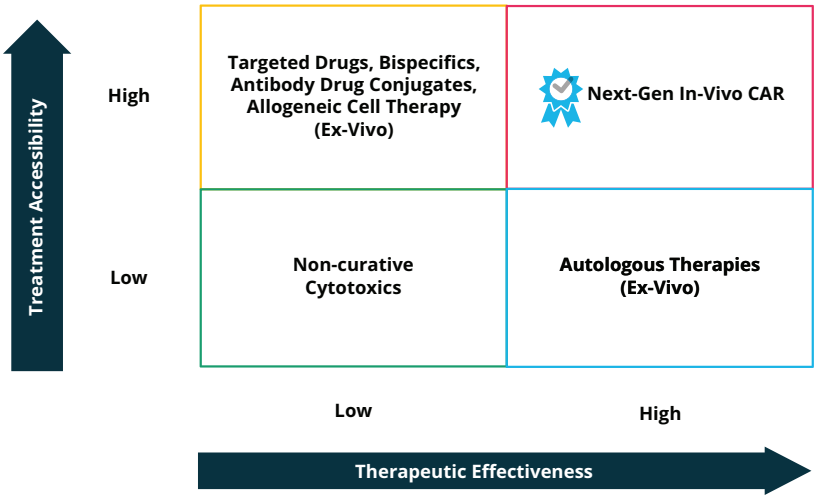
By merging cost efficiency, accessibility, and clinical effectiveness, in-vivo CAR-T therapy holds immense promise for transforming cancer treatment. However, its commercial success depends on further advancements in vector engineering, precise immune targeting, and regulatory adaptation.

In addition, combining in-vivo CAR-T therapies with adjuncts like cancer vaccines, cytokines, immune checkpoint inhibitors, and oncolytic viruses may further enhance efficacy and durability. For example, Cancer vaccines and nano vaccines support precise tumor targeting by presenting tumor-associated epitopes and priming adaptive immune responses. Likewise, cytokines can shift the tumor microenvironment from immunosuppressive to pro-inflammatory, amplifying CAR-T cell activity.

Checkpoint inhibitors on the other hand extend CAR-T persistence by preventing T-cell exhaustion, while oncolytic viruses facilitate tumor infiltration, activate dendritic cells, and promote M1 macrophage polarization. Together, these synergistic strategies have the potential to unlock broader indications, improve therapeutic durability, and optimize both clinical and commercial outcomes.

With the increasing demand for accessible, next-generation immunotherapies, in-vivo CAR-T therapy is well-positioned to reshape the treatment landscape for hematologic malignancies, autoimmune disorders, and beyond, offering a scalable and commercially sustainable alternative to traditional CAR-T therapies. [4,8,12, 27]

Figure 10: Treatment access versus therapeutic efficacy



COMMERCIAL VIABILITY OF IN-VIVO CAR-T

Targeting and Biodistribution: Optimizing Precision for Therapeutic Success		
Strategic Focus	**Key Developments**	**Challenges to Address**
Efficient targeting and biodistribution are critical for the success of in-vivo CAR-T therapies, ensuring high specificity while minimizing off-target effects. Unlike traditional ex-vivo approaches that rely on extracted and engineered T cells, in-vivo gene delivery requires precise T-cell targeting mechanisms to ensure robust CAR expression, expansion, and persistence in patients.	Vector-Based Precision: Most in-vivo CAR-T studies employ targeted lentiviral (LV) and non-viral vectors, primarily selecting CD3 as a pan T-cell marker for efficient gene transfer. *Subset-Specific Targeting for Improved Outcomes:* • CD4-LV selectively generates CD4+ CAR-T cells, contributing to rapid tumor clearance and enhanced persistence. • CD8-LV drives selective CD8+ CAR-T generation, engaging both natural killer (NK) and NKT cells, which significantly contribute to antitumor responses. • Combining CD4 and CD8-targeted vectors in controlled ratios optimizes the therapeutic balance, mimicking the natural CD4/CD8 synergy observed in long-term responding patients. *Biodistribution Optimization:* • Targeted nanocarriers (NCs) and LVs reduce off-target transduction in B cells and monocytes, improving localization to lymph nodes, spleen, and bone marrow. • Bispecific and pseudotyped vectors (e.g., SINV, NiV-LVs) refine targeting, enabling T-cell activation and proliferation without external stimulatory cytokines.	• Vector particle retention in non-lymphoid tissues (e.g., liver accumulation) necessitates engineering solutions to enhance lymphoid homing. • Off-target expression risks (e.g., in macrophages, dendritic cells) require next-generation targeting strategies to refine delivery efficiency

Dosing and Expression Kinetics: Balancing Durability and Control

Strategic Focus	Key Considerations in Expression Kinetics	Challenges to Address
One of the major distinctions between ex-vivo and in-vivo CAR-T therapies is the pharmacokinetics of T-cell expansion. Unlike ex-vivo CAR-T cells, which are infused at therapeutic levels, in-vivo CAR-T generation occurs progressively, requiring fine-tuned dosing strategies.	Key Considerations in Expression Kinetics: *Permanent vs. Transient Gene Expression:* • Lentiviral (LV), AAVs and transposon-based NCs enable stable CAR integration, leading to persistent CAR-T cell activity with a single dose. • Non-integrating vectors (mRNA NCs) require multiple doses to maintain sufficient CAR-T levels due to transient expression. *Dosing Optimization:* • LVs require lower doses (e.g., 4×10^{10} to 2.5×10^{11} particles per mouse) due to high transduction efficiency and stable integration. • Nanoparticle-based strategies often require higher vector particle numbers or repeat administrations to sustain CAR-T presence. • mRNA-based CAR-T expression peaks within 48 hours but diminishes rapidly, necessitating multiple infusions over several weeks. *Pharmacokinetics in Different Models:* • In PBMC-humanized NSG mice, CAR-T expansion occurs rapidly, reaching peak levels in 1–2 weeks. • In CD34+ humanized mice, CAR-T cell emergence takes longer (3–4 weeks) but persists for up to 18 weeks post-injection. *Solid tumors* *Immunosuppressive Tumor Microenvironment (TME):* • Solid tumors create an immunosuppressive TME that inhibits CAR-T cell activation and persistence, reducing therapeutic efficacy. • Solid tumor models show relapses following treatment cessation, reinforcing the need for durable CAR-T cell persistence. Limited CAR-T Cell Homing to Tumor Sites: Poor CAR-T cell trafficking and retention in solid tumors hinders antitumor activity. Antigen Heterogeneity in Solid Tumors: Tumors display high antigenic diversity, making it difficult for CAR-T cells to target all cancerous cells, leading to escape mechanisms and relapse. Inadequate CAR-T Expansion In-vivo: Limited CAR-T proliferation against heterogeneous tumor-associated antigens reduces long-term efficacy. Overcoming these barriers is essential for improving the efficacy and durability of in-vivo CAR-T therapy in solid tumors	• Achieving optimal CAR-T expansion without overstimulation remains a major hurdle, requiring fine-tuned dose escalation strategies. • Long-term CAR expression must balance efficacy and safety, as uncontrolled CAR-T expansion may increase toxicity risks. For solid tumors • Remodeling the vasculature of solid tumors using agents like Bevacizumab to enhance the infiltration of CAR-T cells. • Improving CAR-T cell entry into tumors by altering tumor vasculature or applying chemokine receptor engineering techniques. • Equipping a single T cell with multiple CAR constructs to enable recognition of a broader range of tumor-associated antigens. • Designing CARs that incorporate multiple binding domains to increase antigen specificity and expand targeting capabilities. • Developing next-generation CAR designs that boost T-cell persistence and support more effective engagement of the adaptive immune system.

Translating Dosing and Route of Administration from Animal Models to Clinical Use in In Vivo CAR Therapy

Strategic Focus	Key Safety Concerns and Solutions	Challenges to Address
Intravenous (IV) dosing for broader accessibility	Broad systemic exposure and volume challenges; close monitoring and optimization of IV formulations	Difficulties in defining effective and safe dose range from animal models
Refining preclinical-to-clinical dose translation	Significant differences in PK/PD profiles between humans and NHPs (such as macaques, chimpanzees); highlight need for accurate models	Potential misleading data from preclinical species; poor translational accuracy
Flexibility in dosing routes and levels	High tolerability formulations can allow for dose variation; requires balancing efficacy with safety	Evaluating value and cost of animal models for future in vivo CAR therapies

Safety Considerations: Mitigating Genotoxicity and Immune Reactions

Strategic Focus	Key Safety Concerns and Solutions	Challenges to Address
For in-vivo CAR-T therapy to be clinically viable, ensuring long-term safety is just as important as demonstrating efficacy. Recent setbacks in gene therapy highlight the need for rigorous safety monitoring, particularly in vectors with permanent genetic modifications.	*Genotoxicity Risks:* • Lentiviral and transposon-mediated CAR-T cells risk transcriptional dysregulation, potentially activating oncogenic pathways. • Recent cases of lymphoma following transposon-based gene transfer highlight the need for improved safety controls. *Immune-Related Toxicities:* • Cytokine release syndrome (CRS) and neurotoxicity (ICANS) remain major clinical risks, particularly in patients with aggressive malignancies. • In-vivo CAR-T studies show elevated IL-6, GM-CSF, and TNFa, correlating with T-cell infiltrates in lung, brain, and liver, requiring better pharmacokinetic control mechanisms. *Strategies for Safety Enhancements:* • Dasatinib-mediated CAR-T switching can be used to temporarily halt CAR-T activation in severe CRS cases. • UniCAR platforms offer drug-controlled activation, allowing precise regulation of CAR-T activity. • Suicide gene integration (e.g., inducible caspase-9) provides an emergency safety switch, enabling the selective elimination of CAR-T cells	• Regulatory guidelines for in-vivo CAR-T safety monitoring remain in development, necessitating long-term clinical surveillance. • Inflammatory reactions and complement activation risks from repeated vector dosing must be addressed to improve immune tolerance.

Host Immunity & Vector Persistence: Overcoming Immune Barriers

Strategic Focus	Key Insights from Research	Challenges to Address
The host immune system plays a dual role—essential for CAR-T function but also capable of compromising vector delivery. Developing immune-evasive strategies is key to enhancing durability and repeat dosing potential.	*Vector Immunogenicity & Neutralizing Antibodies (NAbs):* • LVs and AAVs induce strong immune responses, limiting repeated administration due to neutralizing antibody formation. • NiV-pseudotyped vectors are being explored as alternatives to reduce pre-existing immunity. *Macrophage-Mediated Clearance & Liver Uptake:* • Studies show that up to 20% of vector particles are sequestered by macrophages and monocytes. • Strategies like CD47 incorporation into LV particles can reduce phagocytosis, improving transduction efficiency. *Overcoming Dose-Dependent Clearance:* • Higher doses of LVs and adenoviral vectors saturate liver-resident Kupffer cells, enabling increased gene transfer. • Phagocytosis-resistant vectors improve transduction efficiency, reducing dose-dependent clearance.	• Repeated dosing strategies must account for pre-existing immunity, requiring vector modifications or immunosuppressive preconditioning. • The impact of host immune responses on long-term CAR persistence needs further study to ensure durable therapeutic effects. [3,7,8]

REGULATORY CONSIDERATIONS FOR ADVANCING IN-VIVO CAR-T THERAPY

Gene Therapy as a Regulatory Guidepost for In-vivo CAR Therapies

While in-vivo gene-modified cell therapies are a novel drug class, they are closely related to direct gene therapies—several of which have already received regulatory approval in the U.S., EU, and globally. These approvals offer a valuable roadmap for evaluating in-vivo CAR therapies. Although each platform presents unique considerations based on its indication, existing FDA guidance on gene therapy provides helpful direction for sponsors.

As with regulatory reviews of retroviral vectors in non-T cell applications, developers of in-vivo therapies using retroviral vectors must demonstrate that gene transfer is specific to the intended target cell type (e.g., T cells), avoiding transduction of unintended cells like hematopoietic stem cells or gametes. Special attention must be paid to the tropism of the chosen delivery system, whether viral or non-viral.[1] The evolving regulatory landscape for in-vivo CAR-Therapies demands careful attention to safety, vector selection, trial design, and approval strategy. The table below outlines key regulatory considerations across different stages of development.

Host Immunity & Vector Persistence: Overcoming Immune Barriers	
Key Safety Concerns and Solutions	**Key Considerations**
Preclinical Safety & Translational Challenges	- In-vivo CAR-T therapies must meet strict safety and efficacy standards before clinical translation.
	- Regulatory agencies like FDA and EMA require rigorous toxicology evaluations to assess potential ad-verse effects in human studies.
Vector Safety & Compliance	- Lipid nanocarriers must undergo extensive toxicity testing to prevent accumulation in healthy tissues.
	- Viral vectors need validation for purity, stability, and oncogenic risks to avoid insertional mutagenesis and inflammatory responses.
Gene Editing Risks	- CRISPR/Cas9-based gene editing raises concerns about off-target mutations, gene disruption, and immune responses.
	- High-fidelity Cas9 variants are being explored to reduce unintended modifications and improve therapeutic precision.
Clinical Trial Considerations	- Establishing clear patient eligibility criteria is essential to selecting candidates with the best risk-benefit profile.
	- Clinical trials must assess dose levels, safety markers (CRS, neurotoxicity, dose-limiting toxicities), and efficacy outcomes.
	- Long-term follow-up data is required to monitor delayed adverse effects and treatment durability.
Preclinical Safety & Translational Challenges	- Regulatory approval depends on therapy classification (e.g., cell-based, gene therapy, biologics).
	- Approvals may require submission via FDA's Biologics License Application (BLA) or EMA's Marketing Authorization Application (MAA).
	- Complex multi-step reviews demand substantial supporting clinical evidence, increasing regulatory timelines. [3]

Advancing In-vivo CAR-T Therapy – From Proof Of Concept To Clinical Adoption

The progress in in-vivo CAR-T cell generation highlights the potential of targeted vector technology in cancer immunotherapy and gene therapy. While these advancements demonstrate tumor-clearing capabilities, critical questions remain regarding the short-, medium-, and long-term safety and efficacy of both permanent and transient gene transfer approaches.

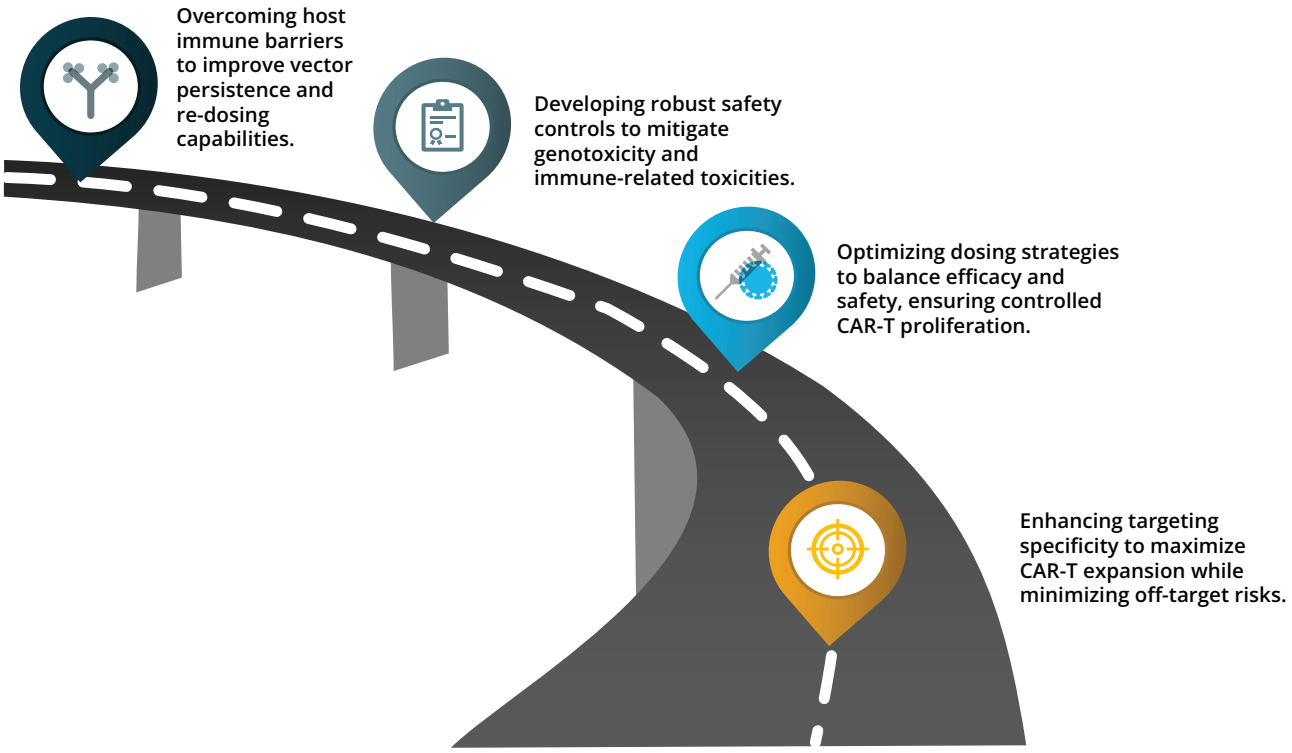
To ensure patient safety and regulatory readiness, long-term preclinical studies are essential to assess vector interactions with the host immune system, potential toxicity risks, and immune-mediated vector clearance. Unlike other gene therapies, broad immunosuppression or lymphodepletion is not viable for targeting T cells, necessitating precision-engineered conditioning regimens that minimize inflammation while preserving T-cell function.

Future research must leverage fully immunocompetent syngeneic models to refine vector designs, optimize immune modulation strategies, and evaluate host immunity responses. The availability of LVs and AAVs engineered for mouse T-cell targeting, such as CD8-directed DARPins, provides a foundation for advancing in-vivo CAR-T therapies toward clinical application. Table 5 provides a comparative overview of vector platforms used for in-vivo CAR therapies.[7]

Features	LV	AAV	mRNA-NC	mRNA-LNP
T cell targeting demonstrated	+	+	+	+
Synthetic product	-	-	+	+
Permanent gene transfer	+	-	-	-
Clinical experience	+	+	-	+
Immunogenic	+	+	Not yet determined	Not yet determined

The rapid progress in in-vivo CAR-T therapy demonstrates its potential to transform immunotherapy, yet several hurdles remain before broad clinical adoption is achieved. Key priorities are summarized in figure 11 below. With continued vector innovation, immune shielding strategies, and regulatory advancements, in-vivo CAR-T therapy is on the path to becoming a scalable, cost-effective, and transformative treatment approach. Moreover, the ability to engineer a diverse range of CAR-modified immune cells—such as CAR-NK, CAR-M, CAR-DC, and CAR-Tregs—offers a significant advantage, providing flexibility to tailor treatments for different disease settings while improving safety, durability, and therapeutic reach beyond traditional CAR-T approaches.

Figure 11: Key strategic priorities for clinical adoption of in-vivo CAR-T



Strategic Considerations for Global Regulatory Planning in in-vivo CAR Trials

As in-vivo CAR therapies move into early clinical development, biotech innovators are adopting global regulatory strategies to accelerate timelines and reduce costs. One strategic example involves leveraging Australia's regulatory ecosystem, which offers rapid ethics and regulatory approvals through the TGA, as well as significant R&D incentives and access to world-class clinical infrastructure. This has made Australia an attractive entry point for first-in-human studies, while maintaining compatibility with U.S. FDA and EMA requirements.

To support global expansion, sponsors are increasingly aligning early trials with multinational expectations — ensuring that clinical protocols, data collection methods, and quality standards meet the threshold for multi-region submissions. Data generated in Australia is commonly accepted in the U.S. and Europe, creating a foundation for broader regulatory filings.

One notable success using this approach includes the initiation of first-in-human dosing in the INVISE trial of an in-vivo lentiviral-based CAR therapy, conducted at leading institutions in Melbourne and Sydney (Interius BioTherapeutics). The strategy also enabled regulatory submissions to the EU and a pre-IND meeting with the U.S. FDA, supported by high-quality Australian-generated clinical data—demonstrating how early international trials can build a strong platform for global advancement.

This model is highly applicable to other developers in the in-vivo CAR space due to several factors: the need for rapid proof-of-concept, the high costs associated with U.S.-based early-phase trials, and the importance of generating globally recognized data to attract further funding and partnerships. As a result, a globally harmonized yet regionally optimized regulatory pathway—starting in Australia and expanding outward—offers a compelling blueprint for biotech companies aiming to scale novel in-vivo CAR programs efficiently and effectively. [26]

BEYOND CAR-T AND BEYOND ONCOLOGY

Diversifying CAR Modalities beyond T cells: In-vivo Generation of CAR-NK, CAR-M, CAR-DC and CAR-Treg

While CAR-T cells have led the field of cellular immunotherapy, scientific advancements are rapidly expanding this frontier. Alternative immune cell types—such as Natural Killer (NK) cells, macrophages (M), dendritic cells (DCs), and regulatory T cells (Tregs)—are now being engineered with CARs to overcome key limitations of CAR-T therapies, including safety, tumor accessibility, and applicability across disease types. Table 6 summarizes these emerging CAR platforms, highlighting their biological features, engineering approaches, clinical progress, and safety advantages.

Table 6: Emerging CAR platforms and indications

Cell Type	Mechanism and Advantages	Sources	Engineering Strategies	Clinical Trials*	Safety Profile	Indications
CAR-NK	Innate anti-tumor activity, HLA-independent killing, low GvHD risk, lower CRS/ICANS risk	PBMCs, iPSCs, cord blood, NK92 cell line	Incorporate NK-specific domains like NKG2D, DAP10/12; 4th-gen CARs produce IL-2/IL-15 for persistence	70+ trials underway	Minimal GvHD, reduced CRS/ICANS risk due to short persistence	Hematologic malignancies, solid tumors
CAR-M	Strong infiltration into solid tumors, antigen presentation, reversal of immuno-suppressive TME	PBMCs, iPSCs, THP-1 cell line	Engineered to express CARs; HER2-targeted CAR-Ms induce M1 polari-zation	4 trials ongoing	Low GvHD, limited expansion, mild side effects (fever, weight loss)	Primarily solid tumors (e.g., HER2+ cancers)
CAR-DC	Cross-presentation of antigens, effective T-cell priming, overcoming antigen escape	Conven-tional dendritic cells (cDC1 subset)	Engineered to prime naïve T cells; activate strong de novo immune responses	3 trials ongoing	Data limited, but favorable immune modulation profile	Solid tumors, potential for broader immune activation
CAR-Treg	Suppress immune responses, used in autoimmune diseases and transplant rejection	Natural Treg pool, engi-neered ex-vivo	MHC-independent antigen targeting; lower IL-2 de-pendency vs TCR-Tregs	Ongoing trials in transplant rejection, RA, UC, and other autoimmune dis-eases	Ex-vivo expan-sion risk of phenotype shift; in-vivo may mitigate this risk	Autoimmune diseases, transplant tolerance

**These figures are based on data published in a 2023 review article and may evolve with the rapid pace of clinical advancements; source: International Journal of Molecular Sciences, 2023*

As research advances, non-CAR-T cell therapies are poised to complement or even replace traditional CAR-T approaches in specific clinical settings. Their potential for off-the-shelf manufacturing, improved safety profiles, and unique immunological mechanisms may enable broader patient access and expanded indications, including solid tumors and autoimmune diseases. Continued progress in in-vivo engineering and combination strategies will be critical to unlocking their full therapeutic potential. [5,6]

In-vivo CAR Therapy Beyond Cancer

Beyond oncology, CAR-based immunotherapies are being explored for a range of conditions including autoimmune and inflammatory disorders, infectious diseases, fibrosis, and aging-related pathologies.

In-vivo CAR therapies for autoimmune diseases

CAR immunotherapy for autoimmune diseases is currently advancing along two main strategies: one involves using CAR-T cells to eliminate auto-reactive immune cells and achieve a deep immune reset, while the other focuses on employing CAR-T regulatory cells (CAR-Tregs) to suppress these harmful immune responses.

To date, treatment of autoimmune diseases with CAR-T has shown a lower incidence of ICANS and CRS than what has been seen in the treatment of cancer. While the exact reasons for the apparent lower levels of toxicity are still under investigation, factors such as dosing at lower levels in autoimmune disease, the design of the CAR products, and an overall lower disease burden are likely factors, What has been noted is a different toxicity known as Local Immune Cell-Associated Toxicity Syndrome (LICATS) which occurs often in CAR-T treated autoimmune patients and usually affects the areas of the body where the particular disease has affected the patient. LICATS can present as a skin rash, oral mucosal lesions and muscle inflammation/pain and kidney function impact. While most LICATS symptoms resolve on their own within a few weeks, treatment with corticosteroids can help accelerate recovery. LICATS may be related to the clearing of the B-Cells that the T-cells have targeted and killed.

A potential further advantage of CAR-T in autoimmune disease is the ability of the CAR-T cells to work well within tissues where other B-cell depleting therapies have not shown similar results. In studies comparing rituximab to CAR-T, deeper B-cell depletion was observed with the CAR-T treatment. [24]

A few pioneering companies are also leveraging RNA-based platforms to translate these strategies into in-vivo CAR therapies for autoimmune and inflammatory diseases—for example, Orna Therapeutics and Orbital Therapeutics.

Orna Therapeutics is advancing in-vivo CAR therapies using its circular RNA (oRNA®) platform combined with LNP delivery. Its panCAR™ programs aim to generate CAR-T, CAR-NK, and CAR-M cells directly in the body, enabling re-dosable, off-the-shelf treatment. Orna is actively exploring autoimmune indications and B-cell–mediated diseases, with early preclinical data (poster presented at the 66th American Society of Hematology (ASH) Annual Meeting, San Diego, December 2024) showing potent B-cell depletion in non-human primates. [21]

Similarly, Orbital Therapeutics is developing RNA-based medicines for immunomodulation, including in-vivo CAR-T therapies. The company focuses on targeting immune pathways relevant to autoimmune and inflammatory disorders, using selective RNA delivery to modulate immune responses. Backed by US$270M in Series A funding, Orbital is building a pipeline of in-vivo immunotherapies beyond oncology.[22]

In addition, Capstan's lead in-vivo CAR-T candidate, CPTX2309, targets CD19 in B-cell–mediated autoimmune diseases including SLE. Preclinical studies presented at ACR Convergence 2024 demonstrated successful in-vivo generation of CAR-T cells and robust B-cell depletion in non-human primates—without the need for lymphodepletion.[23]

Building on this momentum, Umoja Biopharma is developing UB-VV400, an in-vivo CD22-targeted CAR-T therapy for autoimmune and oncology indications. Preclinical data published in Blood (June 2024) showed complete and durable B-cell depletion in non-human primates without lymphodepletion. The therapy is powered by Umoja's VivoVec™ platform, enabling direct in-body CAR-T generation. In January 2025, Umoja raised $100M in Series C funding to advance UB-VV400 into clinical trials. Future plans include initiating multiple clinical studies targeting B-cell–mediated autoimmune diseases.[25]

These developments highlight a clear trend toward in-vivo CAR technologies as a promising therapeutic approach beyond cancer. Following oncology, autoimmune diseases have emerged as the second most actively explored area for in-vivo CAR applications. By enabling direct, in-body immune cell engineering, these platforms may overcome key limitations of conventional CAR therapies, offering broader accessibility and more durable outcomes for patients with immune-driven conditions.

Additional ongoing clinical investigations exploring CAR therapies for autoimmune and inflammatory diseases are summarized in the Appendix, based on data from ClinicalTrials.gov. [17]

The following table summarizes the emerging applications and CAR antigens across these non-cancer indications.

Table 7: Emerging CAR antigens, therapies and indications

Therapeutic Area	Target/Antigen	Indication	Therapy
Autoimmunity	CD19	Lupus	CAR-T
Autoimmunity	BCMA	Lupus/SLE	CAR-T
Autoimmunity	Dsg3	PV	CAAR T
Autoimmunity	MuSK	MG	CAAR T
Autoimmunity	La/SSB	SS	CAAR T
Autoimmunity	Insulin	T1D	CAR-Treg
Autoimmunity	GAD65	T1D	CAR-Treg
Autoimmunity	TNP	IBD	CAR-Treg
Autoimmunity	CV	RA	CAR-Treg
Autoimmunity	CEA	IBD	CAR-Treg
Autoimmunity	GD3	Vitiligo	CAR-Treg
Autoimmunity	HPi2	T1D	CAR-Treg
Autoimmunity	MOG	MS	CAR-Treg
Autoimmunity	Flic	IBD	CAR-Treg
Autoimmunity	MBP	MS	CAR-Treg
Inflammation	IL-5R a	Asthma	CAR-T

Therapeutic Area	Target/Antigen	Indication	Therapy
Infection	HBsAg	HBV	CAR-T
Infection	PD-1	HIV/SIV	CAR-T
Infection	gp120	HIV/SIV	CAR-T
Infection	gB	CMV	CAR-T
Infection	E2 protein	HCV	CAR-T
Infection	LMP1	EBV	CAR-T
Infection	gp350	EBV	CAR-T
Infection	spike protein	Covid-19	CAR NK
Infection	GXM	Cryptococcus neoformans	CAR-T
Infection	B-glucan	Aspergillus fumigatus	CAR-T
Fibrosis	FAP	cardiac fibrosis	CAR-T
Fibrosis	uPAR	hepatic fibrosis	CAR-T
Senescence	NKG2DL	senescence	CAR-T
Senescence	uPAR	senescence	CAR-T
Transplantation	OX40	GvHD	CAR-T
Transplantation	HLA-A2	GvHD	CAR-Treg
Bleeding disorders	FVIII	Hemophilia	CAR-Treg

Source: Biomark Res 13, 23 (2025). https://doi.org/10.1186/s40364-025-00736-8

Notes: RA, rheumatoid arthritis; SS, Sjögren's syndrome; CV, citrullinated vimentin; MS, multiple sclerosis; EAE: encephalomyelitis; MuSK, muscle-specific tyrosine kinase; TNP, 2,4,6-trinitrophenol; MG, myasthenia gravis; PV, pemphigus vulgaris; T1D, type 1 diabetes; IBD, inflammatory bowel diseases; MBP, myelin basic protein; MOG, myelin oligoden-drocyte glycoprotein; Dsg, desmoglein; CEA, carcinoembryonic antigen; FliC, flagellin derived from Escherichia coli H18; uPAR, urokinase-type plasminogen activator receptor; gB, glycoprotein B; gp350, envelope glycoprotein 350; Treg, CAR-regulatory T cells; CAR NK, CAR natural killer; CAAR, chimeric autoantibody receptor - CAAR T cells represent a promising and adaptable approach for selectively targeting autoreactive B cells involved in antibody-driven autoimmune disorders.

There are also notable studies described below, that have particularly explored in-vivo CAR-T therapy for diseases, such as cardiac fibrosis. These findings highlight the potential of in-vivo CAR-T therapy beyond cancer, offering a novel approach to treating fibrosis-related diseases.

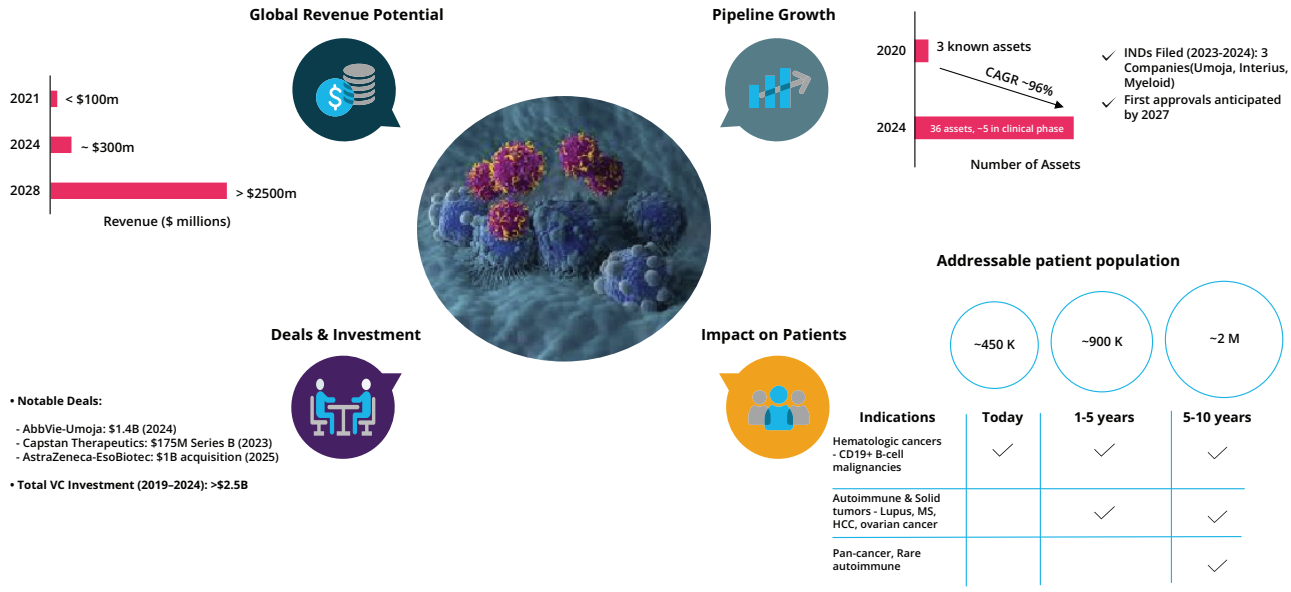
• Aghajanian et al. demonstrated that FAP-targeting CAR-T cells reduced cardiac fibrosis and restored myocardial function in a mouse model of AngII/PE-induced cardiac injury.

• Rurik et al. engineered circulating T cells using CD5-targeted lipid nanoparticles (LNPs) loaded with FAP-specific CAR mRNA, leading to CAR-T accumulation near activated fibroblasts and improved cardiac function within 14 days of a single injection.[5]

FUTURE DIRECTIONS AND OUTLOOK

In-vivo CAR therapy is gaining significant momentum, with its potential to offer scalable, off-the-shelf immunotherapy solutions. Between 2020 and 2024, the number of pipeline assets has expanded over ten-fold, driven by innovation in delivery systems and strong investor confidence. High-profile partnerships—such as AbbVie-Umoja and AstraZeneca's $1B acquisition of EsoBiotec—reflect the strategic importance of this modality. Market forecasts estimate that revenues could surpass $2.5 billion by 2028, with an addressable patient population projected to potentially reach nearly 2 million globally by 2032, spanning both oncology and autoimmune indications. (Figure 12)

Figure 12: In-vivo CAR therapies - emerging assets, milestones, and addressable populations



In-vivo CAR therapy, by uniquely addressing the manufacturing, cost, and safety challenges of ex-vivo approaches, is poised to revolutionize the field of immunotherapy. By eliminating the need for cell harvesting and re-infusion, this approach reduces production time, simplifies logistics, and removes the risk of GvHD by utilizing the patient's own immune system. This has opened the door to broader clinical applications, including solid tumors and autoimmune diseases, where early data from CAR-T studies in lupus and multiple sclerosis are encouraging. Developers are now actively evaluating whether to prioritize highly competitive oncology spaces or underserved autoimmune niches where in-vivo approaches could offer a differentiated edge.

As the field evolves, dosing strategies and route of administration—particularly intravenous (IV) delivery—have become critical to ensuring both efficacy and safety. Translating effective doses from preclinical animal models to humans remains challenging, especially due to species-specific differences in pharmacokinetics and vector clearance. Studies have highlighted the need for improved predictive models and careful dose optimization to mitigate systemic toxicities. Moving forward, refining these parameters will be essential to achieving consistent clinical outcomes across diverse patient populations.

Looking ahead, delivery optimization remains a central challenge and opportunity. Viral vectors such as lentivirus offer the potential for durable expression, while non-viral systems like lipid nanoparticles (LNPs) promise modularity and repeat dosing. Companies like Myeloid Therapeutics and Umoja Biopharma are testing both ends of this delivery spectrum, and pharmacokinetic data from these trials will likely shape future platform strategies.

Importantly, autologous, allogeneic, and in-vivo CAR-T formats are expected to coexist, with their use tailored to the strengths of each platform and the needs of specific diseases. In-vivo CAR-T platforms are also expected to influence the broader gene therapy field—particularly through innovations that address host immune response, targeted delivery, and scalable manufacturing. These advancements may accelerate in-vivo gene therapies for hematologic, autoimmune, infectious, cardiac, and developmental disorders. If current research and clinical momentum are sustained, the number of in-vivo CAR assets is projected to exceed 100 by 2025, signaling a transition into what many now view as the "Age of In-vivo". [7,13,14,15,16]

FEEDBACK FROM KEY OPINION LEADERS

Novotech has had the opportunity to interact with several experts in the field of CAR-T treatments and specifically question them about the potential of in-vivo CAR-T.

In speaking with a key investigator of CAR-T for autoimmune diseases, the investigator noted that the deep B-cell depletion from CAR-T into tissue is the real game changer as compared to BiTES or monoclonal antibodies. They also noted that few patients overall have yet been treated with In-vivo CAR-T and as such we do not know much about the off-target effects of these treatments yet, regardless of whether it is viral vector or LNP delivery. For this reason, the investigator noted that early studies of In-vivo CAR-T products should be conducted at sites capable of treating the associated toxicities known to CAR-T such as ICANS and CRS. Novotech asked if the expected safety profile of in-vivo CAR-T would be more similar to cellular-based ex-vivo CAR-T treatments or to the safety profile of a MAB or other B-cell depleting treatment. In general, in autoimmune patients it would be expected that the toxicity will be as low or lower for In-vivo CAR-T (just as it has been shown to be lower in Ex-vivo CAR-T treatments for autoimmune disease patients) than the toxicity seen in patients undergoing CAR-T treatments for oncology.

In-vivo CAR-T trials are of significant interest to both investigators and patients. Novotech experience from outreach to investigators and sites when approached about participating in clinical trials of in-vivo CAR-T in oncology and autoimmune indications has been largely positive. Notable limitations to participation in clinical trials have been the ability or experience to treat toxicity-related complications such as CRS and ICANS or a lack of experience with ATMP/GMO products.

CURRENT DISCUSSIONS AND OPINION ON IN-VIVO CAR-T

In recent conferences such as the ISCT 2025 Annual Meeting and the ASGCT 2025 Annual meeting, there has been significant discussion regarding the evolution of CAR-T and the role in-vivo therapies will play.

Along with the afore-mentioned potential for in-vivo CAR-T to reduce costs, provide more patient access and simplify the patient journey for CAR-T treatment, in-vivo CAR-T was noted to alleviate the need for apheresis and lymphodepletion in many applications. Apheresis was noted as one of the main limiting factors in scheduling patients for autologous CAR-T treatments due to a lack of capacity at many sites and a lack of training for others. Apheresis was also noted to play a crucial factor in the quality of the CAR-T products, where poor quality cells were due to a lack of standardization for cell collection and poor cell quality for late-stage disease patients.

Additionally, in-vivo CAR-T was noted to be on a parallel path with technologies enabling faster and cheaper production of ex-vivo CAR-T products such as automated and closed-system manufacturing. In-vivo CAR-T has some inherent advantages related to the ability to treat patients off-the-shelf. Although universal/allogenic products are also considered off-the-shelf, and while the in-vivo CAR-T product is subject to robust release testing, the burden of testing is much lower with in-vivo CAR-T therefore reducing production costs and time.

Lastly, many companies are focusing on enhancements to the concept of in-vivo CAR-T, such as targeted LNP particles to enhance CAR delivery to specific cells. Some examples from abstracts presented at the ASGCT 2025 conference include:

- Antibody-based ligand targeting strategy to direct lipid nanoparticles (LNPs) carrying mRNA encoding CD19 CAR to specific T cell populations in vivo. (Aera Therapeutics)
- Cell-targeted LNP (ctLNP) platform to enable the in vivo generation of mRNA-based CAR-T cells. Because CAR expression is driven by ctLNP-delivered mRNA, the dosing can be flexibly adjusted and repeated, allowing efficient regimens that minimize the risk of CRS while maximizing therapeutic efficacy. (Nitto Denko Corporation)
- tLNP-circCAR, a novel in vivo anti-CD19-CAR circular RNA product delivered via targeted lipid nanoparticles (tLNPs). (RiboX Therapeutics)
- Targeted lipid nanoparticles (tLNPs) are a product of the CellSeekerTM platform technology and comprise a proprietary LNP formulation, conjugated to a recombinant antibody that binds to a specific surface marker on HSPCs, enabling efficient delivery of CRISPR-Cas components to genetically edit HSPCs in vivo. (Capstan Therapeutics)

APPENDIX

Top startups, mid and large biotech companies driving in-vivo CAR therapy innovations include Abintus, Myeloid, Cellinfinity Bio, CytoMed Therapeutics Limited, Precision BioSciences, Inc, Interius BioTherapeutics and Umoja Biopharma to name a few. The infographic below shows the In-Vivo CAR-T competitive landscape.

Company	Intended Target Cells	CAR Targets	Route of Administration
Lentiviral			
ABINTUS BIO	CD4+ T cells. CD8+ T cells, NK cells, NKT cells, and γδ T cells	CD19	IV
EXUMA Biotech	CD3+ Tcells	Undisclosed	ILN
interius	CD7+ cells (T cells and NK cells)	CD20 BCMA+ BCMA undisclosed CD19 target	IV
kelonia	T cells	BCMA	ILN
Sana Biotechnology	CD8+ Tcells CD8+ Tcells	CD19 CD22 BCMA	IV
Umoja BIOPHARMA	CD3+ Tcells	CD 19 FAP PSMA CA IX	ILN
	Activated T Cells	CD20	ID+ILN
Lipid Nanoparticle (LNP)			
capstan™ therapeutics	CD8 T + cells CD2+ cells (T and NK cells) CD5+ cells T NK and Macs)	CD19 CD20 BCMA FAP Other undisclosed targets	IV
carisma THERAPEUTICS moderna	Macrophages	Undisclosed	Undisclosed
ORNA	Multiple immune cells	Undisclosed	Undisclosed
TESSERA	CD4+ and CD8+ T cells	Undisclosed	ILN
MYELOID	Myeloid cells	TROP2 GPC3	Undisclosed
Lipid Nanoparticle (LNP)			
ixaka	CD3+cells	CD19	Undisclosed
sanofi	Undisclosed	Undisclosed	Undisclosed
Adenovirus- Based Particles			
Vector BioPharma Precision Gene Delivery	CD3+cells	CD19	Undisclosed
ensoma	Undisclosed	Undisclosed	Undisclosed
Other/Undisclosed Technology			
EsoBiotec	Undisclosed	Undisclosed	Undisclosed
GIGAMUNE	Undisclosed	Undisclosed	Undisclosed
ImmTune Therapies	Undisclosed	Undisclosed	Undisclosed

Sources: Spencer Knight; Company Reports; William Blair Equity Research

Ongoing clinical trials of CAR-T cell therapy in autoimmune and inflammatory diseases

Disease	NCT no.	Antigen	Phase	Status	T cell source	Sponsor
SLE	NCT06350110	CD19/BCMA	Phase 1/2	Recruiting	Autologous	Essen Biotech
	NCT06340750	BAFF-R	Early Phase 1	Recruiting	Autologous	Luminary Therapeutics
	NCT03030976	CD19	Phase 1	Unknown	Autologous	Shanghai GeneChem Company; RenJi Hospital
	NCT06333483	CD19	Phase 1	Recruiting	Autologous	Autolus Limited
	NCT06189157	CD19	Phase 1/2	Recruiting	Autologous	Miltenyi Biomedicine GmbH
	NCT05474885	CD19/BCMA	Phase 1	Recruiting	Autologous	iCell Gene Therapeutics; iCAR Bio Therapeutics Ltd China
	NCT05869955	CD19	Phase 1	Recruiting	Autologous	Juno Therapeutics, Inc., a Bristol Myers Squibb Company
	NCT05798117	CD19	Phase 1/2	Recruiting	Autologous	Novartis Pharmaceuticals
	NCT05858684	CD19/BCMA	Phase 1	Recruiting	Autologous	RenJi Hospital; Gracell Biotechnology Shanghai Company Ltd
	NCT06121297	CD19	Phase 1/2	Recruiting	Autologous	Cabaletta Bio
	NCT06297408	CD19	Phase 1	Not yet recruiting	Autologous	Shanghai Ming Ju Biotechnology Company Ltd
	NCT05846347	CD19/BCMA	Phase 1	Recruiting	Autologous	RenJi Hospital; Gracell Biotechnology Shanghai Company Ltd
immunological nephritis	NCT06342960	CD19	Phase 1/2	Recruiting	Autologous	Kyverna Therapeutics
MS	NCT05938725	CD19	Phase 1	Recruiting	Autologous	Kyverna Therapeutics
	NCT06138132	CD19	Phase 1	Recruiting	Autologous	Stanford University; Kyverna Therapeutics
	NCT06220201	CD19	Phase 1	Recruiting	Autologous	Juno Therapeutics, Inc., a Bristol Myers Squibb Company; Celgene Corporation
	NCT06384976	CD19	Phase 2	Not yet recruiting	Autologous	Kyverna Therapeutics
	NCT06193889	CD19	Phase 2	Recruiting	Autologous	Kyverna Therapeutics
MG	NCT05451212	MuSK	Phase 1	Recruiting	Autologous	Cabaletta Bio
	NCT04146051	BCMA	Phase 1/2	Recruiting	Autologous	Cartesian Therapeutics
	NCT06154252	CD19	Phase 1/2	Recruiting	Autologous	Cabaletta Bio
IIM	NCT06298019	CD19	Phase 1	Not yet recruiting	Autologous	Stanford University; Kyverna Therapeutics
autoimmune hemolytic anemia	NCT06231368	CD19	Phase 1	Recruiting	Autologous	Regenerative Medical Center, Institute of Hematology & Blood Diseases Hospital, China; Juventas Cell Therapy Ltd
PV	NCT04422912	DSG3/CD19	Phase 1	Recruiting	Autologous	Cabaletta Bio
dermatomyositis	NCT06298019	CD19	Phase 1	Not yet recruiting	Autologous	Stanford University; Kyverna Therapeutics

SLE, systemic lupus erythematosus; MS, multiple sclerosis; MG, myasthenia gravis; AIHA, autoimmune hemolytic anemia; PV, pemphigus vulgaris; IIM, Idiopathic inflammatory myopathies

Source: Biomark Res 13, 23 (2025). https://doi.org/10.1186/s40364-025-00736-8

NOVOTECH OVERVIEW

Founded in 1997, Novotech is a global full-service clinical Contract Research Organization (CRO) focused on partnering with biotech companies to accelerate the development of advanced and novel therapeutics at every phase.

Novotech has direct experience helping companies develop In-vivo CAR products, including LNP mRNA-based CAR-T therapies for oncology and autoimmune indications. Novotech has conducted over 100 ATMP studies including gene therapies, gene modified cell therapies, and mRNA products.

Recognized for its industry-leading contributions, Novotech has received numerous prestigious awards, including the Frost &Sullivan 2024 Global Biotech CRO Award, 2024 Employer of Choice, 2024 Great Place to Work in the US, 2024 Brandon Hall Gold Award, CRO Leadership Award 2023, the Asia-Pacific Cell & Gene Therapy Clinical Trials Excellence 2023, the Asia-Pacific Contract Research Organization Company of the Year Award since 2006.

The Company offers a comprehensive suite of services including laboratories, Phase I facilities, drug development consulting, regulatory expertise, and has experience with over 5,000 clinical projects, including Phase I to Phase IV clinical trials and bioequivalence studies. With a presence in 34 office locations and a dedicated team of 3,000+ professionals worldwide, Novotech is a trusted end-to-end strategic partner of choice.

For more information or to speak to an expert team member visit www.Novotech-CRO.com

Sources: Spencer Knight; Company Reports; William Blair Equity Research

REFERENCES

1. Challener, C. A. (2023, January 30). Technology for in vivo CAR T-cell therapy advances. BioPharm International.

2. Hung Trinh, Vertex Biopharm Consulting, February 2025; ASGCT.org

3. Bui, T. A., Mei, H., Sang, R., Ortega, D. G., & Deng, W. (2024). Advancements and challenges in developing in vivo CAR T cell therapies for cancer treatment. EBioMedicine, 106, 105266.

4. Policano, J. (2025, January 15). Our science. Umoja Biopharma.

5. Pinto, E., Lione, L., Compagnone, M., Paccagnella, M., Salvatori, E., Greco, M., Frezza, V., Marra, E., Aurisicchio, L., Roscilli, G., & Conforti, A. (2025). From ex vivo to in vivo chimeric antigen T cells manufacturing: new horizons for CAR T-cell based therapy. Journal of Translational Medicine, 23(1).

6. Uscanga-Palomeque, A. C., Chávez-Escamilla, A. K., Alvizo-Báez, C. A., Saavedra-Alonso, S., Terrazas-Armendáriz, L. D., Tamez-Guerra, R. S., Rodríguez-Padilla, C., & Alcocer-González, J. M. (2023). CAR-T cell therapy: From the shop to cancer therapy. International Journal of Molecular Sciences, 24(21), 15688.

7. Michels, A., Ho, N., & Buchholz, C. J. (2022). Precision medicine: In vivo CAR therapy as a showcase for receptor-targeted vector plat-forms. Molecular Therapy, 30(7), 2401–2415.

8. Abintus Bio, Inc. (2024, October 3). Abintus Bio, Inc. | LinkedIn [Online forum post].

9. In vivo Car-T gains traction. (n.d.). ApexOnco - Clinical Trials News and Analysis.

10. BioSpace. (2025, January 24). EXUMA Biotech Announces Phase 1 Results for its Autologous HER2 Directed, Tumor Metabolism Regu-lated (TMR) CAR-T Product at ASCO GI 2025.

11. Alliance for Regenerative Medicine. (2024). Sector snapshot: Review of the 2024 cell and gene therapy approvals and notable achieve-ments.

12. Recent trends of CAR-T cell therapies for cancer Treatment - AAPS Newsmagazine. (n.d.). Higher Logic, LLC.

13. Beacon. (2024, December 12). A deep dive into the in vivo CAR therapies landscape.

14. Myeloid Therapeutics, Inc. (2024, November 8). Myeloid Therapeutics Announces SITC 2024 Oral Presentation on MT-303, the First in vivo GPC3 Targeting mRNA CAR in Human Studies for Advanced Hepatocellular Carcinoma (HCC). Prnewswire.

15. The Guardian. (2025, March 2). AstraZeneca to acquire EsoBiotec in $1bn deal to boost in vivo CAR-T pipeline.

16. Fierce Biotech. (2024, January 9). Umoja Biopharma grabs $210M AbbVie deal to expand CAR-T tech.

17. Huang, Q., Zhu, X. & Zhang, Y. Advances in engineered T cell immunotherapy for autoimmune and other non-oncological diseas-es. Biomark Res 13, 23 (2025).

18. Rossi, M., & Breman, E. (2024). Engineering strategies to safely drive CAR T-cells into the future. Frontiers in Immunology, 15.

19. Drug Target Review. (2024, February 15). In vivo CAR-T: faster, cheaper and more effective cancer care.

20. Timmers, M., Roex, G., Campillo-Davo, D., Flament, H., & Van Tendeloo, V. F. I. (2022). In vivo CAR T cell therapy: A step closer to the clinic. Frontiers in Immunology, 13, 887406.

21. BioSpace. (2024, December 10). ORNa Therapeutics presents data highlighting novel PANCARTM approach to unlock LNP-Mediated RNA delivery for B cell mediated diseases at the 66th ASH annual meeting.

22. BioSpace. (2024a, September 9). Orbital Therapeutics.

23. Business Wire. (2024, November 18). Capstan Therapeutics presents preclinical data on lead in vivo CAR-T candidate CPTX2309 at American College of Rheumatology (ACR) Convergence 2024.

24. Smith, J., Lee, A. & Kumar, R. B cell depletion therapy for systemic autoimmune diseases: efficacy, mechanisms, and biomarkers. Ann Rheum Dis 84, 106–114 (2025).

25. Umoja Biopharma. (2025, January 18). Umoja Biopharma announces oversubscribed $100 million Series C financing to advance in vivo CAR-T pipeline through key oncology clinical milestones.

26. Cell & Gene. (2024, April 3). Inside Interius' global regulatory strategy.

27. Song, Z., Zhou, Y., Wang, B., Geng, Y., Tang, G., Wang, Y., & Yang, J. (2025). In vivo production of CAR T cell: Opportunities and chal-lenges. Genes & Diseases, 101612.

28. Myeloid Therapeutics. Myeloid Therapeutics Unveils First-in-Human In Vivo mRNA CAR Data, Marking a Breakthrough in RNA-Based Immuno-Oncology at the 2025 ASCO Annual Meeting. Published May 31, 2025.

NOVOTECH™

Biotech's Partner at Every Phase

Talk to a Novotech expert about your clinical trial needs

Founded in 1997, Novotech is a global full-service clinical Contract Research Organization (CRO) focused on partnering with biotech companies to accelerate the development of advanced and novel therapeutics at every phase.

Recognized for its industry-leading contributions, Novotech has received numerous prestigious awards, including the Frost & Sullivan 2024 Global Biotech CRO Award, 2024 Clinical Trials Arena Award for Excellence in Business Expansion, Marketing, and Innovation, 2024 Employer of Choice, 2024 Great Place to Work in the US, 2024 Brandon Hall Gold Award, CRO Leadership Award 2023, the Asia Pacific Cell & Gene Therapy Clinical Trials Excellence 2023, the Asia-Pacific Contract Research Organization Company of the Year Award since 2006.

The Company offers a comprehensive suite of services including laboratories, Phase I facilities, drug development consulting, regulatory expertise, and has experience with over 5,000 clinical projects, including Phase I to Phase IV clinical trials and bioequivalence studies. With a presence in 34 office locations and a dedicated team of 3,000+ professionals worldwide, Novotech is a trusted end-to-end strategic partner of choice.

For more information or to speak to an expert team member visit

novotech-cro.com/our-company