Genetic engineering strategies for cell-based therapy: viral, non-viral, and precision genome editing technologies

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ABSTRACT

Objective: To review the existing genetic engineering strategies commonly used for generating chimeric antigen receptor (CAR)-T cell therapy. **Methods:** This article provides a comprehensive literature review and compares different approaches on viral, non-viral and precision genome editing technologies, aiming to provide an overview and guidelines for methods selection during clinical CAR-T cell manufacturing. **Results:** CAR-T cell therapy has revolutionized cancer treatment for hematological malignancies. The manufacturing of CAR-T cell therapy relies on the genetic modification of T cells to achieve ectopic CAR gene expression. Depending on the evolving CAR designs, viral, non-viral, and genome editing platforms are used for optimal CAR expression, which could determine the clinical efficacy and safety profiles of these products. Efforts have been ongoing to empower CAR-T cell efficacy while minimizing toxicity over the past decades. **Conclusion:** Successful CAR-T cell therapy depends on rational platform selection and optimization of gene delivery methods based on clinical needs and context.

Keywords: Chimeric antigen receptor; Genome editing; Cell-based therapy; In vivo CAR-T therapy.

INTRODUCTION

Chimeric antigen receptor (CAR)-T cell therapy is a programmable cell therapy achieved through genetic modification.[1] These modifications enable antigen recognition, cell activation, persistence, resistance to immunosuppression, and safety control. Three gene delivery platforms dominate: viral vectors, non-viral delivery, and precision genome editing. In this article, we discuss the differences among these platforms and their common applications.

Viral vector

Viral delivery systems use engineered viruses to introduce foreign genetic material of interest into target cells. The virus serves as a biological vector, in which the gene of interest is designed to be packaged and pseudotyped for delivery with specific cell tropism, with pathogenic genes removed for safety. Common viruses include lentivirus,[2] γ-retrovirus,[3] and adeno-associated virus (AAV).[4] Lentivirus for clinical use is a replication-defective or self-inactivating system split into multiple plasmids to reduce the risk of replication-competent virus formation and improve safety. In clinical manufacturing, this platform is referred to as a third-generation lentiviral system, in which the necessary viral components are supplied in trans by separate plasmids: a transfer plasmid containing the therapeutic gene flanked by long terminal repeats and packaging signals in a self-inactivating design, packaging plasmids encoding gag, pol, and rev, and an envelope plasmid that determines vector tropism. γ-retrovirus is derived from Moloney murine leukemia virus and is usually made using stable producer

cell lines such as Phoenix-Ampho or Eco, which stably express gag and pol.[5] Both vector systems are clinically proven, regulatory-accepted, and scalable for commercial CAR-T manufacturing. Lentiviral vectors are used in Kymriah™, Breyanzi™, and Abecma™. In contrast, γ-retroviral vectors are used in products such as Yescarta™ and Tecartus™, both developed by Kite Pharma. AAV is often used in *in vivo* gene therapy for genetic diseases such as hemophilia and muscular dystrophy.[4] The episomal expression and limited packaging size make this system ideal for CRISPR-mediated insertion of the CAR construct and enzyme/protein replacement purposes.

There is an intermediate platform derived from the viral system called a virus-like particle (VLP). VLPs are self-assembling structures that mimic the architecture of a virus but lack the viral genome, so they cannot replicate.[6] Essentially, VLPs are made by expressing one or more viral structural proteins, such as capsid or envelope proteins, in a host cell. These proteins spontaneously assemble into particles that have the same size, shape, and surface features as the native virus, which allows them to interact with target cells or the immune system as a virus would. Because they are non-replicating and non-infectious, VLPs are widely used as safe vaccine platforms (e.g., hepatitis B and HPV vaccines) and as delivery vehicles for proteins, RNA, or small molecules. In gene therapy or cell therapy research, VLPs can sometimes be engineered to carry functional cargo without integrating viral genomes, providing a temporary effect with reduced safety concerns compared with conventional viral vectors. To date, there are no widely reported VLP-based therapies that have reached later-stage clinical trials in human CAR-T or other *ex vivo* cell therapy applications, but multiple platforms are actively advancing through preclinical validation prior to clinical testing.[7]

Non-viral-based gene delivery

Non-viral gene delivery systems comprise a variety of approaches, including physical and chemical-based methods to package the genetic material for transfer into target cells. Common non-viral gene delivery approaches include using naked DNA electroporation, lipid nanoparticles (LNPs), and exosome vesicles[8] (Table 1). Currently, no FDA-approved CAR-T or engineered cell therapy uses a fully non-viral gene delivery platform, and all approved products rely on lentiviral or γ-retroviral vectors for stable gene delivery. However, several emerging non-viral approaches in clinical development aim to reduce manufacturing costs, simplify chemistry, manufacturing, and controls, and mitigate insertional safety concerns. Regardless of the specific non-viral delivery methodology, three principal vector platforms are commonly engineered for efficient delivery: minicircle/nanoplasmid DNA, DNA transposon systems, and CRISPR/Cas9-based systems (Table 2). Manufacturing scalability has been enabled by flow electroporation platforms, which allow current good manufacturing practice (cGMP)-compatible delivery of DNA, mRNA, or protein into large cell batches with improved reproducibility and cell viability. The key differences between viral and non-viral delivery systems are summarized in Table 3.

Table 1. Approaches to non-viral gene delivery.

Technology	Mechanism	Key advantages	Key limitations	Best use cases
Electroporation with DNA, RNA, or RNP (e.g., conventional plasmids, mRNA, self-amplifying RNA, and circRNA)	An electric pulse transiently opens the target cell membrane to deliver nucleic acids or protein complexes	High delivery efficiency across many primary cell types; flexible cargo (DNA/mRNA/RNP)	Trade-off between efficiency and viability; optimization is heavy; cell stress	Knockouts/edits (RNP), transient expression, multiplex editing
LNPs	Lipid vesicles deliver RNA/DNA to the cytosol	Scalable chemistry; tunable composition; low immunogenicity	Endosomal escape as efficiency key bottleneck; cell-type variation; durability low	mRNA delivery; *in vivo* editing; systemic delivery targets
Exosomes/extracellular vesicles	Membrane-bound nanoparticles to deliver nucleic acids and RNPs	Biocompatible; intrinsic targeting	Loading efficiency low; manufacturing challenge	Signal delivery; immuno-modulation, *in vivo* editing

circRNA: circular DNA; RNP: ribonucleoprotein. Source: Elaborated by the authors.

Table 2. Vector systems for non-viral gene delivery.

Vector system	Mechanism of action	Key advantages	Key limitations	Best use cases
Transposon systems (piggyBac, Sleeping Beauty)	Sleeping Beauty: a reconstructed Tc1/mariner transposon originally derived from fish, in which a transposase enzyme mediates stable integration of a transgene flanked by ITRs into TA dinucleotide sites in the genome; piggyBac: originally identified in insects, integrates at TTAA sites of genome	Stable integration; large cargo capacity; simpler than viral mechanism	Semi-random integration; careful safety control required	Stable multi-gene payloads
Minicircle DNA; miniplasmids; nanoplasmids; Doggybone DNA	Supercoiled minimal plasmid delivered via carrier	Higher expression than conventional plasmid; reduced bacterial DNA	Still relatively transient; intracellular barriers remain	Sustained non-viral expression
CRISPR/Cas9; base/prime editors (RNP + template)	Direct edits via RNP complexes and repair templates	Precision edits with or without double-strand breaks	Homology-directed repair efficiency low in primary cells; potential creation of random insertions or deletions (indels) in non-homologous end joining process for gene knockout; optimization is heavy	Precise gene correction, knock-in, gene knockout while knock-in editing

Source: Elaborated by the authors.

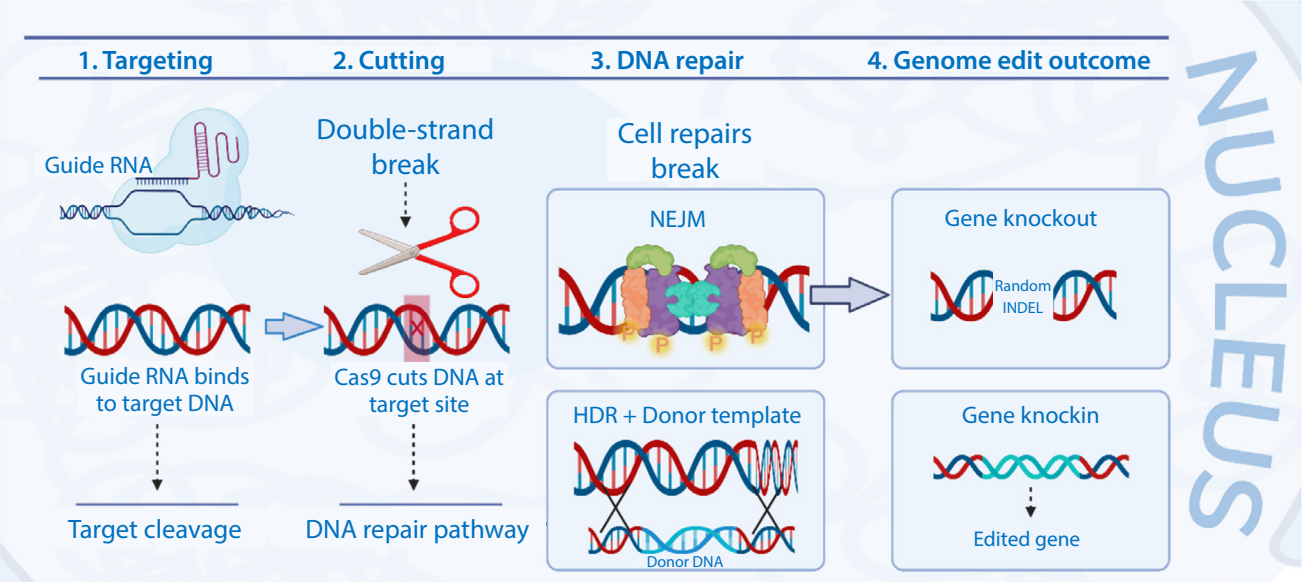
Table 3. Comparison between viral and non-viral gene delivery systems.

	Viral delivery systems	Non-viral delivery systems
Common modalities	Lentivirus, γ-retrovirus, AAV	Electroporation (DNA/RNA), transposons (Sleeping Beauty, piggyBac), LNP, polymer nanoparticles, CRISPR RNP
Transduction/transfection efficiency	High and consistent, especially in primary T cells	Moderate to high (cell-type dependent); often lower than the viral method
Expression durability	Long-term, stable (integrating viruses)	Transient (mRNA/RNP) or stable (transposons)
Genome integration	Yes (lentivirus, γ-retrovirus); semi-random (AAV)	Optional (transposons integrate; mRNA/RNP non-integrating)
Insertional mutagenesis risk	Present (low but non-zero; regulatory concern)	None for mRNA/RNP; controlled for transposons
Cargo size limit	Limited (e.g., ~8–9 kb for lentivirus; ~7-8 kb for γ-retrovirus; ≤ ~ 4.5 kb for AAV)	Large payloads possible (multi-gene circuits)
Multiplex engineering	Difficult (multiple vectors or bi/tri-cistronic)	Straightforward (simultaneous delivery of multiple RNAs/DNAs)
Cell viability impact	Generally low	Electroporation can reduce viability unless optimized
Manufacturing complexity	High (cell-based production, multiple plasmids, viral harvest)	Low to moderate (synthetic reagents, simpler workflows)
Scalability	Feasible at a large scale with optimization	Highly scalable and parallelizable
Batch-to-batch variability	Moderate–high	Low
Cost of goods	High (GMP viral vector production dominates cost)	Low to moderate
Release testing burden	Heavy (RCL/RCR, vector copy number, infectivity)	Lighter (residual DNA/RNA, off-target analysis)
Regulatory maturity	Very high (many approved products)	Growing rapidly
Turnaround time	Longer (vector production lead time)	Short (days vs. weeks)
Re-dosing flexibility	Limited (anti-viral immunity)	Excellent
Indication	Cancer indications: hematological malignancies and solid tumors	Non-cancer indications: autoimmune, enzyme deficiency, fibrosis, hyperinflammation, infection, transplantation

Source: Elaborated by the authors.

Genome editing for cell therapy

Genome editing has revolutionized the development of next-generation cell therapies by enabling precise manipulation of immune cells to enhance efficacy and safety. Over the past decades, genome editing methods have evolved from meganucleases for highly specific DNA recognition and modification.[9] To zinc-finger nucleases[10] and TALENs for protein-guided site-specific cleavage.[11] Recent technologies include CRISPR/Cas systems for targeted double-strand breaks, base editors for precise single-nucleotide changes without inducing double-strand breaks, and prime editors for programmable insertions, deletions, and substitutions.[11] Together, these platforms enable precise, efficient, and versatile genetic modifications in diverse cell types. In CAR-T manufacturing, precise genome editing has been used to enhance CAR-T cell persistence or potency, such as Tet2,[12] and the creation of allogeneic CAR-T cell therapy by knocking out β2M and TRAC. The mechanism of action for CRISPR/Cas9 in genome editing is shown in Fig. 1. For gene knock-in, the most common donor DNA templates are delivered by transient gene delivery methods such as AAV, DNA, and DNA-RNA hybrids.



Source: Elaborated by the authors.

Figure 1. Future outlook: toward in vivo CAR-T cell therapy.

Looking forward, the convergence of *in vivo* CAR-T cell therapy over conventional *ex vivo* CAR-T cell therapy (Table 4) and virus-free genome engineering promises to transform cell therapy. These next-generation strategies aim to make treatments more accessible, safer, and more precisely controllable, reducing manufacturing complexity and immune-related risks. While *in vivo* CAR-T cell therapy offers advantages in scalability and accessibility, potential challenges include regulatory paradigm changes and safety uncertainties because it shifts from a controlled *ex vivo* manufacturing environment to direct *in vivo* gene editing. Regulators consider risks such as off-target transduction and unintended tissue exposure associated with viral or LNP delivery systems. In addition, while critical quality attributes can be defined for the delivery platform itself (e.g., lentivirus or LNP), the absence of a fully characterized final engineered cell product complicates release testing, comparability, and long-term safety monitoring. Besides key concerns around bioavailability (immune clearance of lentivirus vs hepatic uptake of LNP), biodistribution, and dose control, developers will need to address safety concerns with measures such as transient expression systems or switchable safety switches (e.g., induced caspase 9 or EGFR kill switch). While multiple first-in-human studies are actively recruiting patients, early clinical evidence supports lentivirus- and LNP-based *in vivo* CAR-T strategies, including a Phase 1 multiple myeloma study (inMMyCAR/KLN-1010, NCT07075185) demonstrating de novo CAR-T generation

and early clinical responses without *ex vivo* manufacturing or lymphodepletion,[13] as well as LNP-based CD19 CAR mRNA programs showing rapid *in vivo* B-cell depletion and preliminary activity in refractory systemic lupus erythematosus (NCT06801119).[14]

Table 4. Comparison between *ex vivo* and *in vivo* CAR-T cell therapies.

Feature	*Ex vivo* CAR-T	*In vivo* CAR-T
Major formats	Lentiviral or γ-retroviral transduced autologous or allogeneic T cells; sometimes transposon-based (Sleeping Beauty, piggyBac)	Lentiviral-based and LNP-based (mRNA or circRNA)
Manufacturing	Cells collected, engineered, expanded, and reinfused	No cell collection; direct delivery to patient
Targeting/specificity	High; modified cells can be selected and characterized before infusion	Depends on delivery vector tropism; less controllable *ex vivo*; specificity usually redirected using a cell-specific antibody, e.g., anti-CD3
Expression duration	Long-term; stable integration possible	Transient (mRNA or circRNA) or increased persistence when a viral vector is used
Key benefits	Proven clinical efficacy; controlled expansion; established regulatory track record	Simplified logistics; off-the-shelf potential; avoids complex cell processing; rapid deployment; no chemotherapy preconditioning needed.
Potential safety/toxicity	Cytokine release syndrome, neurotoxicity, and insertional mutagenesis with integrating vectors	Potential off-target transduction; inflammatory toxicity; vector-related toxicity; transient expression may reduce long-term risk

Source: Elaborated by the authors.

Key points

Regardless of the gene delivery or modification method, successful genome engineering depends on carefully designing the plasmid, including the gene cargo and the accessory components. For example, the codon-optimized DNA sequence of target genes should be used for efficient expression in the species of choice. Promoters should be carefully selected for constitutive gene expression with different strengths. Depending on the targeted cell type, envelope protein and LNP surface in viral and non-viral gene delivery systems, respectively, can be engineered with an antibody for redirecting to specific tropism or cell of interest (e.g., anti-CD3 for CAR-T cell targeting).

Real-world data highlights how the platform selection is tightly linked to clinical context. Established CAR-T cell products such as Kymriah and Yescarta demonstrate a mature autologous manufacturing platform, providing the most predictable option for indications such as B-cell lymphoma, acute lymphoblastic leukemia, and multiple myeloma, where patients retain relatively functional and non-exhausted T cells. Adoption of an autologous CAR-T manufacturing platform offers a more controlled safety framework when targeting novel or high-risk antigens, where off-tumor toxicity remains uncertain. In cases where patient T-cell fitness is compromised, such as in chronic lymphocytic leukemia, intrinsic T-cell dysfunction may limit the quality and consistency of autologous products. In these settings, allogeneic CAR-T or CAR-NK strategies may provide functionally "reset" effector cells, less affected by tumor-induced T-cell exhaustion or the tumor microenvironment. When autologous products are still required, genome engineering approaches such as CRISPR can be used to restore or enhance T-cell fitness and overcome dysfunction-related limitations by knocking out genes related to T-cell exhaustion or effector function inhibition.[15] In non-oncology indications such as autoimmune disease, the priorities shift toward scalability, repeat dosing potential, and cost efficiency, leaning toward off-the-shelf or even *in vivo* CAR engineering strategies over individualized manufacturing. Similarly, in regenerative medicine or fibrosis, manufacturing cost, scalability, and fine-tuned durability often become more important than achieving the extreme cytotoxic potency required in cancer therapy. Overall, platform selection reflects a balance between T-cell biology, clinical context, safety risk, and the practical constraints of manufacturing and global clinical deployment.

CONFLICT OF INTEREST

De Lima M has a research collaboration with Miltenyi Biotec, is a consultant of AbbVie and Priothera, and is the chief medical officer of Kure Cells. Chan WK has no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Chan WK. **Investigation:** Chan WK. **Methodology:** Chan WK. **Formal Analysis:** Chan WK, De Lima M. **Data Curation:** Chan WK. **Project Administration:** Chan WK, De Lima M. **Funding Acquisition:** Chan WK. **Writing:** Chan WK, De Lima M. **Supervision:** Chan WK, De Lima M. **Final Approval:** Chan WK, De Lima, M.

DATA AVAILABILITY STATEMENT

Not applicable.

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DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE TOOLS

The authors declare that no artificial intelligence tools were used in the preparation, writing, data analysis, or review of this manuscript.

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