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Emerging Therapies – CAR-T Cell Therapy, mRNA Technology Beyond COVID and Gene Editing (CRISPR)

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Abstract: The past decade has witnessed remarkable advances in emerging therapeutic modalities, including CAR-T cell therapy, mRNA-based therapeutics, and CRISPR/Cas gene editing, each revolutionizing treatment paradigms across oncology, genetic disorders, and infectious diseases. CAR-T therapies leverage engineered T cells for targeted cytotoxicity, while mRNA therapeutics enable rapid, transient protein expression for vaccination and personalized cancer immunotherapy. CRISPR/Cas systems provide precise genomic and transcriptomic editing, with next-generation approaches—base editing, prime editing, and epigenome modulation—offering enhanced specificity and therapeutic versatility. These modalities differ mechanistically yet exhibit complementary potential, particularly when integrated synergistically, as in CRISPR-enhanced CAR-T or mRNA-facilitated in vivo gene editing. Despite unprecedented promise, challenges remain, including off-target effects, ethical and regulatory considerations, manufacturing complexity, cost, and global accessibility. Advances in delivery technologies, artificial intelligence-driven target design, and interdisciplinary collaboration are critical to overcoming these barriers. Looking forward, the integration of cellular, nucleic acid, and genomic therapeutics is poised to redefine precision medicine, enabling highly personalized, curative interventions and shaping the next decade of translational innovation in healthcare.

Keywords: CAR-T cell therapy, mRNA therapeutics, CRISPR/Cas gene editing, precision medicine, emerging therapies, translational innovation

1. Introduction

The landscape of modern medicine is rapidly evolving, shifting from conventional pharmacotherapy—dominated by small molecules and biologics—to therapies that act directly at the molecular and genetic levels. Traditional drugs, though invaluable, often target disease symptoms rather than the root molecular causes, leading to incomplete efficacy and adverse effects in complex or genetically driven diseases [1]. This limitation has driven the emergence of next-generation therapeutics designed to correct, replace, or reprogram dysfunctional biological

systems [2]. At the core of this transformation lie the principles of personalized, precision, and regenerative medicine, which together emphasize tailoring treatment to an individual's genetic and molecular profile. Personalized medicine uses genomic data to design patient-specific interventions, while precision medicine categorizes patients into molecularly defined subgroups to optimize therapeutic outcomes [3]. Regenerative medicine, on the other hand, focuses on restoring or replacing damaged tissues and organs through cell-based or gene-based interventions. The convergence of these disciplines has led to major strides in immune engineering, RNA therapeutics, and genome editing technologies [4]. Among the most promising of these innovations are CAR-T cell therapy, mRNA-based therapeutics, and CRISPR-mediated gene editing—three platforms that have fundamentally redefined how diseases can be treated.

CAR-T cell therapy (Chimeric Antigen Receptor T-cell therapy) involves engineering a patient's own T cells to express synthetic receptors that recognize tumor-specific antigens, enabling potent and selective destruction of cancer cells. Clinical success in hematologic malignancies has been remarkable, with several FDA-approved therapies (e.g., Kymriah®, Yescarta®, Breyanzi®) demonstrating durable remission rates [5]. However, challenges such as cytokine release syndrome, neurotoxicity, and limited efficacy in solid tumors remain active areas of research. mRNA technology, propelled into prominence by the success of COVID-19 vaccines, has since evolved far beyond infectious disease prevention. Synthetic mRNA can transiently express therapeutic proteins or antigens without genomic integration, enabling broad applications in cancer immunotherapy, protein replacement, and regenerative medicine. Advances such as self-amplifying and circular mRNA have improved expression durability and stability, while lipid nanoparticle (LNP) delivery has revolutionized its clinical feasibility [6].

CRISPR gene editing represents another transformative modality, allowing precise modification of DNA sequences in living cells through the programmable CRISPR-Cas system. Since its first application in mammalian cells, CRISPR has become a cornerstone of genetic medicine, facilitating correction of monogenic disorders such as sickle cell anemia and β-thalassemia. Ongoing refinements such as base editing, prime editing, and CRISPR-Cas12/13 variants have expanded precision, reduced off-target effects, and introduced new avenues for epigenetic and RNA editing [7]. Together, these technologies mark a paradigm shift toward curative, patient-specific, and regenerative therapies, representing a new frontier in biomedicine. This review aims to provide a comprehensive synthesis of the mechanisms, applications, and future directions of CAR-T cell therapy, mRNA therapeutics beyond COVID-19, and CRISPR-based gene editing, with emphasis on their translational potential, challenges, and the evolving ethical and regulatory landscape.

2. CAR-T Cell Therapy

2.1 Overview and Mechanism

Chimeric Antigen Receptor T-cell (CAR-T) therapy represents one of the most transformative innovations in cancer immunotherapy. It involves engineering a patient's own T lymphocytes to recognize and destroy malignant cells through synthetic receptors that bypass the natural constraints of the T-cell receptor (TCR)–major histocompatibility complex (MHC) interaction [8]. The chimeric antigen receptor (CAR) is a modular construct typically composed of an extracellular antigen-binding domain, a transmembrane domain, and intracellular signaling domains that trigger T-cell activation and cytotoxicity [9]. The process of CAR-T cell generation involves several distinct steps. Initially, T cells are collected from the patient via leukapheresis. These cells are then genetically modified, most commonly using viral vectors such as lentivirus or retrovirus, to introduce the CAR gene construct [3]. The modified T cells are subsequently expanded ex vivo under controlled culture conditions to achieve sufficient cell numbers before being reinfused into the patient. Prior to infusion, patients often undergo lymphodepleting chemotherapy to enhance CAR-T cell persistence and engraftment [10].

CAR-T cell therapy has undergone multiple generational advancements, each improving upon the structure and functionality of its predecessors.

- First-generation CARs incorporated only the CD3ζ signaling domain, leading to limited T-cell persistence and poor therapeutic durability.
- Second-generation CARs added a single costimulatory domain such as CD28 or 4-1BB, significantly improving proliferation, persistence, and antitumor activity.
- Third-generation CARs integrated two costimulatory domains (e.g., CD28 and 4-1BB), further enhancing cytokine production and cytotoxic potential.
- Fourth-generation CARs, also termed TRUCKs (T cells Redirected for Universal Cytokine Killing), were designed to secrete cytokines like IL-12 at tumor sites, amplifying local immune activation [11].
- Fifth-generation CARs represent the latest evolution, featuring truncated cytokine receptor domains (such as IL-2Rβ) that engage the JAK/STAT signaling pathway, thereby improving proliferation, persistence, and control over cell fate [12].

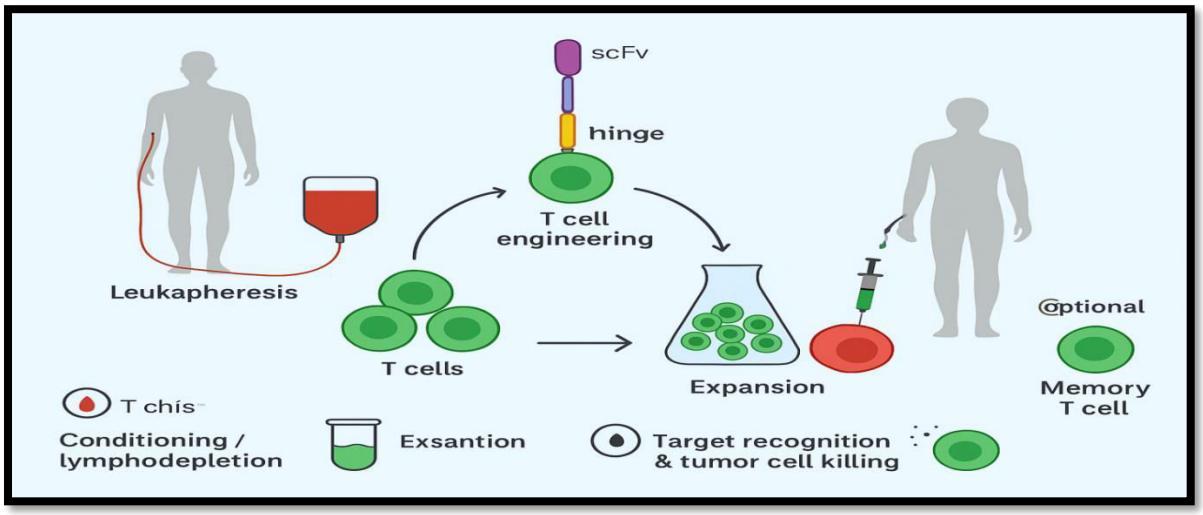

Fig 1. Mechanism of CAR-T cell therapy

2.2 Clinical Applications and Approved Products

CAR-T cell therapy has achieved groundbreaking success in hematologic malignancies, particularly B-cell leukemias and lymphomas. The first FDA-approved CAR-T product, Tisagenlecleucel (Kymriah®), developed by Novartis, targets CD19 and was approved in 2017 for pediatric and young adult patients with relapsed or refractory B-cell acute lymphoblastic leukemia (ALL). Shortly thereafter, Axicabtagene ciloleucel (Yescarta®) and Lisocabtagene maraleucel (Breyanzi®) were approved for large B-cell lymphomas, expanding the therapeutic landscape. These therapies demonstrated unprecedented remission rates, with overall response rates exceeding 80% in certain patient populations [13].

Beyond hematologic cancers, CAR-T therapy is being actively explored for solid tumors, though challenges such as antigen heterogeneity, poor trafficking, and immunosuppressive tumor microenvironments have limited efficacy. Novel targets like HER2, GD2, mesothelin, and PSMA are under clinical evaluation, and advanced CAR designs incorporating "logic-gated" recognition or cytokine-secreting modules aim to overcome these barriers [14]. Furthermore, CAR-T cells are now being adapted for non-oncologic diseases, including autoimmune disorders. In 2024, a landmark study demonstrated that CD19-directed CAR-T therapy induced durable remission in patients with refractory systemic lupus erythematosus (SLE), highlighting its potential to reprogram autoreactive immune responses [15]. Trials are also ongoing in multiple sclerosis and myasthenia gravis, illustrating the therapy's expansion beyond oncology.

From a regulatory and manufacturing perspective, the commercial CAR-T landscape continues to grow, with several approved therapies across the globe. Apart from Kymriah®, Yescarta®, and Breyanzi®, Idecabtagene vicleucel (Abecma®) and Ciltacabtagene autoleucel (Carvykti®) have been approved for multiple myeloma,

targeting the B-cell maturation antigen (BCMA). These approvals have validated the scalability of autologous CAR-T production platforms while also motivating efforts toward off-the-shelf allogeneic CAR-T products that can reduce cost, turnaround time, and manufacturing complexity [16]. The clinical milestones achieved by CAR-T therapy underscore its position as a cornerstone of precision cellular immunotherapy. Continuous improvements in CAR design, manufacturing logistics, and clinical management of toxicities are expected to further expand its indications and accessibility.

2.3 Advantages and Challenges

- **Advantages**

CAR-T cell therapy provides unparalleled antigen specificity and the potential for long-term remission in hematologic malignancies. The synthetic chimeric receptor allows T cells to recognize target antigens such as CD19 or BCMA independent of the MHC complex, thereby overcoming mechanisms of tumor immune evasion. Clinical studies in refractory acute lymphoblastic leukemia and large B-cell lymphoma have shown complete remission rates exceeding 80%, with some patients maintaining durable responses beyond five years after a single infusion. These durable remissions highlight the self-renewing capacity of CAR-T cells, which can persist as memory populations that continue immune surveillance against residual malignant cells [17].

- **Challenges**

Despite its success, CAR-T therapy presents several significant clinical and logistical challenges.

i. Cytokine Release Syndrome (CRS)

CRS is the most frequent acute toxicity, characterized by fever, hypotension, and organ dysfunction due to massive cytokine secretion—primarily interleukin-6, interferon-γ, and GM-CSF—following robust CAR-T activation. While most cases are manageable, severe CRS can be life-threatening. Tocilizumab, an IL-6 receptor antagonist, together with corticosteroids, has become standard for treatment, and risk-stratified step-up dosing regimens have reduced severe events [18].

ii. Neurotoxicity (ICANS)

Immune Effector Cell–Associated Neurotoxicity Syndrome manifests with confusion, aphasia, and seizures, resulting from endothelial activation and blood–brain barrier disruption [19]. Management includes close neurologic monitoring, corticosteroids for severe grades, and supportive measures. Although reversible in most cases, its unpredictable onset remains a safety concern.

iii. Manufacturing Complexity and Cost

Autologous CAR-T manufacturing requires individualized leukapheresis, viral gene transfer, and ex vivo expansion under GMP conditions, leading to production times of 2–4 weeks and costs exceeding USD 350 000 per dose. Delays may render some patients ineligible due to rapid disease progression, highlighting the need for process automation and scalable off-the-shelf models [20].

iv. Antigen Escape and Relapse:

Loss or down-regulation of target antigens—such as CD19 loss after anti-CD19 therapy—can drive relapse. Mechanisms include alternative splicing, lineage switch, and selection of antigen-negative clones. Multi-target CARs and sequential or combination immunotherapies are being developed to address this limitation [21].

v. Strategies to Improve Safety and Efficacy

Multiple engineering strategies are being implemented to enhance therapeutic precision:

- Next-generation CAR designs incorporating optimized co-stimulatory domains and inducible "suicide switches" allow external control of CAR-T activity, mitigating severe CRS or off-tumor toxicity.
- Armored CAR-T cells capable of secreting cytokines such as IL-12 or checkpoint inhibitors (e.g., anti-PD-1 scFv) remodel the tumor microenvironment to improve infiltration and cytotoxicity.
- CRISPR/Cas9-edited CAR-T cells enable deletion of inhibitory receptors (PD-1, LAG-3) or endogenous TCRs to create universal allogeneic products with reduced risk of graft-versus-host disease and improved persistence.
- Pharmacologic modulation, including prophylactic IL-6 or IL-1 blockade, is also under investigation to pre-empt severe inflammatory toxicities without impairing efficacy [22].

3. mRNA Technology Beyond COVID-19

3.1 Principles of mRNA Therapeutics

i. mRNA Structure and Translation Mechanism

Messenger RNA (mRNA) therapeutics utilizes synthetic transcripts to instruct host cells to produce a desired protein. A typical mRNA construct contains a 5' cap, a 5' untranslated region (UTR), an open reading frame (ORF) encoding the protein of interest, a 3' UTR, and a poly(A) tail. Upon delivery into the cytoplasm, mRNA engages the host ribosomal machinery to produce the encoded protein, which can act as an antigen for vaccination, a therapeutic enzyme, or a signaling molecule [23].

ii. Delivery Systems

Efficient cellular delivery is critical for mRNA therapeutic efficacy. The two primary strategies include:

- **Lipid Nanoparticles (LNPs):** LNPs are currently the most advanced delivery platform. They encapsulate mRNA, protect it from extracellular RNases, and facilitate endosomal uptake. Ionizable lipids in LNPs promote endosomal escape, releasing mRNA into the cytoplasm.
- **Polymer-Based Carriers:** Polymers such as poly(beta-amino esters) and polyethyleneimine (PEI) offer alternative delivery strategies, enabling controlled release, tissue targeting, and reduced immunogenicity. Polymer carriers are under active investigation to complement or improve on LNP technology, particularly for repeated dosing or localized administration [24].

iii. Stability and Immune Evasion Strategies

Unmodified mRNA is inherently unstable and immunostimulatory. Strategies to improve stability and reduce innate immune activation include:

- Nucleoside modifications such as pseudouridine or 1-methylpseudouridine reduce recognition by Toll-like receptors (TLRs) and RIG-I, decreasing unwanted interferon responses [25].
- Optimized UTR sequences enhance translational efficiency and transcript half-life.
- Codon optimization can improve ribosomal translation without altering protein sequence.
- Formulation with protective carriers such as LNPs or PEGylated lipids shields mRNA from extracellular RNases and prolongs circulation time [26].

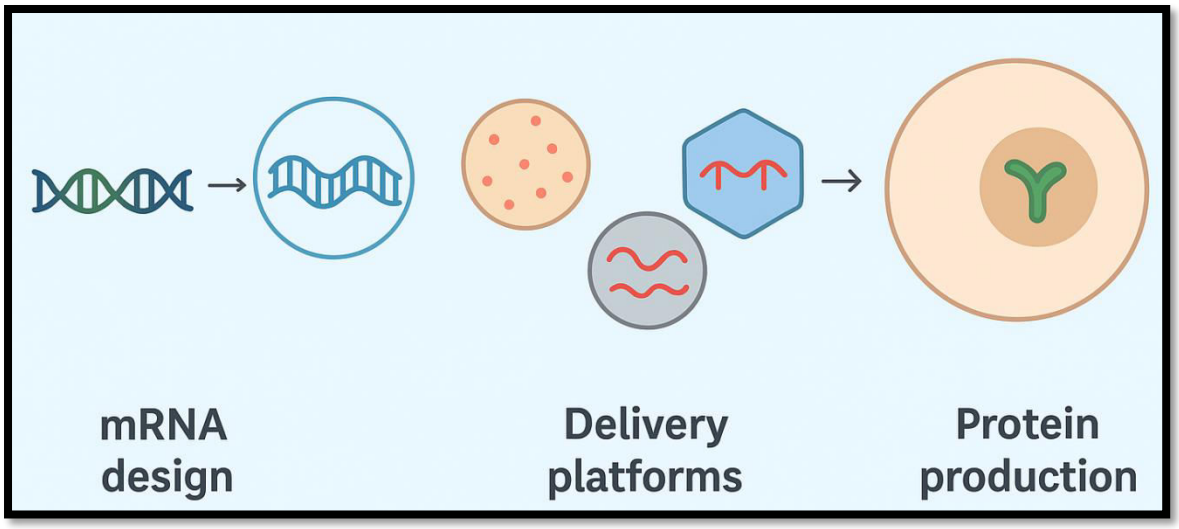

Fig 2. mRNA therapeutic design and delivery platforms

3.2 Applications beyond Vaccines

i. Cancer Immunotherapy

mRNA therapeutics have emerged as a promising platform for personalized cancer immunotherapy. Synthetic mRNA encoding tumor-associated antigens (TAAs) can be delivered to dendritic cells in vitro or in vivo, leading to antigen presentation and activation of cytotoxic T lymphocytes (CTLs) capable of targeting malignant cells [27]. Early-phase clinical trials in melanoma, prostate cancer, and glioblastoma have demonstrated induction of antigen-specific immune responses with favorable safety profiles. Additionally, mRNA-based neoantigen vaccines, tailored to individual tumor mutations, have shown enhanced immunogenicity and the ability to generate durable T-cell memory [28].

ii. Protein Replacement Therapy

Beyond oncology, mRNA therapeutics offer a platform for protein replacement in rare genetic disorders. For example, mRNA encoding functional versions of enzymes deficient in diseases such as ornithine transcarbamylase deficiency or cystic fibrosis can restore protein activity transiently, providing a reversible and controllable therapeutic approach [29]. Advantages include avoidance of viral vectors, transient expression limiting long-term off-target effects, and the potential for repeat dosing.

iii. Infectious Diseases

The success of COVID-19 mRNA vaccines has accelerated exploration of mRNA therapeutics for other pathogens. Clinical and preclinical studies are investigating mRNA vaccines for influenza, Zika virus, respiratory syncytial virus (RSV), and cytomegalovirus (CMV), demonstrating rapid immunogenicity, favorable safety, and scalable manufacturing [30]. mRNA allows rapid adaptation to emerging viral strains, a major advantage for seasonal or pandemic-prone pathogens.

iv. Cardiovascular and Regenerative Medicine

mRNA therapeutics are being explored in cardiovascular and regenerative applications, where delivery of mRNA encoding growth factors or transcription factors can induce tissue repair or angiogenesis. For instance, VEGF-A mRNA has been evaluated in clinical trials to promote neovascularization in ischemic heart disease, and mRNA encoding transcription factors has been investigated to reprogram fibroblasts into functional cardiomyocytes [31]. Such strategies demonstrate the versatility of mRNA beyond traditional vaccination or protein replacement, offering a platform for precision regenerative medicine. These applications illustrate the broad potential of mRNA therapeutics beyond prophylactic vaccines, encompassing oncology, rare disease therapy, infectious

diseases, and regenerative medicine, with ongoing clinical translation and technological refinements enhancing efficacy and safety.

3.3 Key Advancements

The success of mRNA-based COVID-19 vaccines has accelerated innovation across multiple fronts of mRNA therapeutics, leading to the development of next-generation platforms designed for greater stability, potency, and tissue specificity.

i. Self-amplifying mRNA (saRNA)

Self-amplifying mRNA (saRNA) is an evolution of conventional mRNA constructs that incorporates replicase machinery derived from alphaviruses, enabling intracellular amplification of RNA and antigen expression at significantly lower doses. This approach enhances immunogenicity while reducing production costs and reactogenicity. Several saRNA vaccines, such as those targeting influenza and SARS-CoV-2 variants, have entered early-phase clinical trials, demonstrating strong antibody titers and T-cell responses [32].

ii. Circular RNA (circRNA) for Prolonged Expression

Circular RNA (circRNA) technology has emerged as a promising alternative to linear mRNA due to its resistance to exonuclease degradation and prolonged translational activity [6]. Unlike linear mRNA, circRNA lacks free ends, resulting in greater molecular stability and sustained protein production. Recent preclinical studies have shown that engineered circRNAs can elicit durable immune responses and maintain expression for several days post-delivery, offering potential for chronic disease treatment and next-generation vaccines [33].

iii. Targeted Delivery and Tissue-Specific Expression

Advances in delivery systems, particularly lipid nanoparticle (LNP) engineering, have enabled more precise targeting of mRNA therapeutics to specific tissues and cell types. Modifications in lipid composition, PEGylation, and ionizable lipids allow fine-tuning of biodistribution, minimizing off-target effects and systemic toxicity. Furthermore, conjugation strategies using antibodies or ligands are being developed to achieve cell-selective delivery—for instance, liver-targeted mRNA for metabolic disorders or cardiac-specific mRNA for myocardial regeneration.

3.4 Limitations and Future Prospects

Despite the transformative success of mRNA therapeutics, several limitations persist that must be addressed to enable their broader clinical utility. Key challenges include storage stability, large-scale manufacturing, and potential immunogenicity, each posing significant hurdles for global deployment and long-term use.

i. Storage and Stability Challenges

The requirement for ultra-cold storage temperatures remains a major logistical barrier, particularly in low- and middle-income regions. mRNA molecules are inherently unstable and susceptible to hydrolysis and oxidation, which can compromise vaccine potency. Efforts to improve thermostability through optimized lipid nanoparticle (LNP) formulations, lyophilization techniques, and modified nucleosides are actively being pursued to support easier distribution and long-term shelf life [34].

ii. Scalability and Manufacturing Constraints

The large-scale production of mRNA vaccines and therapeutics requires stringent control of in vitro transcription, purification, and encapsulation processes [4]. Batch-to-batch variability and cost of cGMP manufacturing pose challenges for consistent quality and affordability. Automation and continuous manufacturing technologies are emerging to enhance yield and reproducibility [5].

iii. Immunogenicity and Safety Concerns

Although nucleoside modifications and delivery optimization have significantly reduced innate immune activation, some degree of reactogenicity and inflammatory response persists. Long-term safety data are still limited, especially for chronic or repeat-dose mRNA therapies. Continuous post-marketing surveillance and pharmacovigilance are critical to understand potential risks.

iv. Integration with Nanotechnology and Gene Editing for Next-Generation Therapeutics

Looking forward, the integration of mRNA technology with nanotechnology and gene-editing tools such as CRISPR-Cas systems heralds a new therapeutic frontier. Engineered nanoparticles enable organ- or cell-specific mRNA delivery, while CRISPR components encoded via mRNA could facilitate transient, non-integrative genome editing, reducing off-target risks [35]. Moreover, synergistic combinations—such as mRNA-guided expression of therapeutic proteins, gene editors, or immunomodulators—are poised to redefine precision medicine and regenerative therapies [10, 11].

4. Gene Editing and CRISPR Technology

4.1 Overview and Mechanism

The advent of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and its associated nucleases (Cas proteins) has revolutionized the field of genetic engineering, providing an efficient, programmable, and versatile tool for precise genomic modification. Originally discovered as part of the adaptive immune defense system in bacteria and archaea, CRISPR enables microorganisms to

recognize and cleave foreign nucleic acids from invading bacteriophages [36]. This bacterial mechanism has been successfully repurposed for targeted genome editing in eukaryotic cells, marking a paradigm shift in biomedical research and therapeutics.

i. Discovery of the CRISPR-Cas System

The CRISPR-Cas system was first identified in Escherichia coli in 1987 by Ishino and colleagues, who described unusual repeat sequences within the bacterial genome. Subsequent work by Mojica et al. and Bolotin et al. in the early 2000s established that these repeat-spacer arrays serve as an adaptive immune archive against viral infections. The pivotal breakthrough occurred in 2012 when Jennifer Doudna and Emmanuelle Charpentier demonstrated that the CRISPR-Cas9 system from Streptococcus pyogenes could be reprogrammed with synthetic guide RNA (gRNA) to induce site-specific DNA cleavage in vitro [37]. This discovery laid the foundation for modern genome editing and earned them the 2020 Nobel Prize in Chemistry.

ii. Types of Cas Enzymes

Several classes and subtypes of Cas nucleases have since been characterized, each offering unique editing capabilities. Cas9, derived from S. pyogenes, is the most widely used nuclease, recognizing specific DNA sequences via a protospacer adjacent motif (PAM) and generating double-strand breaks (DSBs) [8]. Cas12 (Cpf1) exhibits distinct PAM requirements and produces staggered DNA cuts, which can be advantageous for certain gene knock-in strategies. Meanwhile, Cas13 represents a unique RNA-targeting nuclease, enabling transcriptome editing, RNA interference, and diagnostic applications [38]. The discovery of miniature and high-fidelity Cas variants, such as SaCas9, SpCas9-HF1, and CasMINI, has further improved delivery efficiency and editing precision.

iii. Mechanism of Action

The canonical CRISPR-Cas9 system operates through a simple yet powerful mechanism involving three main components: a Cas nuclease, a guide RNA (gRNA), and the target DNA. The gRNA is designed to be complementary to the desired DNA sequence, directing Cas9 to bind and induce a DSB at the targeted locus. Following cleavage, cellular repair pathways are activated—primarily non-homologous end joining (NHEJ), which often results in insertions or deletions (indels) that disrupt gene function, or homology-directed repair (HDR), which enables precise sequence correction or insertion using an exogenous DNA template [39].

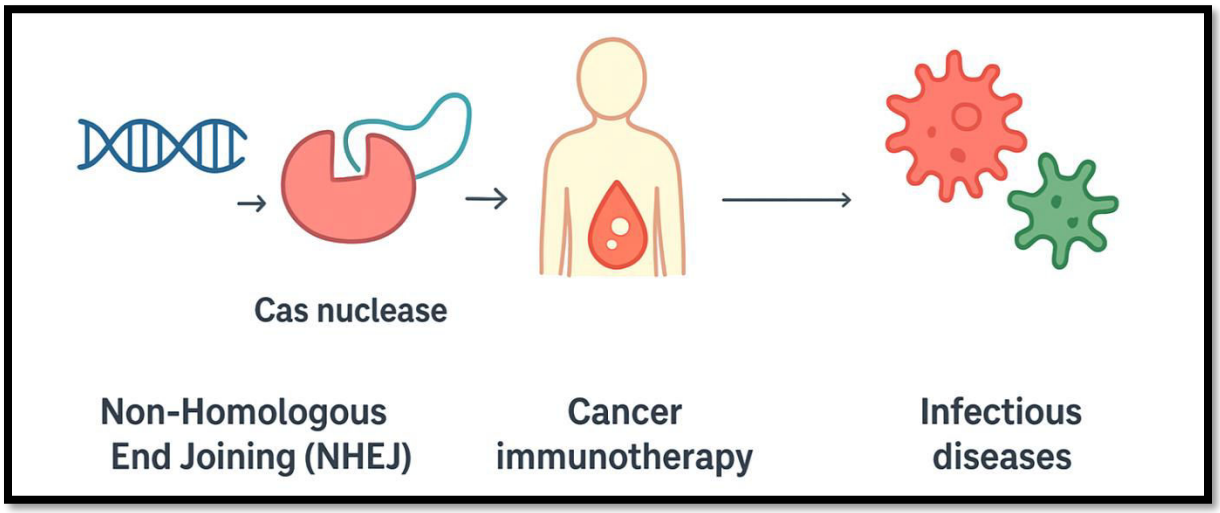

Fig 3. CRISPR editing mechanisms and therapeutic applications

4.2 Therapeutic Applications

CRISPR-based gene editing has emerged as one of the most transformative therapeutic tools of the 21st century, enabling precise genetic alterations with wide-ranging biomedical and biotechnological implications.

i. Treatment of Genetic Diseases

CRISPR-Cas9 has shown remarkable success in the correction of monogenic disorders such as sickle cell anemia and β-thalassemia. By targeting the HBB gene mutation responsible for defective hemoglobin, CRISPR facilitates reactivation of fetal hemoglobin (HbF) synthesis or direct repair of the mutant allele, restoring normal erythropoiesis. Clinical trials by Vertex Pharmaceuticals and CRISPR Therapeutics (CTX001, now exa-cel) have demonstrated curative outcomes in both diseases, representing the first FDA-approved CRISPR-based therapy in 2024.

ii. Cancer Immunotherapy

CRISPR technology is revolutionizing cell-based cancer immunotherapy, particularly in the development of next-generation CAR-T cells. Through targeted disruption of inhibitory receptors such as PD-1 or CTLA-4, CRISPR enhances T-cell persistence and tumor cytotoxicity. Moreover, multiplex editing allows for the creation of "off-the-shelf" universal donor CAR-T cells, eliminating the need for patient-derived cells. Trials in hematologic malignancies and solid tumors underscore its potential to improve tumor specificity and reduce relapse rates [40].

iii. Infectious Disease Control

CRISPR-Cas systems are also being exploited for antiviral therapy. Engineered Cas13 enzymes can specifically target RNA viruses such as HIV, SARS-CoV-2, and influenza by degrading viral RNA transcripts. Similarly, CRISPR-based excision of

integrated HIV proviral DNA from host genomes has shown promise in preclinical models, offering a potential functional cure. CRISPR is also being tested for the elimination of oncogenic viruses like HPV through targeted genome disruption [41].

iv. Agricultural and Microbiome Applications

Beyond human therapeutics, CRISPR is revolutionizing agricultural biotechnology. It enables the development of disease-resistant, nutrient-enriched, and climate-resilient crops without transgenic DNA insertion, which enhances regulatory acceptance. In the microbiome, CRISPR is used to engineer beneficial microbial strains and selectively eliminate pathogenic species, with implications for metabolic disorders, antimicrobial resistance, and gut-brain axis modulation [42].

4.3 Ethical and Safety Considerations

While CRISPR-Cas technology has ushered in a new age of genomic precision, its rapid evolution has raised profound ethical, safety, and societal concerns that require deliberate scrutiny before full-scale clinical integration.

i. Off-Target Effects and Mosaicism

Despite its precision, CRISPR is not infallible. Unintended off-target mutations—where the Cas nuclease cleaves DNA sequences resembling the intended target—pose a significant safety challenge. Such alterations can disrupt essential genes or activate oncogenes, potentially leading to tumorigenesis or other pathologies. Advances like high-fidelity Cas9 variants (e.g., SpCas9-HF1, eSpCas9, HypaCas9) and base/prime editors have substantially improved specificity. However, mosaicism, the occurrence of both edited and unedited cells within the same organism, remains problematic, particularly in embryonic editing where timing and delivery efficiency are critical [43].

ii. Germline Editing and Ethical Boundaries

Perhaps the most contentious issue lies in germline gene editing—alterations in sperm, ova, or embryos that are heritable. The 2018 birth of CRISPR-edited twins in China triggered global condemnation, emphasizing the ethical dangers of manipulating human heredity without comprehensive safety validation or societal consensus. Germline editing raises moral questions surrounding consent, eugenics, and equity in genetic enhancement. International regulatory bodies, including the World Health Organization (WHO) and UNESCO, have since called for stringent moratoria on heritable genome editing, urging focus on somatic cell applications that do not affect future generations [44].

iii. Regulatory Landscape and Public Perception

The regulatory framework for CRISPR applications remains heterogeneous across jurisdictions. Agencies such as the US FDA, European Medicines Agency (EMA), and

Indian Council of Medical Research (ICMR) have outlined cautious pathways emphasizing risk-benefit assessment, ethical oversight, and transparency in clinical trials. Nonetheless, public perception remains divided—while patients and advocacy groups view CRISPR as a potential cure for incurable diseases, broader society expresses concern over "designer babies," genetic inequality, and misuse for non-therapeutic enhancement. Transparent communication, participatory policymaking, and global governance will therefore be pivotal in maintaining public trust [45].

4.4 Emerging Trends

The landscape of gene editing is evolving rapidly beyond the conventional CRISPR-Cas9 system, giving rise to next-generation platforms that expand the precision, safety, and scope of genome manipulation. Three key frontiers—base editing, prime editing, and epigenome editing—alongside innovations in CRISPR-dCas systems and AI-driven design algorithms, are redefining the potential of genetic medicine.

i. Base Editing, Prime Editing, and Epigenome Editing

Base editing, first developed by David Liu's group at Harvard in 2016, allows direct and irreversible conversion of one nucleotide to another (e.g., C•G to T•A) without inducing double-strand DNA breaks. This approach minimizes undesired mutations and has shown success in correcting pathogenic single-nucleotide variants responsible for conditions such as sickle cell disease and Tay–Sachs disease [2].

Prime editing, a later innovation, merges a Cas9 nickase with a reverse transcriptase and a prime editing guide RNA (pegRNA), enabling insertion, deletion, or substitution of DNA sequences with exceptional accuracy and minimal off-target activity. Meanwhile, epigenome editing employs catalytically dead Cas variants (dCas) fused with chromatin modifiers to reversibly regulate gene expression without altering the DNA sequence—an approach promising for diseases where transcriptional dysregulation plays a key role [46].

ii. CRISPR-d Cas Systems for Gene Regulation

Catalytically inactive Cas proteins (dCas9, dCas12a) are now being harnessed for programmable control of gene expression. When fused with transcriptional activators or repressors (e.g., VP64, KRAB, p300), these systems enable precise modulation of endogenous gene activity [47]. The CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) platforms are finding broad utility in studying gene networks, synthetic biology, and functional genomics, offering reversible and tunable control that traditional editing lacks.

iii. Integration with Artificial Intelligence for Precision Target Design

Artificial intelligence (AI) and machine learning (ML) are revolutionizing CRISPR-based genome engineering by predicting off-target sites, optimizing guide RNA (gRNA) design, and improving delivery strategies. AI-driven platforms such as DeepCRISPR and CRISPR-Net integrate large-scale genomic datasets to enhance the specificity and efficacy of editing. In therapeutic development, AI is increasingly used to simulate on-target/off-target effects and to accelerate candidate validation, drastically reducing preclinical timelines [48]. The convergence of AI and CRISPR represents a pivotal step toward predictive, precise, and personalized genome editing.

5. Comparative Analysis of Emerging Modalities

5.1 Mechanistic differences and therapeutic targets

CAR-T cell therapy, mRNA therapeutics, and CRISPR gene-editing operate at different biological layers and therefore target distinct therapeutic problems:

CAR-T cell therapy is a cellular immunotherapy that reprograms patient or donor T cells to express chimeric antigen receptors directed at surface antigens (e.g., CD19, BCMA). Its main strength is direct cellular cytotoxicity against antigen-expressing malignancies, especially hematologic cancers, but it is inherently limited to targets presented on cell surfaces. mRNA therapeutics deliver transient genetic instructions (mRNA) to host cells, enabling de novo production of proteins — used as prophylactic or therapeutic vaccines, cancer neoantigen vaccines, or for protein replacement. mRNA acts at the translational level and is especially powerful for diseases where transient protein expression is therapeutic. CRISPR/Cas systems effect genomic change (permanent or semi-permanent) by directly editing DNA (or RNA for Cas13). This modality is suited for monogenic disorders, ex vivo cell engineering (e.g., HSCs or T cells), and durable corrections where long-term change is desired. Because they act on different biological substrates (cell, mRNA/protein, genome), the three modalities can address complementary clinical needs — immediate immune activation (mRNA), targeted cell-mediated killing (CAR-T), or durable genetic cure (CRISPR) [49].

5.2 Synergistic potential between CAR-T, mRNA, and CRISPR

Combination and platform integration are a major trend

CRISPR + CAR-T: CRISPR editing can improve CAR-T safety/efficacy — e.g., PD-1 knockout to reduce exhaustion, multiplex edits to remove endogenous TCRs (create universal allogeneic CAR-T), or to remove inhibitory pathways and enhance persistence. Preclinical and early clinical studies show that CRISPR-boosted CAR-T can improve potency and broaden indications.

mRNA + CAR-T/CRISPR: mRNA offers transient expression of CAR constructs or CRISPR components (e.g., Cas mRNA + gRNA) to enable in vivo engineering or to 'arm' immune cells without genomic integration. Self-amplifying and targeted LNPs allow lower doses and organ-selective delivery, opening possibilities for in vivo CAR expression or transient gene editing that avoids long-term nuclease exposure [50].

Table 1. Comparative Summary of CAR-T, mRNA, and CRISPR Therapies

Parameter	CAR-T Cell Therapy	mRNA Therapeutics	CRISPR Gene Editing
Core Mechanism	Ex vivo modification of patient's T cells to express a chimeric antigen receptor (CAR) that recognizes tumor-associated antigens and triggers immune-mediated cytotoxicity [12].	Delivery of synthetic messenger RNA encoding a therapeutic or immunogenic protein, translated transiently within host cells [29].	Utilizes CRISPR-associated (Cas) nucleases guided by RNA sequences to introduce targeted genome edits via double-strand break and repair mechanisms (NHEJ or HDR) [45].
Therapeutic Focus	Primarily hematologic malignancies (e.g., ALL, lymphoma); expanding to solid tumors and autoimmune diseases [18].	Infectious diseases, oncology, rare genetic disorders, regenerative medicine, and protein replacement therapy [33].	Monogenic diseases (e.g., sickle cell disease, β-thalassemia), cancer immunotherapy, infectious disease resistance, and agricultural biotechnology [46].
Mode of Administration	Autologous or allogeneic T-cell infusion following lymphodepletion [19].	Intramuscular, intravenous, or local injection of lipid nanoparticle (LNP)-encapsulated mRNA [35].	Ex vivo edited cells reintroduced into patients, or in vivo delivery using viral/non-viral vectors (AAV, LNPs) [46].
Duration of Effect	Long-term or potentially curative; persistence of memory CAR-T cells provides lasting remission [19].	Transient expression; requires repeat dosing or modified self-amplifying/circular mRNA for durability [35].	Permanent genomic modification (irreversible); long-term effects depend on target locus and repair pathway [47].
Major Advantages	Precision immune activation, high specificity, durable responses in refractory	Rapid design and production, non-integrating, tunable protein expression,	Precise genome correction, versatile platform, potential for single-dose cure [48].

	cancers [21].	and broad applicability [36].	
Key Limitations	Cytokine release syndrome (CRS), neurotoxicity, high cost, and limited efficacy in solid tumors [21].	Instability, cold-chain requirements, innate immune activation, limited tissue targeting.	Off-target edits, mosaicism, delivery inefficiency, and ethical concerns regarding germline editing.
Regulatory & Ethical Challenges	High manufacturing and regulatory complexity for patient-specific products.	Need for standardized quality control and scalable production for global distribution.	Ethical boundaries for germline modification, long-term biosafety, and international governance gaps.
Current Clinical Examples	FDA-approved: Kymriah® (tisagenlecleucel), Yescarta® (axicabtagene ciloleucel), Breyanzi® (lisocabtagene maraleucel).	mRNA-1273 (Moderna COVID-19 vaccine), BNT162b2 (Pfizer-Bio NTech), mRNA-4157 (Moderna personalized cancer vaccine).	Exa-cel (exa-cel for sickle cell and β-thalassemia, Vertex/CRISPR Therapeutics); NTLA-2001 (for transthyretin amyloidosis).
Future Directions	Allogeneic "off-the-shelf" CAR-Ts, dual-antigen targeting, armored CARs, and AI-driven antigen discovery.	Self-amplifying mRNA, circular RNA, tissue-specific delivery, and combination with CRISPR or CAR-T platforms.	Base editing, prime editing, epigenome editing, and AI-guided target optimization for precision therapeutics.

5.3 Challenges in manufacturing, regulation, and cost

Shared and modality-specific bottlenecks limit broad clinical access

Manufacturing / scalability: Autologous CAR-T manufacturing is complex, individualized, and time-consuming (vein-to-vein delays), creating scale and capacity constraints. Centralized processes increase logistics burden; decentralization and automated closed systems are active solutions but not yet ubiquitous.

Cost: Acquisition and total treatment costs for CAR-T are high (hundreds of thousands of USD per patient when inpatient care and complication management are included), posing reimbursement and equity challenges. mRNA vaccines scaled rapidly during the pandemic, but individualized mRNA applications (e.g., neoantigen vaccines) still face manufacturing cost and throughput issues. CRISPR therapies, especially ex vivo edits (HSCs, T cells), similarly incur high manufacturing and clinical costs.

Regulation and quality control: Each modality requires stringent, modality-specific analytic assays (potency, purity, off-target profiling for CRISPR, residual DNA, endotoxin, LNP characterisation for mRNA). Regulatory pathways are evolving, and heterogeneity between jurisdictions complicates global deployment. Post-approval safety surveillance and standardized potency metrics remain priorities [51].

5.4 Personalized medicine implications

All three modalities accelerate personalization but in different ways:

Rapid personalization: mRNA platforms allow short design-to-manufacture cycles (useful for individualized cancer vaccines and emerging pathogens). This supports highly personalized regimens on clinically relevant timelines.

Durable cures: CRISPR offers the potential for one-time, curative interventions for monogenic diseases (e.g., ex vivo editing of HSCs), shifting care paradigms from lifelong therapy to single interventions.

Adaptive cellular therapies: CAR-T can be personalized through autologous products or adapted using genomic edits to reduce rejection and improve applicability across patients. Combined approaches (e.g., CRISPR edited, mRNA-primed CAR-T) further enhance patient-tailored strategies.

However, personalization magnifies supply-chain, regulatory and cost challenges; equitable access will require innovations in manufacturing (automation, decentralized production), payer models (outcomes-based pricing), and global regulatory harmonization [52].

6. Ethical, Regulatory, and Societal Perspectives

Rapid advances in CAR-T, mRNA therapeutics and CRISPR gene editing have outpaced many existing governance frameworks, raising interlinked ethical, regulatory and societal questions that must be addressed to ensure safe, equitable and trustworthy translation.

6.1 Global regulatory frameworks

Regulatory agencies and international bodies are adapting existing pathways and producing targeted guidance for advanced therapies. The World Health Organization's governance framework for human genome editing outlines principles for oversight, transparency, a global registry, and mechanisms to coordinate national policies on somatic versus germline applications [1]. National regulators—most notably the U.S. Food and Drug Administration (FDA)—have published multiple guidance documents addressing preclinical testing, CMC (chemistry, manufacturing and controls), potency assays and clinical trial design for cellular and gene

therapies, reflecting heightened emphasis on product-specific comparability and long-term safety monitoring [53]. The European Medicines Agency (EMA) similarly maintains a suite of multidisciplinary guidelines for advanced therapy medicinal products (ATMPs), while regional initiatives are pushing to modernize definitions, evidentiary standards and regulatory sandboxes to accommodate novel modalities.

6.2 Ethical dilemmas and informed consent

Ethical concerns span immediate clinical safety (off-target effects, immunogenicity, insertional mutagenesis) to long-term and intergenerational risks—the latter especially salient for germline interventions. The literature and expert bodies caution against heritable genome editing outside strict, internationally coordinated research frameworks; calls for moratoria or precautionary pauses followed the first reported germline edits in humans [6,7]. Informed consent for somatic cell therapies also presents challenges: patients may face complex risk–benefit calculations (e.g., one-time curative prospect vs unknown late effects), and consent processes must clearly communicate uncertainty, potential for irreversible outcomes, and options for long-term follow-up.

6.3 Accessibility and affordability in LMICs

High prices, complex manufacturing and cold-chain requirements threaten equitable access. Recent analyses of CAR-T and other ATMPs document prohibitive per-patient costs, centralized manufacturing bottlenecks, and uneven reimbursement policies—factors that limit availability in low- and middle-income countries (LMICs) [9]. Nonetheless, emerging decentralised manufacturing models (regionally based GMP facilities), technology transfer, cost-reducing innovations (e.g., allogeneic "off-the-shelf" products, lower-dose saRNA approaches) and adaptive financing (outcomes-based payments, tiered pricing) offer realistic pathways to improve access.

6.4 Public trust, engagement and communication

Public perception shapes technology adoption. Surveys and case studies show that transparency, stakeholder engagement, and early, two-way communication reduce misunderstanding and build legitimacy—especially when technologies touch on heritability, enhancement or ecological release (e.g., gene drives) [54]. Proactive science communication should explain both promises and uncertainties, make trial results and adverse events publicly accessible, and involve patient groups, ethicists and civil society in policy deliberations.

7. Conclusion

Emerging therapeutic modalities—CAR-T cell therapy, mRNA-based therapeutics, and CRISPR/Cas gene editing—collectively represent a transformative shift in

modern medicine, each operating at distinct biological levels to address previously intractable diseases. CAR-T therapies exemplify the power of engineered cellular immunity for hematologic malignancies and are evolving toward allogeneic and multiplex-edited platforms. mRNA therapeutics have demonstrated unprecedented adaptability and speed, not only in pandemic vaccines but increasingly in personalized cancer immunotherapy and protein replacement strategies. CRISPR technology offers precise, potentially curative genomic interventions for monogenic disorders, cancer immunotherapy enhancement, and transcriptome modulation, while next-generation approaches such as base editing, prime editing, and epigenome modulation promise improved safety and expanded applicability. Despite remarkable progress, these modalities face shared challenges including manufacturing complexity, regulatory hurdles, cost, off-target risks, and ethical concerns that necessitate robust oversight, equitable access strategies, and informed public engagement. The synergistic integration of these platforms, supported by advances in nanotechnology, artificial intelligence, and systems biology, underscores the potential for combinatorial and personalized interventions. Looking forward, the next decade is likely to witness increasingly interdisciplinary collaboration, translating these innovations from bench to bedside and ushering in an era of precision therapeutics capable of addressing diverse genetic, infectious, and oncologic diseases at an unprecedented scale.

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