Journal of Pharmacology, Genetics and Molecular Biology (JPGMB)
ISSN: 3049-3757 | Vol. 02, Issue-02, March-April, 2026

Gene-Editing and Drug Delivery Convergence: Pharmaceutical Strategies for Precision Gene Therapy

Sanjeev Kumar[1*], Kumar Harsheet Raj[2], Kirti Rani[3], Manoj Kumawat[4], Priyanshi Srivastava[1]

[1]ESIC Medical College and Hospital, Bihta, Patna,801103
[2]Netaji Subhas Medical College & Hospital, Bihta, Patna
[3]Department of Biochemistry, IGIMS, Patna
[4]Indian Institute of Science Education and Research, Bhopal-462066, Madhya Pradesh, India

***Corresponding Author E-mail: drsanjeevscb@gmail.com**

Abstract:

Gene-editing therapeutics, powered by transformative molecular tools such as CRISPR/Cas, TALENs, and zinc-finger nucleases, offer unprecedented precision in treating genetic and acquired diseases. Successful clinical translation is profoundly dependent on efficient, safe, and targeted delivery vectors that navigate biological barriers to deliver gene-editing machinery intracellularly while minimizing off-target effects and immunogenicity. This review discusses viral, non-viral, and hybrid biomimetic delivery platforms—highlighting their mechanisms of cellular uptake, intracellular trafficking, and controlled release—for enhanced gene-editing efficiency and safety. It explores advanced formulation strategies incorporating stabilization, stimuli-responsiveness, and active targeting, alongside pharmacokinetic and biodistribution considerations. Clinical applications span monogenic disorders, oncology, infectious diseases, and neurodegenerative conditions, demonstrating therapeutic promise. Despite significant challenges including off-target editing, vector immunogenicity, manufacturing reproducibility, and regulatory hurdles, ongoing advances in gene editor design, AI-driven optimization, and personalized medicine forecast a new era in precision gene therapy. Integration of multi-modal therapies and real-time monitoring will further enhance safety and efficacy, establishing gene-editing therapeutics as a cornerstone of modern precision medicine.

Keywords: Gene editing, CRISPR/Cas, TALENs, zinc-finger nucleases, gene delivery vectors, viral vectors, non-viral vectors, biomimetic nanoparticles, intracellular trafficking, controlled release, off-target effects, immunogenicity, pharmacokinetics, personalized medicine, precision gene therapy, AI-driven optimization.

Received: Jan. 10, 2026 **Revised: Feb. 19, 2026** **Accepted: March. 28, 2026** **Published: April 8, 2026**

DOI: https://doi.org/10.64062/JPGMB.Vol2.Issue2.1

https://jpgmb.com/1/issue/archive

1. Introduction

Gene therapy has emerged as one of the most transformative paradigms in modern medicine, offering the potential to treat, cure, or even prevent a wide range of genetic and acquired disorders at their molecular root. Unlike conventional therapeutics that primarily focus on symptom management, gene therapy targets the underlying genetic aberrations, providing precision interventions that can permanently correct defective genes or modulate gene expression. Over the past two decades, significant advances in molecular biology and genomics have paved the way for increasingly sophisticated approaches, culminating in the development of gene-editing technologies such as CRISPR/Cas9, transcription activator-like effector nucleases (TALENs), and zinc-finger nucleases (ZFNs) [1-2]. These tools enable targeted modifications at precise genomic loci, allowing for unparalleled specificity in correcting mutations, knocking out deleterious genes, or introducing therapeutic sequences. The clinical relevance of these gene-editing platforms has been demonstrated across a spectrum of disorders, including monogenic diseases, cancers, and viral infections, showcasing their immense potential to revolutionize patient care.

Despite these advances, the successful translation of gene-editing strategies into clinically viable therapies remains constrained by significant challenges in delivery. The intracellular and nuclear localization requirements of gene-editing components necessitate highly efficient delivery vehicles capable of navigating biological barriers while maintaining payload integrity. Naked nucleic acids, such as CRISPR-associated guide RNAs or plasmid DNA, are prone to rapid degradation by nucleases in the bloodstream, limited cellular uptake, and potential immunogenicity. Furthermore, off-target effects, unwanted immune activation, and dose-limiting toxicity present critical hurdles that must be addressed to ensure both safety and efficacy. These limitations underscore the urgent need for sophisticated drug delivery systems that can protect gene-editing cargo, facilitate targeted cellular uptake, and enable controlled release, all while minimizing adverse effects [3-4].

In response to these challenges, the integration of advanced drug delivery strategies with gene-editing technologies has emerged as a pivotal approach in precision medicine. Nanocarriers, viral vectors, polymeric systems, and hybrid biomimetic platforms are increasingly being explored as vehicles for the efficient transport of gene-editing tools to target tissues. These delivery systems not only enhance the stability and bioavailability of the payload but also allow for tissue-specific targeting, reduced immunogenicity, and improved intracellular trafficking, thus bridging the gap between molecular innovation and clinical application. By leveraging these strategies, researchers aim to achieve precise, controlled, and safe gene modifications, expanding the therapeutic horizon of gene therapy beyond monogenic disorders to complex diseases, including oncology, neurodegenerative conditions, and cardiovascular pathologies.

The objective of this review is to provide a comprehensive examination of the convergence between gene-editing technologies and drug delivery systems, emphasizing pharmaceutical strategies that optimize therapeutic outcomes in precision medicine. This paper explores the current landscape of gene-editing platforms, vector systems, and nanocarrier-based delivery

approaches, alongside considerations of pharmacokinetics, biodistribution, safety, and regulatory challenges. By highlighting both the potential and the limitations of existing methodologies, the review aims to outline future directions for integrating innovative delivery strategies with gene-editing therapeutics, ultimately advancing the clinical translation of precision gene therapy [5-6].

2. Gene-Editing Technologies

Gene-editing technologies have fundamentally reshaped the landscape of molecular therapeutics, offering unprecedented precision in modifying the genome and correcting genetic abnormalities. Among the most widely adopted and versatile tools is the CRISPR/Cas system, which has rapidly become a cornerstone in both basic research and therapeutic development. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and its associated nucleases, such as Cas9 and Cas12, leverage an RNA-guided mechanism to introduce site-specific double-strand breaks in DNA, enabling targeted gene knockout, insertion, or correction. The simplicity and programmability of CRISPR/Cas systems lie in the use of a single-guide RNA (sgRNA) that directs the nuclease to the complementary genomic locus, allowing precise editing with minimal labor-intensive customization. Beyond the canonical CRISPR/Cas9, advancements such as base editors and prime editors have further expanded the toolkit, enabling single-nucleotide modifications or precise insertions without creating double-strand breaks, thereby reducing the risks of undesired mutations or chromosomal rearrangements. These refinements have broadened the therapeutic applicability of CRISPR-based systems to monogenic disorders, oncogenes, viral infections, and metabolic diseases, underscoring their transformative potential in personalized medicine [7-8].

Complementing the CRISPR/Cas paradigm, transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs) represent early-generation, protein-based gene-editing platforms that remain clinically relevant due to their high specificity and unique mechanisms of action. TALENs consist of programmable DNA-binding domains fused to a FokI nuclease, where each repeat domain recognizes a specific nucleotide sequence, allowing targeted cleavage when paired with another TALEN on the opposite DNA strand. Similarly, ZFNs employ zinc-finger motifs, each capable of binding a triplet nucleotide sequence, coupled to the FokI nuclease for site-specific DNA cleavage. These engineered nucleases operate through a dimerization-dependent cleavage mechanism, inducing double-strand breaks that stimulate endogenous DNA repair pathways, including non-homologous end joining or homology-directed repair. While TALENs and ZFNs require more complex protein engineering than CRISPR systems, they offer advantages such as reduced off-target activity in certain contexts and the ability to target genomic regions inaccessible to RNA-guided nucleases. Therapeutic applications of TALENs and ZFNs have been demonstrated in clinical trials for hemoglobinopathies, immune cell engineering, and viral resistance, reflecting their enduring value in precision gene therapy [9-10].

A key distinction between RNA-guided and protein-based editing platforms lies in their mode of target recognition and ease of customization. CRISPR/Cas systems rely on the complementary base pairing of sgRNA to the DNA target, making them highly adaptable to virtually any genomic locus with minimal redesign, whereas TALENs and ZFNs require the

labor-intensive assembly of customized protein-DNA recognition domains for each target site. However, protein-based platforms may exhibit lower immunogenicity in certain cell types and reduced dependence on endogenous cellular machinery, factors that can be advantageous for therapeutic applications. RNA-guided editors, particularly CRISPR-based systems, benefit from rapid iteration, multiplexing potential, and integration with emerging delivery platforms, including lipid nanoparticles, viral vectors, and hybrid nanocarriers. Moreover, innovations in CRISPR technology, such as catalytically inactive "dead" Cas nucleases fused to transcriptional modulators, epigenetic modifiers, or fluorescent tags, allow precise regulation of gene expression and real-time tracking of genomic loci, expanding their utility beyond simple genome editing into functional genomics and therapeutic modulation [11].

Despite their transformative potential, each gene-editing platform carries inherent advantages and limitations that inform their selection for specific therapeutic contexts. CRISPR/Cas systems are lauded for simplicity, scalability, and versatility but may elicit off-target mutations, immune responses against bacterial nucleases, or unintended genomic rearrangements, necessitating careful optimization and delivery strategies. TALENs and ZFNs, while highly specific, require more extensive engineering, which can limit throughput and increase production costs. The choice of editing platform often depends on the disease target, tissue type, delivery constraints, and regulatory considerations, with an increasing emphasis on minimizing genotoxicity and maximizing safety. Furthermore, the development of next-generation editors, including prime editors, base editors, and RNA-targeting CRISPR variants, seeks to overcome these limitations by offering higher precision, reduced off-target effects, and the ability to edit otherwise intractable genomic sequences.

Therapeutically, gene-editing technologies have been applied across a wide spectrum of diseases. Hematological disorders such as sickle cell anemia and beta-thalassemia have been successfully targeted using ex vivo editing of hematopoietic stem cells with CRISPR/Cas9 or ZFNs, followed by autologous transplantation. In oncology, CRISPR-mediated knockout or modulation of immune checkpoint genes in T-cells is being explored to enhance the efficacy of CAR-T therapies. Viral infections, including HIV, have been addressed through targeted excision or inactivation of viral genomes using CRISPR-based approaches. Furthermore, metabolic and rare genetic disorders are increasingly considered viable targets for in vivo gene-editing interventions, particularly when combined with advanced delivery systems capable of tissue-specific targeting and sustained activity. As these technologies continue to evolve, their integration with computational modeling, high-throughput screening, and predictive pharmacology is poised to optimize editing efficiency, minimize risks, and accelerate translation into clinically approved therapies [12-13].

In summary, the landscape of gene-editing technologies encompasses a diverse array of RNA-guided and protein-based platforms, each with unique mechanisms, advantages, and limitations. CRISPR/Cas systems provide unparalleled versatility and ease of use, enabling rapid adaptation to new targets and the development of sophisticated therapeutic strategies. TALENs and ZFNs, while more labor-intensive, maintain a critical role due to their specificity and well-characterized performance in clinical applications. Understanding the strengths and weaknesses of each platform is essential for designing effective gene therapies, particularly when integrated with advanced drug delivery systems that enhance cellular uptake, reduce

immunogenicity, and improve overall therapeutic outcomes. The continued refinement and convergence of these gene-editing technologies with precision delivery strategies herald a new era in molecular medicine, where targeted, safe, and efficient genome modification can be translated into transformative clinical interventions [15-16].

3. Vectors for Gene Delivery

The successful translation of gene-editing technologies into clinical therapies hinges not only on the precision of the nucleases but also on the development of efficient, safe, and target-specific delivery vectors. Gene-editing tools, whether CRISPR/Cas systems, TALENs, or ZFNs, require carriers that protect them from degradation, facilitate cellular uptake, and direct them to specific tissues or cell types. Broadly, vectors for gene delivery are categorized into viral, non-viral, and hybrid/biomimetic systems, each with distinct advantages, limitations, and applications in precision medicine [17-18].

Viral vectors have long been the cornerstone of gene therapy due to their inherent efficiency in transducing mammalian cells and their natural ability to deliver genetic material into the nucleus. Among these, adeno-associated viruses (AAVs) have gained prominence for their relatively low immunogenicity, broad tropism, and capacity to mediate long-term gene expression without integrating into the host genome, reducing the risk of insertional mutagenesis. AAVs have been successfully employed in clinical trials targeting monogenic diseases, including retinal dystrophies, hemophilia, and spinal muscular atrophy. Lentiviral vectors, derived from human immunodeficiency virus (HIV), offer stable genomic integration and sustained expression in dividing and non-dividing cells, making them suitable for ex vivo gene-editing applications such as hematopoietic stem cell modification. Retroviral vectors, though historically important, are limited by preferential integration into dividing cells and potential insertional oncogenesis, prompting the development of safer, self-inactivating variants. Viral vectors are highly efficient due to their evolved mechanisms for cellular entry, nuclear trafficking, and transcriptional activation. However, their clinical use is constrained by immunogenicity, limited cargo capacity, potential for off-target integration, and manufacturing challenges that require strict biosafety protocols and high production costs [19-20].

Non-viral vectors have emerged as a versatile alternative, offering greater flexibility, safety, and scalability than viral systems, albeit generally with lower intrinsic transfection efficiency. Lipid nanoparticles (LNPs) are among the most widely studied non-viral carriers, composed of cationic or ionizable lipids that encapsulate nucleic acids and facilitate cellular uptake via endocytosis. LNPs protect gene-editing components from nuclease degradation and can be functionalized with targeting ligands for tissue-specific delivery, as demonstrated by the successful deployment of mRNA vaccines during the COVID-19 pandemic. Polymeric carriers, including biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA), polyethyleneimine (PEI), chitosan, and PEGylated derivatives, provide tunable surface chemistry, controlled release properties, and reduced immunogenicity. Dendrimers, with their highly branched architecture and multivalent surface groups, enable efficient nucleic acid condensation, cellular internalization, and endosomal escape. Inorganic nanoparticles, such as gold, silica, and magnetic particles, offer additional advantages, including imaging compatibility, photothermal responsiveness, and theranostic potential. Non-viral vectors can

accommodate larger cargoes than viral systems and are less likely to trigger immune responses, enabling repeated administration and combination therapies. However, challenges remain, including optimizing endosomal escape, improving nuclear delivery efficiency, and ensuring consistent performance across diverse cell types [21-22].

Hybrid and biomimetic delivery systems represent the latest evolution in vector design, combining the efficiency of natural systems with the safety and tunability of synthetic platforms. Exosome-mimetic carriers leverage the biology of extracellular vesicles to achieve efficient cellular uptake, immune evasion, and inherent tropism toward specific tissues. These systems can encapsulate gene-editing components, including CRISPR ribonucleoproteins (RNPs), mRNA, or plasmid DNA, while maintaining the biocompatibility and low immunogenicity of endogenous vesicles. Cell membrane-coated nanoparticles represent another innovative strategy, wherein synthetic carriers are cloaked with membranes derived from red blood cells, platelets, or cancer cells, conferring extended circulation, immune evasion, and homotypic targeting capabilities. Lipid–polymer hybrids combine the structural stability of polymeric cores with the biocompatibility and membrane-fusion properties of lipids, enabling efficient cytosolic delivery and controlled release. These biomimetic systems hold great promise for precision gene therapy, particularly for in vivo applications where targeted delivery and immune modulation are critical [23-24].

The choice of vector is dictated by multiple factors, including the nature of the therapeutic cargo, the target tissue or cell type, the desired duration of gene expression, and safety considerations. For ex vivo therapies, where cells can be modified outside the body and then reintroduced, integrating viral vectors such as lentiviruses provides sustained expression, while non-viral vectors can be optimized for transient editing with minimal genomic disruption. In contrast, in vivo applications necessitate vectors capable of navigating physiological barriers, evading immune detection, and achieving targeted tissue accumulation; here, non-viral and biomimetic systems offer distinct advantages due to their modifiable surface properties and reduced immunogenicity. Critical parameters such as vector size, charge, and surface functionality influence biodistribution, cellular uptake, and intracellular trafficking, underscoring the importance of rational design and predictive modeling in vector development.

Clinical translation of gene-editing therapies is increasingly reliant on the convergence of vector innovation and delivery strategies. Viral vectors have already achieved regulatory approval in a limited number of therapies, demonstrating efficacy in rare genetic disorders. Non-viral and hybrid systems are rapidly advancing through preclinical and early clinical stages, with the potential to address broader patient populations and repeated dosing requirements. Furthermore, the integration of these vectors with stimuli-responsive or targeted nanocarriers enhances their therapeutic index, allowing spatiotemporal control over gene-editing activity. Safety remains a paramount concern, necessitating rigorous evaluation of cytotoxicity, immunogenicity, off-target effects, and long-term persistence, particularly for in vivo applications [25-26].

In summary, vectors for gene delivery form the backbone of precision gene therapy, mediating the efficient and safe transport of gene-editing machinery to the intended cells. Viral vectors, including AAVs, lentiviruses, and retroviruses, provide high transduction efficiency and

established clinical applicability but are constrained by immunogenicity, cargo limitations, and production complexity. Non-viral vectors, such as lipid nanoparticles, polymeric systems, dendrimers, and inorganic nanoparticles, offer safety, tunability, and scalability, though optimization is required to match viral efficiency. Hybrid and biomimetic systems, including exosome-mimetic and cell membrane-coated carriers, represent the next frontier, combining the advantages of natural and synthetic platforms to achieve targeted, safe, and efficient gene delivery. The rational selection and design of delivery vectors, tailored to specific therapeutic goals, are essential to realizing the full potential of gene-editing technologies in precision medicine, enabling effective, controlled, and clinically translatable interventions [27-28].

4. Mechanisms of Cellular Uptake and Intracellular Delivery

The efficacy of gene-editing therapeutics is critically dependent on the ability of delivery vectors to traverse cellular barriers and release their cargo at the appropriate intracellular site. Cellular uptake and intracellular delivery mechanisms determine not only the efficiency of gene-editing activity but also the specificity, safety, and overall therapeutic outcome. A comprehensive understanding of these processes is essential for designing vectors that can successfully navigate complex cellular environments while minimizing off-target effects and cytotoxicity. Multiple endocytic pathways, vector properties, and intracellular trafficking routes collectively influence how nucleic acids, proteins, or ribonucleoprotein complexes are internalized and processed within target cells.

Endocytosis represents the predominant route for the internalization of both viral and non-viral vectors. This energy-dependent process allows cells to engulf extracellular materials via invaginations of the plasma membrane, forming endocytic vesicles. Endocytosis can be further subdivided into clathrin-mediated, caveolae-mediated, clathrin- and caveolae-independent pathways, and macropinocytosis. Clathrin-mediated endocytosis involves the formation of clathrin-coated pits that concentrate ligands bound to specific receptors, facilitating targeted uptake of ligand-conjugated vectors. This pathway is particularly relevant for vectors designed with targeting moieties, such as antibodies, peptides, or aptamers, that recognize cell surface receptors. Caveolae-mediated endocytosis, by contrast, relies on lipid raft domains rich in cholesterol and caveolin proteins and often avoids lysosomal degradation, providing a more direct route to the cytoplasm or specific organelles. Macropinocytosis involves the non-specific uptake of large volumes of extracellular fluid, forming macropinosomes that can encapsulate bulk nanocarriers. While this pathway is less selective, it is particularly exploited by larger nanoparticles and viral vectors to achieve high internalization rates [29-30].

Once internalized, vectors are often trafficked to endosomes, which serve as sorting compartments that determine their intracellular fate. Efficient endosomal escape is a critical bottleneck in gene-editing delivery, as entrapment in endosomes can lead to degradation of nucleic acids by lysosomal enzymes. Various strategies have been developed to facilitate endosomal escape, depending on the vector type. Lipid-based vectors, such as cationic liposomes or lipid nanoparticles, often leverage the proton-sponge effect or pH-responsive fusogenic lipids to destabilize the endosomal membrane under acidic conditions. Polymeric carriers, particularly those with amine-rich or pH-sensitive polymers, can swell in acidic endosomes, generating osmotic pressure that disrupts vesicles and releases the cargo. In viral

vectors, naturally evolved fusion proteins mediate endosomal escape and nuclear entry, highlighting the importance of structural motifs in efficient intracellular delivery. Emerging biomimetic and hybrid systems combine these strategies, incorporating cell-penetrating peptides, fusogenic lipids, or membrane-disruptive polymers to optimize cytoplasmic or nuclear release.

Intracellular trafficking extends beyond endosomal escape and involves navigation toward the appropriate subcellular compartment, depending on the therapeutic target. For gene-editing applications, nuclear delivery is essential for DNA- or plasmid-based systems, while mRNA or ribonucleoprotein complexes may require only cytoplasmic localization. Microtubule-mediated transport is a critical component of intracellular trafficking, particularly for larger nanoparticles and viral vectors, which often utilize dynein or kinesin motor proteins to traverse the cytoplasm. Vectors can also be designed with nuclear localization signals (NLS) or organelle-targeting ligands to enhance specificity and efficiency. Targeting to mitochondria, lysosomes, or endoplasmic reticulum is also feasible through specific peptide sequences or small molecule conjugates, enabling precision therapeutic interventions for organelle-specific disorders [31-32].

The physicochemical properties of delivery vectors strongly influence cellular uptake, trafficking, and eventual therapeutic efficacy. Particle size is a key determinant, as nanoparticles within the 20–200 nm range are generally optimal for receptor-mediated endocytosis and efficient intracellular transport, whereas larger particles may rely on macropinocytosis or phagocytosis. Surface charge modulates interactions with the negatively charged cell membrane: cationic vectors enhance adsorption and uptake but may induce cytotoxicity, while neutral or slightly anionic vectors offer improved biocompatibility and reduced immunogenicity. Surface modifications, including polyethylene glycol (PEG) coatings, targeting ligands, or hydrophobic moieties, further dictate uptake efficiency, circulation time, and intracellular trafficking pathways. Ligand conjugation enables active targeting to specific receptors, promoting selective internalization by diseased cells and minimizing off-target effects.

Intracellular fate is also affected by vector architecture, including shape, rigidity, and hydrophobicity. Spherical nanoparticles are generally internalized more efficiently, whereas rod-shaped or filamentous vectors may exhibit prolonged circulation and altered endocytic pathways. Flexible or deformable carriers can navigate the dense extracellular matrix and cellular microenvironments more effectively, improving intracellular delivery in tissues such as tumors or fibrotic regions. Moreover, stimuli-responsive carriers that respond to pH, redox potential, or enzymatic activity can release their cargo in a controlled manner within specific subcellular compartments, enhancing therapeutic efficacy while reducing systemic exposure.

The interplay between vector design and cellular mechanisms has been demonstrated in numerous preclinical studies. For instance, lipid nanoparticles functionalized with targeting ligands and endosome-disruptive elements achieve high cytoplasmic delivery of CRISPR/Cas9 RNPs, resulting in efficient gene editing with minimal off-target effects. Similarly, polymeric nanoparticles engineered with pH-sensitive linkers and NLS motifs have been shown to facilitate nuclear entry of plasmid DNA in stem cells, overcoming traditional barriers

associated with nuclear envelope transport. Viral vectors continue to exploit natural intracellular trafficking machinery to achieve high transduction efficiencies, highlighting the evolutionary advantage of these systems. Hybrid systems combining polymeric cores with lipid shells or cell membrane coatings have demonstrated improved uptake, endosomal escape, and targeting specificity, underscoring the potential of biomimetic approaches.

Despite significant advances, challenges remain in fully elucidating and controlling intracellular delivery processes. The heterogeneity of cellular uptake pathways, differences in endosomal maturation rates, and variability across cell types complicate the prediction of vector behavior. Additionally, the potential for cytotoxicity, immune activation, or unintended interactions with intracellular organelles necessitates careful vector design and thorough preclinical evaluation. Computational modeling, high-content imaging, and single-cell analyses are increasingly employed to understand uptake kinetics, trafficking routes, and subcellular localization, guiding the rational design of next-generation delivery systems [32-34].

In conclusion, the mechanisms of cellular uptake and intracellular delivery are central to the success of gene-editing therapeutics. Endocytosis, micropinocytosis, and receptor-mediated pathways determine the initial internalization of vectors, while endosomal escape and intracellular trafficking govern the availability of the therapeutic cargo at its target site. Vector properties, including size, charge, surface modifications, ligand conjugation, and architecture, critically influence these processes, dictating efficiency, specificity, and safety. A deep understanding of these mechanisms enables the rational design of viral, non-viral, and hybrid vectors capable of precise, effective, and clinically translatable gene-editing interventions, laying the foundation for the next generation of precision medicine therapies. Figure 1

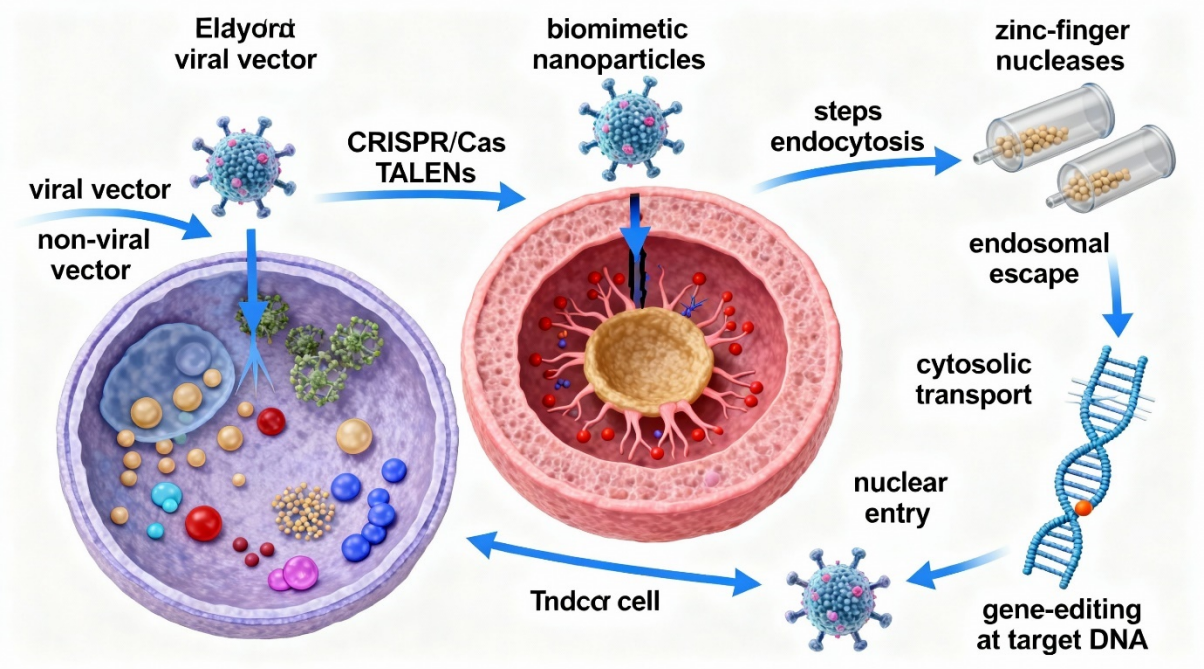

Figure 1: Illustration of gene-editing payload delivery and intracellular trafficking

5. Formulation Strategies for Gene-Editing Therapeutics

The formulation of gene-editing therapeutics represents a critical determinant of their efficacy, specificity, and safety. At the core of these formulations lies the need to efficiently encapsulate and protect the active biomolecules—be it plasmid DNA, messenger RNA (mRNA), single-guide RNA (sgRNA), or ribonucleoprotein (RNP) complexes—while ensuring their delivery to the target cell and subcellular compartment. Nucleic acids are inherently unstable in biological environments, prone to degradation by nucleases, and can trigger immunogenic responses if unshielded. Therefore, robust encapsulation strategies are essential, with lipid-based nanoparticles, polymeric carriers, and hybrid systems being the most widely employed. Lipid nanoparticles, for example, provide a hydrophobic shell that protects nucleic acids and facilitates membrane fusion, whereas polymeric systems such as poly(β-amino esters), chitosan derivatives, and PEGylated polymers offer tunable release profiles, surface functionalization, and enhanced stability. The selection of vector composition is often guided by the size, charge, and hydrophobicity of the cargo, as well as the intended tissue or cellular target [35-36].

Stabilization of the nucleic acid payload is another critical aspect, with chemical modifications employed to improve resistance to enzymatic degradation, reduce immunogenicity, and prolong systemic circulation. Modifications such as 2'-O-methyl or 2'-fluoro substitutions in RNA, phosphorothioate linkages, and end-capping strategies enhance nuclease resistance while maintaining biological activity. Additionally, PEGylation of nanoparticles or direct conjugation to nucleic acids improves colloidal stability, reduces opsonization, and minimizes rapid clearance by the mononuclear phagocyte system. Lipid coatings or amphiphilic molecules can further shield gene-editing components from premature degradation, ensuring that the therapeutic payload reaches the target tissue intact.

Controlled and stimuli-responsive release is a key strategy in optimizing gene-editing therapeutics. By designing carriers that respond to specific triggers—such as pH shifts in the endosomal environment, enzymatic activity in target tissues, redox potential in the cytoplasm, or external stimuli like light and ultrasound—therapeutic molecules can be released at precise intracellular locations. For instance, pH-sensitive lipids or polymers can destabilize under acidic endosomal conditions, promoting endosomal escape and cytoplasmic availability of RNP complexes. Redox-responsive linkers degrade in the presence of intracellular glutathione, while enzyme-sensitive polymers release payloads in tissues overexpressing specific proteases. Such precision-controlled release strategies reduce off-target activity and systemic exposure, which is critical for the safety of gene-editing therapeutics.

Targeted delivery remains central to the design of gene-editing formulations, enhancing both efficacy and safety. Surface modification with ligands, such as antibodies, aptamers, peptides, or small molecules, allows vectors to bind selectively to receptors on target cells, facilitating receptor-mediated endocytosis. This active targeting approach complements passive targeting strategies based on particle size, charge, and tissue-specific permeability. Moreover, co-delivery approaches, where gene-editing components are combined with therapeutic drugs, chemotherapeutic agents, or immunomodulators within the same carrier, offer synergistic benefits. Such multi-functional formulations enable simultaneous genome editing and pharmacological intervention, enhancing treatment outcomes in complex diseases such as cancer or genetic disorders with inflammatory components [37-38].

Formulation strategies are further refined through advanced techniques like microfluidic-assisted nanoparticle assembly, layer-by-layer deposition, and self-assembling nanostructures, which offer precise control over size distribution, surface charge, and encapsulation efficiency. These approaches also allow scalable production, batch reproducibility, and integration with quality-by-design (QbD) principles, ensuring consistent performance and regulatory compliance. Computational modeling and machine learning are increasingly employed to predict optimal formulation parameters, facilitating rational design and reducing reliance on empirical trial-and-error methods [39].

6. Pharmacokinetics, Biodistribution, and Safety

The pharmacokinetics, biodistribution, and safety profile of gene-editing therapeutics are heavily influenced by the design of the delivery vector and its physicochemical properties. Following administration, the systemic circulation, tissue accumulation, and clearance of the vector determine both the efficacy and the potential off-target effects. Particle size, surface charge, hydrophobicity, and surface modifications dictate interactions with serum proteins, immune cells, and the vascular endothelium, affecting circulation time and organ-specific accumulation. Small nanoparticles (<100 nm) generally achieve better tissue penetration, whereas larger particles may exhibit prolonged retention in reticuloendothelial organs such as the liver and spleen. PEGylation and other stealth coatings minimize opsonization and phagocytic uptake, enhancing systemic availability and reducing immune-mediated clearance.

Biodistribution is critically important in ensuring that gene-editing tools reach their intended cellular targets while minimizing exposure to non-target tissues. Targeted delivery strategies, including ligand-mediated recognition and tissue-specific promoters, enhance localization and activity in diseased cells, thereby improving therapeutic outcomes and reducing toxicity. For example, lipid nanoparticles delivering CRISPR/Cas mRNA specifically to hepatocytes can achieve efficient gene editing in the liver with minimal systemic exposure. Non-viral polymeric and inorganic vectors are designed to exploit specific transport mechanisms, including receptor-mediated uptake or enhanced permeation in pathological tissues such as tumors, to achieve site-specific delivery [40-41].

Immunogenicity and off-target effects remain significant concerns in gene-editing therapies. Both viral and non-viral vectors can trigger innate and adaptive immune responses, leading to clearance of the therapeutic payload or adverse reactions. Chemical modifications, such as nucleic acid base alterations or use of synthetic RNPs, can mitigate immune recognition. Additionally, vector design strategies that minimize cationic surface charge or incorporate immunologically inert coatings further reduce the risk of inflammation or complement activation. Preclinical evaluation in animal models, including comprehensive toxicology studies, is essential to assess both acute and chronic safety, covering endpoints such as cytotoxicity, hematology, organ function, and genotoxicity.

Toxicity assessment also encompasses off-target gene editing, which may result in unintended mutations or dysregulation of non-target genes. High-fidelity Cas variants, truncated guide RNAs, or ribonucleoprotein delivery reduce off-target activity, while computational modeling and genome-wide off-target prediction tools inform design choices. In parallel, vector

optimization for controlled intracellular release and tissue-specific targeting minimizes systemic exposure and enhances the therapeutic index [42].

Pharmacokinetic improvements are achieved through strategies that prolong systemic circulation, enhance tissue accumulation, and facilitate controlled intracellular release. Encapsulation within nanoparticles shields nucleic acids from degradation, while surface functionalization modulates interaction with blood components and target cells. Stimuli-responsive carriers allow for the spatially and temporally precise release of cargo, ensuring high local concentration in target tissues with minimal systemic diffusion. Integration of pharmacokinetic studies with in vitro–in vivo correlation models enables predictive evaluation of bioavailability, therapeutic efficacy, and safety, guiding clinical translation.

Preclinical studies have demonstrated that rationally designed gene-editing formulations can achieve high editing efficiency while maintaining favorable pharmacokinetics and safety. For instance, lipid nanoparticle delivery of Cas9 mRNA and sgRNA to liver cells achieved >80% gene editing with minimal immune activation. Polymeric nanoparticles with redox-responsive release exhibited enhanced cytoplasmic delivery and reduced off-target editing in in vivo tumor models. Hybrid systems combining cell membrane coatings with polymeric cores improved circulation time, reduced immunogenicity, and enhanced targeting specificity, highlighting the potential for safe and efficient clinical translation.

In conclusion, formulation strategies and pharmacokinetic considerations are interdependent in determining the success of gene-editing therapeutics. Optimized encapsulation, stabilization, controlled and stimuli-responsive release, and targeted delivery collectively improve bioavailability, efficacy, and safety. Understanding the influence of vector properties on systemic circulation, tissue distribution, and immunogenicity is critical for minimizing off-target effects and toxicity. Together, these strategies lay the foundation for clinically translatable gene-editing therapies capable of precise genome modification with predictable pharmacological profiles and improved patient outcomes [43-44].

7. Therapeutic Applications

The integration of gene-editing technologies with advanced drug delivery systems has revolutionized the treatment landscape for a wide spectrum of diseases, enabling highly precise and potentially curative interventions. Among the most impactful applications are monogenic disorders, where single-gene defects drive pathogenesis. Conditions such as sickle cell disease, cystic fibrosis, hemophilia, and Duchenne muscular dystrophy are prime candidates for gene-editing therapeutics. In these contexts, CRISPR/Cas-based systems, TALENs, and zinc-finger nucleases are employed to either correct the defective gene, restore normal protein function, or disrupt pathological gene expression. The use of lipid nanoparticles, polymeric carriers, or viral vectors allows for efficient delivery of gene-editing components directly to the relevant cell types, such as hematopoietic stem cells in sickle cell disease. Preclinical and early clinical studies have demonstrated successful gene correction ex vivo, followed by autologous transplantation, achieving long-term restoration of functional proteins with minimal off-target effects [46-47].

In oncology, gene-editing therapeutics offer transformative strategies to tackle both solid tumors and hematological malignancies. Gene-editing platforms can be designed to inactivate oncogenes, restore tumor suppressor gene function, or modulate immune checkpoints to enhance anti-tumor immunity. For instance, CRISPR-mediated knockout of PD-1 in T cells has shown promise in enhancing adoptive cell therapy for cancer patients. Advanced delivery strategies ensure that gene editors reach tumor microenvironments while minimizing systemic exposure, thereby reducing cytotoxicity to healthy tissues. Additionally, hybrid nanocarriers that combine imaging and therapeutic functionalities facilitate real-time monitoring of delivery and therapeutic efficacy, creating opportunities for theranostic approaches in precision oncology.

Infectious diseases represent another major arena for gene-editing applications. Viral infections such as HIV, hepatitis B, and emerging viral pathogens can potentially be targeted through the disruption of viral genomes, inactivation of host cell receptors necessary for viral entry, or modulation of antiviral immune responses. Delivery of CRISPR/Cas systems via targeted nanoparticles or viral vectors enables selective gene editing in infected cells, reducing viral load and improving immune clearance. For example, preclinical studies using AAV-mediated CRISPR/Cas delivery have successfully excised integrated viral DNA in hepatocytes for hepatitis B models. Similarly, ex vivo editing of immune cells to enhance antiviral responses is being investigated for chronic viral infections, illustrating the versatility of gene-editing therapeutics in infectious disease management [48-49].

Neurological disorders, particularly inherited neurodegenerative diseases such as Huntington's disease, spinal muscular atrophy, and certain forms of epilepsy, are emerging targets for gene-editing therapeutics. The blood–brain barrier (BBB) presents a formidable delivery challenge; however, lipid nanoparticles, polymeric nanocarriers, and exosome-mimetic systems have demonstrated potential in transporting gene-editing machinery across this barrier. Once delivered, precise genome editing can correct pathogenic mutations or modulate gene expression, potentially halting or reversing neurodegeneration. Early preclinical studies have achieved successful gene editing in neuronal populations, offering hope for conditions previously considered untreatable. In addition to monogenic disorders, polygenic and complex neurological conditions are increasingly explored using combinatorial gene-editing strategies paired with small-molecule therapeutics to maximize therapeutic outcomes.

Case studies highlighting preclinical and clinical successes underscore the feasibility of these approaches. For instance, CRISPR/Cas9-edited hematopoietic stem cells have been used in clinical trials for sickle cell disease and β-thalassemia, achieving functional hemoglobin production and clinical remission in patients. Similarly, engineered CAR-T cells with PD-1 knockouts are being evaluated in oncology trials, demonstrating enhanced anti-tumor activity and persistence. These examples collectively illustrate the immense therapeutic potential when gene-editing technologies are precisely delivered to their targets, offering unprecedented opportunities for disease modification across genetic, infectious, oncological, and neurological domains [50-51].

8. Challenges and Limitations

Despite remarkable progress, significant challenges impede the widespread clinical translation of gene-editing therapeutics. Off-target gene editing remains a paramount concern, as unintended mutations can have deleterious consequences, including tumorigenesis or disruption of essential gene networks. Advances in high-fidelity Cas enzymes, truncated guide RNAs, and base/prime editors have reduced off-target activity, but robust and scalable monitoring strategies are still necessary to ensure safety. Off-target effects are particularly critical in in vivo applications, where systemic distribution of gene editors may expose non-target tissues to potential mutagenesis.

Vector immunogenicity and delivery efficiency present additional barriers. Viral vectors, though highly efficient, can provoke strong immune responses, limiting repeated dosing and raising safety concerns. Non-viral vectors, while generally safer, often exhibit lower transfection efficiencies, necessitating optimization of particle size, charge, and surface chemistry to balance efficacy and biocompatibility. Hybrid systems, such as exosome-mimetic nanoparticles or cell membrane-coated carriers, aim to address these issues, but their scalability and reproducibility are still under investigation [52].

Scalability, reproducibility, and manufacturing challenges further complicate clinical translation. The production of high-quality vectors with consistent encapsulation, particle size distribution, and surface functionality requires precise control and robust quality assurance. Moreover, regulatory oversight demands adherence to stringent guidelines for good manufacturing practice (GMP), complicating large-scale deployment. Economic considerations, including production cost and accessibility, remain critical, particularly for rare diseases where patient populations are small, limiting commercial incentive for investment.

Finally, regulatory and ethical considerations introduce significant hurdles. Human genome editing raises complex ethical questions, particularly for germline interventions or permanent modifications. While somatic gene-editing therapies are progressing in clinical trials under strict regulatory frameworks, long-term monitoring, informed consent, and societal acceptance remain critical factors. Existing guidelines from agencies such as the FDA and EMA provide pathways for approval but require comprehensive safety, efficacy, and reproducibility data. These limitations underscore the need for multidisciplinary strategies that integrate molecular biology, materials science, clinical pharmacology, and bioethics to ensure safe and effective clinical translation [53-54].

9. Future Perspectives

The future of gene-editing therapeutics lies in the convergence of next-generation editors, advanced delivery systems, and personalized medicine approaches. Development of highly specific, low-immunogenicity gene editors—including base editors, prime editors, and epigenetic modulators—promises to enhance both safety and efficacy. Integration with artificial intelligence and predictive modeling is poised to revolutionize delivery optimization, enabling rational design of vectors and real-time assessment of pharmacokinetics, biodistribution, and off-target effects. Machine learning algorithms can predict the best

combination of delivery parameters, vector composition, and patient-specific genomic context to maximize therapeutic outcomes.

Multi-modal therapies combining gene editing with nanopharmaceuticals, immunotherapy, or small-molecule drugs represent a promising direction. Such combinatorial approaches can address complex disease pathologies by providing synergistic effects, improving efficacy while reducing required doses. Personalized gene therapy, guided by patient-specific genomics, epigenomics, and tissue-specific profiles, may allow for precise intervention with minimal systemic exposure. Emerging trends, such as in vivo base editing, RNA editing, and programmable epigenetic modulation, further expand the therapeutic toolkit, allowing transient, reversible, or fine-tuned modifications of gene expression without permanent genomic alteration [56-57].

Advances in vector design, including stimuli-responsive carriers, hybrid biomimetic nanoparticles, and exosome-based delivery systems, are expected to enhance targeting precision and reduce immunogenicity. Integration with real-time imaging, biosensing, and digital health platforms may enable adaptive therapy, monitoring therapeutic outcomes at the cellular level and adjusting dosing in a feedback-controlled manner. The confluence of these innovations heralds a future where gene-editing therapeutics are safe, efficient, and fully personalized, bridging the gap between laboratory research and clinical application [58].

10. Conclusion

The convergence of gene-editing technologies and advanced drug delivery systems has transformed the paradigm of precision medicine, offering the potential for curative therapies across a wide spectrum of genetic, oncological, infectious, and neurological diseases. By leveraging innovative vectors, stabilization strategies, and targeted delivery mechanisms, gene-editing therapeutics can achieve high efficacy while minimizing off-target effects and systemic toxicity. Integration with advanced formulation techniques, predictive modeling, and AI-driven optimization further enhances safety, precision, and patient-specific tailoring.

Clinical translation has already been realized in conditions such as sickle cell disease, β-thalassemia, and adoptive T cell therapies in oncology, demonstrating that the combination of precise gene editing with efficient delivery systems can yield tangible therapeutic benefits. Nonetheless, ongoing challenges—including off-target editing, immunogenicity, manufacturing reproducibility, regulatory compliance, and ethical considerations—must be addressed through multidisciplinary collaboration and innovative engineering solutions.

Looking ahead, next-generation gene editors, multi-modal nanocarrier systems, AI-guided personalization, and advanced monitoring platforms promise to extend the reach and effectiveness of gene-editing therapeutics. Collectively, these strategies point toward a future in which gene therapy is not only highly effective and safe but also fully personalized and clinically accessible, enabling precision intervention for previously untreatable diseases and fulfilling the long-standing promise of genomic medicine.

Reference

1. National Institutes of Health. (2023). *The Human BioMolecular Atlas Program*. https://commonfund.nih.gov/HuBMAP

2. Naldini, L. (2015). Gene therapy returns to centre stage. *Nature, 526*, 351–360.

3. Watts, G. (2012). Gene therapy to be authorised for first time in EU. *BMJ, 345*, e5009.

4. US Food and Drug Administration. (2023). *Approved cellular and gene therapy products*. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products

5. Paul-Ehrlich-Institute. (2023). *Gene therapy medicinal products*. https://www.pei.de/EN/medicinal-products/atmp/gene-therapy-medicinal-products/gene-therapy-node.html

6. Andersson, R., & Sandelin, A. (2020). Determinants of enhancer and promoter activities of regulatory elements. *Nature Reviews Genetics, 21*, 71–87.

7. Vertex. (2023, November 16). *Vertex and CRISPR Therapeutics announce authorization of the first CRISPR/Cas9 gene-edited therapy, CASGEVY™*. https://investors.vrtx.com/news-releases/news-release-details/vertex-and-crispr-therapeutics-announce-authorization-first

8. Porteus, M. H. (2019). A new class of medicines through DNA editing. *New England Journal of Medicine, 380*, 947–959.

9. Diakatou, M., Manes, G., Bocquet, B., Meunier, I., & Kalatzis, V. (2019). Genome editing as a treatment for the most prevalent causative genes of autosomal dominant retinitis pigmentosa. *International Journal of Molecular Sciences, 20*, 2542.

10. Adli, M. (2018). The CRISPR tool kit for genome editing and beyond. *Nature Communications, 9*, 1911.

11. Broeders, M., Herrero-Hernandez, P., Ernst, M. P. T., van der Ploeg, A. T., & Pijnappel, W. W. M. P. (2020). Sharpening the molecular scissors: Advances in gene-editing technology. *iScience, 23*, 100789.

12. González Castro, N., Bjelic, J., Malhotra, G., Huang, C., & Alsaffar, S. H. (2021). Comparison of the feasibility, efficiency, and safety of genome editing technologies. *International Journal of Molecular Sciences, 22*, 10355.

13. Maeder, M. L., & Gersbach, C. A. (2016). Genome-editing technologies for gene and cell therapy. *Molecular Therapy, 24*, 430–446.

14. Tröder, S. E., & Zevnik, B. (2022). History of genome editing: From meganucleases to CRISPR. *Laboratory Animals, 56*, 60–68.

15. Takeuchi, R., Choi, M., & Stoddard, B. L. (2015). Engineering of customized meganucleases via in vitro compartmentalization and in cellulo optimization. In *Methods in Molecular Biology* (Vol. 1239, pp. 105–132).

16. Urnov, F. D., Miller, J. C., Lee, Y. L., et al. (2005). Highly efficient endogenous human gene correction using designed zinc-finger nucleases. *Nature, 435*, 646–651.

17. Urnov, F. D., Rebar, E. J., Holmes, M. C., Zhang, H. S., & Gregory, P. D. (2010). Genome editing with engineered zinc finger nucleases. *Nature Reviews Genetics, 11*, 636–646.

18. Kay, S., Hahn, S., Marois, E., Hause, G., & Bonas, U. (2007). A bacterial effector acts as a plant transcription factor and induces a cell size regulator. *Science, 318*, 648–651.

19. Cong, L., Ran, F. A., Cox, D., et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science, 339*, 819–823.

20. Feng, Z., Zhang, B., Ding, W., et al. (2013). Efficient genome editing in plants using a CRISPR/Cas system. *Cell Research, 23*, 1229–1232.

21. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science, 337*, 816–821.

22. Mussolino, C., Alzubi, J., Pennucci, V., Turchiano, G., & Cathomen, T. (2017). Genome and epigenome editing to treat disorders of the hematopoietic system. *Human Gene Therapy, 28*, 1105–1115.

23. Amabile, A., Migliara, A., Capasso, P., et al. (2016). Inheritable silencing of endogenous genes by hit-and-run targeted epigenetic editing. *Cell, 167*, 219–232.e14.

24. Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A., & Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature, 533*, 420–424.

25. Gaudelli, N. M., Komor, A. C., Rees, H. A., et al. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature, 551*, 464–471.

26. Kurt, I. C., Zhou, R., Iyer, S., et al. (2021). CRISPR C-to-G base editors for inducing targeted DNA transversions in human cells. *Nature Biotechnology, 39*, 41–46.

27. Anzalone, A. V., Randolph, P. B., Davis, J. R., et al. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature, 576*, 149–157.

28. Hou, X., Zaks, T., Langer, R., & Dong, Y. (2021). Lipid nanoparticles for mRNA delivery. *Nature Reviews Materials, 6*, 1078–1094.

29. Li, L., Hu, S., & Chen, X. (2018). Non-viral delivery systems for CRISPR/Cas9-based genome editing: Challenges and opportunities. *Biomaterials, 171*, 207–218.

30. Rurik, J. G., Tombácz, I., Yadegari, A., et al. (2022). CAR T cells produced in vivo to treat cardiac injury. *Science, 375*, 91–96.

31. Leavitt, A. D., Robles, G., Alesandro, N., & Varmus, H. E. (1996). Human immunodeficiency virus type 1 integrase mutants retain in vitro integrase activity yet fail to integrate viral DNA efficiently during infection. *Journal of Virology, 70*, 721–728.

32. Yáñez-Muñoz, R. J., Balaggan, K. S., MacNeil, A., et al. (2006). Effective gene therapy with nonintegrating lentiviral vectors. *Nature Medicine, 12*, 348–352.

33. Biasco, L., Rothe, M., Büning, H., & Schambach, A. (2017). Analyzing the genotoxicity of retroviral vectors in hematopoietic cell gene therapy. *Molecular Therapy – Methods & Clinical Development, 8*, 21–30.

34. Boucher, P., Cui, X., & Curiel, D. T. (2020). Adenoviral vectors for in vivo delivery of CRISPR-Cas gene editors. *Journal of Controlled Release, 327*, 788–800.

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

36. Büning, H., & Srivastava, A. (2019). Capsid modifications for targeting and improving the efficacy of AAV vectors. *Molecular Therapy – Methods & Clinical Development, 12*, 248–265.

37. Brown, C. E., Rodriguez, A., Palmer, J., et al. (2022). Off-the-shelf, steroid-resistant, IL13Rα2-specific CAR T cells for treatment of glioblastoma. *Neuro-Oncology, 24*, 1318–1330.

38. Georgiadis, C., Rasaiyaah, J., Gkazi, S. A., et al. (2021). Base-edited CAR T cells for combinational therapy against T cell malignancies. *Leukemia, 35*, 3466–3481.

39. Stadtmauer, E. A., Fraietta, J. A., Davis, M. M., et al. (2020). CRISPR-engineered T cells in patients with refractory cancer. *Science, 367*, eaba7365.

40. Poirot, L., Philip, B., Schiffer-Mannioui, C., et al. (2015). Multiplex genome-edited T-cell manufacturing platform for "off-the-shelf" adoptive T-cell immunotherapies. *Cancer Research, 75*, 3853–3864.

41. Bak, R. O., & Porteus, M. H. (2017). CRISPR-mediated integration of large gene cassettes using AAV donor vectors. *Cell Reports, 20*, 750–756.

42. Atsavapranee, E. S., Billingsley, M. M., & Mitchell, M. J. (2021). Delivery technologies for T cell gene editing: Applications in cancer immunotherapy. *EBioMedicine, 67*, 103354.

43. Tebas, P., Jadlowsky, J. K., Shaw, P. A., et al. (2021). CCR5-edited CD4+ T cells augment HIV-specific immunity to enable post-rebound control. *Journal of Clinical Investigation, 131*, e144486.

44. Lu, Y., Xue, J., & Deng, T., et al. (2020). Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. *Nature Medicine, 26*, 732–740.

45. He, S. (2020). The first human trial of CRISPR-based cell therapy clears safety concerns as new treatment for late-stage lung cancer. *Signal Transduction and Targeted Therapy, 5*, 168.

46. Palmer, D. C., Webber, B. R., Patel, Y., et al. (2022). Internal checkpoint regulates T cell neoantigen reactivity and susceptibility to PD1 blockade. *Med, 3*, 682–704.

47. Palmer, D. C., Guittard, G. C., Franco, Z., et al. (2015). Cish actively silences TCR signaling in CD8+ T cells to maintain tumor tolerance. *Journal of Experimental Medicine, 212*, 2095–2113.

48. Elisa, A., Krishnendu, C., Lydia, V., et al. (2021). Genetic editing of CISH enhances T cell effector programs. *bioRxiv*. https://doi.org/10.1101/2021.08.17.456714

49. Sawasdikosol, S., & Burakoff, S. (2020). A perspective on HPK1 as a novel immuno-oncology drug target. *eLife, 9*, e55122.

50. Basar, R., Daher, M., & Rezvani, K. (2020). Next-generation cell therapies: The emerging role of CAR-NK cells. *Blood Advances, 4*, 5868–5876.

51. O'Brien, S., Nastoupil, L. J., Essell, J., et al. (2022). A first-in-human phase 1 study of CB-010, a CRISPR-edited anti-CD19 CAR-T cell therapy. *Blood, 140*(Suppl. 1), 9457–9458.

52. Caribou Biosciences. (2023, July 13). *Caribou Biosciences reports positive clinical data from dose escalation of CB-010 ANTLER phase 1 trial*. https://www.globenewswire.com/news-release/2023/07/13/2704702/0/en/Caribou-Biosciences-Reports-Positive-Clinical-Data-from-Dose-Escalation-of-CB-010-ANTLER-Phase-1-Trial-in-r-r-B-NHL.html

53. Benjamin, R., Jain, N., Maus, M. V., et al. (2022). UCART19... phase 1 trial. *Lancet Haematology, 9*, e833–e843.

54. Chiesa, R., Georgiadis, C., Syed, F., et al. (2023). Base-edited CAR7 T cells for relapsed T-cell acute lymphoblastic leukemia. *New England Journal of Medicine, 389*, 899–910.

55. Dai, Z., Mu, W., Zhao, Y., et al. (2021). Development of CD5-targeting biepitopic CARs. *Molecular Therapy, 29*, 2707–2722.

56. Cooper, B. W., Suh, H. C., Koura, D., et al. (2022). Trial in progress: VOR33 allograft with GO treatment. *Blood, 140*(Suppl. 1), 12973–12975.

57. Koehne, G., Tomlinson, B., Suh, H., et al. (2023). CD33-deleted HSPCs display normal engraftment... *EHA 2023 Congress Abstract P1396.* https://library.ehaweb.org

58. Locke, F. L., Miklos, D. B., Tees, M., et al. (2022). CRC-403: Phase 1/2 study of bbT369. *Blood, 140*(Suppl. 1), 12716–12717.