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Review Article

Nano-carrier-based Delivery of CRISPR-Cas9 for Oncolytic Gene Therapy: Insights from Xenograft Models

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ABSTRACT

The CRISPR-Cas9 system has revolutionized genome editing, offering unprecedented precision and efficiency in gene modification. Its potential in cancer therapy, particularly oncolytic gene therapy, has garnered significant attention, especially with the development of advanced delivery platforms. However, effective and safe *in vivo* delivery of CRISPR components remains a major barrier to clinical translation. This review provides a comprehensive overview of viral and non-viral nanocarrier systems for CRISPR-Cas9 delivery, with a particular focus on their application in xenograft models of cancer. The present study aimed to bridge the gap between molecular innovation and therapeutic application by evaluating the efficiency and safety of CRISPR-Cas9 delivery systems in preclinical oncology models. The mechanisms and classifications of viral vectors, including adeno-associated viruses (AAV), lentivirus, and adenovirus, were emphasized, highlighting their strengths in gene transfer efficiency, while addressing concerns over immunogenicity, genome integration, and scalability. Subsequently, non-viral nanocarriers, including lipid nanoparticles (LNPs), polymeric systems, dendrimers, and metallic nanoparticles, have emerged as safer and more customizable alternatives. Key considerations, including stability, endosomal escape, payload capacity, and tumor targeting, are evaluated, supported by findings from recent xenograft-based studies. A direct comparison between viral and non-viral systems was presented, emphasizing differences in transfection efficiency, biosafety, immunological responses, and gene-editing precision in preclinical tumor models. The clinical relevance of CRISPR-based oncolytic strategies was examined, along with their integration with other cancer therapies. Additionally, the emerging challenges of immune evasion, tumor heterogeneity, and delivery barriers were evaluated. In addition, the regulatory and ethical dimensions surrounding genome editing in cancer therapy are addressed, including long-term safety concerns, germline editing considerations, and global disparities in clinical oversight. The discussion concluded with an examination of future perspectives, highlighting strategic improvements in delivery technologies and validation pipelines. Xenograft models were proposed as a means to accelerate clinical translation.

1. Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) have revolutionized the field of gene editing by offering a precise, programmable, and relatively simple method for modifying specific genomic sequences[1]. Originally discovered as a part of the adaptive immune system in bacteria and archaea, CRISPR-Cas9 has been repurposed for targeted gene editing in eukaryotic systems[2]. The system relies on a guide RNA (gRNA) to direct the Cas9 endonuclease to a complementary DNA sequence, where it introduces a double-stranded break. The cell's natural repair mechanisms, non-homologous end joining (NHEJ) or homology-directed repair (HDR), then alter the DNA sequence, enabling knock-out or knock-in of genes. Due to

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its efficiency and flexibility, CRISPR-Cas9 is now a cornerstone of modern genetic therapies, including CAR-T cell engineering, tumor suppressor reactivation, especially in oncology[3].

Despite the potential of CRISPR-Cas9, its clinical translation heavily depends on the efficiency and safety of its delivery system[4]. Efficient delivery ensures adequate gene editing at the target site while minimizing off-target effects and systemic toxicity[4]. Delivery vehicles must overcome several biological barriers, such as degradation by nucleases, immune recognition, and poor cellular uptake[5]. Two main classes of delivery systems have emerged, including viral vectors and non-viral nanocarriers[6]. Viral vectors such as adeno-associated viruses (AAVs), lentiviruses, and adenoviruses offer high transduction efficiency but pose challenges including immunogenicity, limited cargo capacity, and risk of insertional mutagenesis[6]. In contrast, non-viral systems such as lipid nanoparticles (LNPs), polymer-based carriers, and inorganic nanostructures offer customizable surface properties, reduced immunogenicity, and scalable production, but often suffer from lower gene-editing efficiency[7]. Thus, selecting an appropriate delivery system is critical, especially in cancer gene therapy, where both safety and efficacy are paramount (Figure 1).

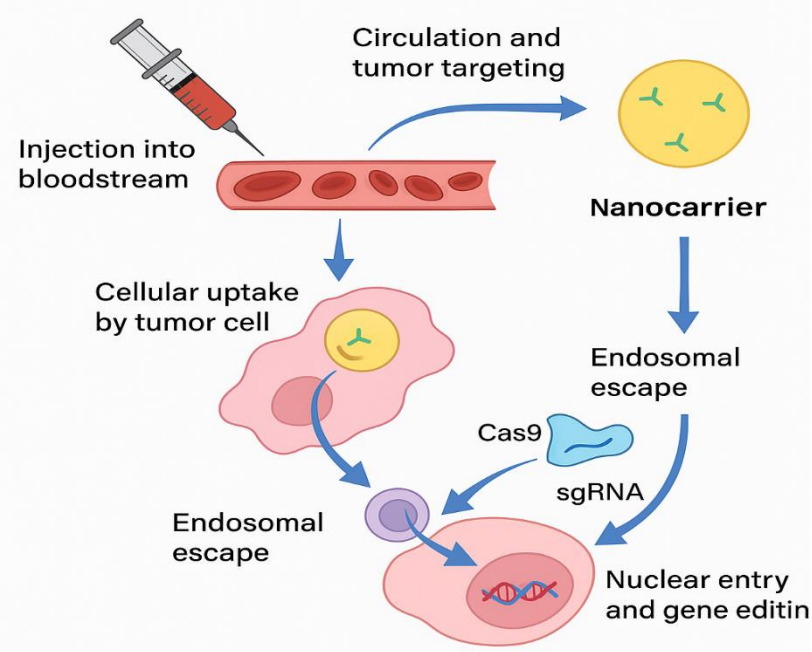

Figure 1. Schematic representation of Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 delivery to tumor cells using nanocarriers. The process involves intravenous injection, systemic circulation, and tumor targeting, cellular uptake by tumor cells, endosomal escape, and subsequent nuclear localization of Cas9-sgRNA complexes leading to targeted gene editing (Figure designed by Biorender).

To evaluate the clinical potential of CRISPR-Cas9-based therapies, preclinical studies often utilize xenograft models, in which human tumor cells are implanted into immunodeficient mice[8]. These models recapitulate key aspects of the human tumor microenvironment, allowing for *in vivo* assessment of delivery efficiency, biodistribution, genome editing activity, and safety profiles[9]. Xenograft models are particularly valuable in cancer research due to their ability to reflect human-specific responses to gene editing tools, especially when testing oncolytic strategies[10]. These models help to identify off-target effects, immune responses, and therapeutic outcomes that may not be evident in *in vitro* systems[9]. Hence, xenograft models serve as an essential bridge between bench research and clinical trials. The present review aimed to evaluate the current strategies for CRISPR-Cas9 delivery using viral and non-viral nanocarriers, with a specific focus on their application in oncolytic gene therapy.

2. Viral nanocarriers for clustered regularly interspaced short palindromic repeats Cas9 delivery

Viral vectors represent one of the most effective platforms for delivering CRISPR-Cas9 components into mammalian cells, particularly for therapeutic gene editing in cancer[11]. Among the most commonly utilized viral carriers are AAV, lentivirus, and adenovirus, each offering unique characteristics in terms of transduction efficiency, cargo capacity, immunogenicity, and genome integration[6].

Adeno-associated virus vectors have emerged as popular candidates due to their low immunogenicity and ability to infect both dividing and non-dividing cells[12]. However, their relatively small packaging capacity, approximately 4.7 kilobases, poses a major limitation, particularly when delivering the full-length *Streptococcus pyogenes* Cas9 (SpCas9) gene along with its associated guide RNA[13]. To circumvent this, alternative strategies as using smaller Cas9 orthologs such as *Staphylococcus aureus*

Cas9 (SaCas9) or employing dual-vector systems have been explored[12]. Lentiviral vectors, derived from Human Immunodeficiency Virus-1 (HIV-1), offer a larger packaging capacity (~8 kb) and stable integration into the host genome, supporting long-term expression of Cas9 and gRNA[14]. While effective, the risk of insertional mutagenesis and oncogene activation has raised safety concerns, especially for therapeutic applications in humans[14]. Adenoviral vectors, on the other hand, do not integrate into the genome and provide a relatively large packaging capacity (up to ~36 kb), making them suitable for delivering full CRISPR systems[15]. Nevertheless, their high immunogenicity and potential to induce strong inflammatory responses present substantial challenges for clinical use[15]. Despite their differences, viral vectors share several advantages for CRISPR-Cas9 delivery. They exhibit high transduction efficiency and can be engineered for tissue-specific tropism, allowing for targeted delivery in tumors[16]. Lentiviral vectors, in particular, provide sustained gene expression that may be beneficial for prolonged gene editing in rapidly proliferating cancer cells[17]. Additionally, the possibility of pseudo-typing viral envelopes or modifying capsids opens avenues for selective targeting of tumor subtypes or specific niches within the tumor microenvironment[16]. However, these advantages must be weighed against inherent limitations[16]. Size constraints, particularly in AAV vectors, restrict the delivery of larger Cas9 variants or multiplexed gRNA arrays[18]. Immunogenicity remains a significant barrier, particularly for adenoviral vectors, which have been associated with systemic toxicity and rapid clearance. Moreover, genome-integrating vectors, such as lentivirus, raise concerns regarding insertional mutagenesis and long-term safety[19]. Consequently, considerable efforts have been directed toward the development of next-generation viral systems, including non-integrating lentiviruses, self-deleting vectors, and engineered AAV capsids with enhanced packaging capacity and reduced immune recognition.

2.1. Evaluation in xenograft models

The performance of viral CRISPR-Cas9 delivery systems has been extensively evaluated in xenograft models, which remain the gold standard for *in vivo* validation in cancer gene therapy[20]. Xenograft models, typically involving the implantation of human tumor cells into immunodeficient mice, offer an *in vivo* platform for assessing delivery efficiency, genome editing precision, therapeutic efficacy, and toxicity[8]. For example, AAV-mediated delivery of SaCas9 targeting E6/E7 oncogenes has demonstrated significant tumor suppression in cervical cancer xenografts, underscoring the potential of CRISPR-based viral systems in oncolytic gene therapy[21]. Similarly, lentiviral vectors encoding Cas9 and guide RNAs directed against oncogenic KRAS mutations have shown successful tumor growth inhibition in lung cancer models, particularly when combined with immunotherapy agents[22]. These results highlight the capacity of lentivirus to induce sustained genetic modulation *in vivo*, although risks associated with genomic integration persist (Figure 2).

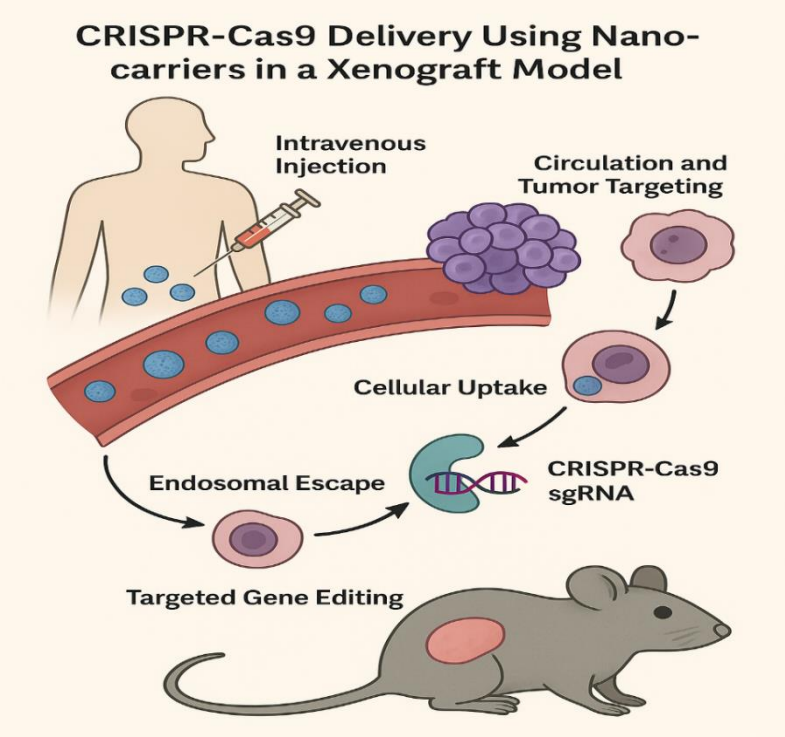

Figure 2. Illustration of Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 nanocarrier delivery in a xenograft mouse model. The figure depicts the intravenous administration of nanocarriers, their circulation and tumor-specific targeting, cellular uptake by human tumor cells engrafted in the mouse, endosomal escape, and release of the CRISPR-Cas9-sgRNA complex, resulting in targeted gene editing within the xenograft tumor tissue (Figure designed by Biorender).

Adenoviral vectors have also been used to deliver CRISPR-Cas9 for transient disruption of oncogenic signaling pathways in xenograft models of glioblastoma and hepatocellular carcinoma[23]. While effective in inducing gene knockdown, these studies often report heightened immune cell infiltration and tissue damage, necessitating optimization of vector dose and delivery route to improve therapeutic windows[23]. Overall, data from xenograft studies affirmed that viral vectors can achieve high genome editing efficiency and notable therapeutic outcomes. Nonetheless, limitations related to immunogenicity, off-target effects, and vector-induced toxicity remain significant hurdles to clinical translation. Continued engineering of viral carriers and refinement of delivery strategies in preclinical xenograft models are essential to balancing efficacy and safety in CRISPR-based oncolytic gene therapies[24].

3. Non-viral nano-carriers for clustered regularly interspaced short palindromic repeats-Cas9 delivery

Non-viral nanocarriers have emerged as versatile and increasingly preferred alternatives to viral vectors for CRISPR-Cas9 delivery, particularly in the context of cancer gene therapy. Unlike viral systems, non-viral nanocarriers offer customizable physicochemical properties, reduced immunogenicity, and scalable production, which are especially important for clinical translation[25]. Among the various types, LNP, polymeric nanoparticles, dendrimers, and inorganic nanomaterials have demonstrated significant promise in delivering CRISPR components with varying degrees of success in both *in vitro* and *in vivo* properties[26].

3.1. Lipid nanoparticles

Lipid nanoparticles are currently the most clinically advanced non-viral vectors, with successful application in mRNA vaccine platforms providing a translational precedent for gene-editing therapies[27]. Lipid nanoparticles typically consist of ionizable lipids, helper lipids, cholesterol, and polyethylene glycol (PEG)-lipids, which together form a stable, biocompatible structure capable of encapsulating and protecting Cas9 mRNA, plasmids, or ribonucleoprotein (RNP) complexes[28]. The efficiency of LNPs relies on protonation under endosomal pH, promoting endosomal escape and cytoplasmic release of cargo[29]. Lipid nanoparticles have demonstrated effective delivery of CRISPR-Cas9 to hepatocytes, and more recently, to solid tumors through intratumoral or systemic administration[28]. Advances in tumor-targeting strategies, such as ligand-functionalized LNPs, have improved their specificity toward cancer cells while minimizing off-target delivery to healthy tissues[28]. Notably, the Food and Drug Administration-approved LNP-based siRNA drug, Patisiran, and mRNA vaccines have accelerated the clinical evaluation of LNPs for gene editing in cancer[29].

3.2. Polymeric nanoparticles

In addition to LNPs, polymeric nanoparticles have attracted attention due to their structural diversity and tunable degradation kinetics[30]. Cationic polymers such as polyethyleneimine (PEI), poly lactic-co-glycolic acid (PLGA), and chitosan have been employed to condense and deliver CRISPR plasmids or RNP[31]. While PEI provides efficient gene condensation and endosomal escape, it often exhibits cytotoxicity at higher molecular weights[32]. Poly lactic-co-glycolic acid, on the other hand, is FDA-approved and biodegradable, making it suitable for sustained delivery applications[32]. These polymers can be engineered to achieve controlled release, pH-responsive behavior, or surface functionalization with targeting ligands, thus enhancing tumor selectivity[33]. Dendrimers, particularly polyamidoamine (PAMAM) dendrimers, offer precise control over molecular architecture and have demonstrated successful delivery of CRISPR components with relatively low toxicity and high cellular uptake[34].

3.3. Inorganic nanocarriers

Inorganic nanocarriers, such as gold nanoparticles, mesoporous silica nanoparticles, and magnetic nanoparticles, represent another class of non-viral delivery systems with distinct advantages[35]. The rigidity of inorganic nanocarriers, ease of functionalization, and responsiveness to external stimuli (such as magnetic fields or light) allow for site-specific delivery and triggered release mechanisms[36]. Gold nanoparticles, in particular, have been used to deliver CRISPR-Cas9 RNP into tumor cells with high precision and minimal toxicity, owing to their biocompatibility and ease of surface modification[37].

The performance of these non-viral nanocarriers has been rigorously evaluated in xenograft models to assess their gene-editing efficiency, safety, and biodistribution. In hepatocellular carcinoma xenografts, LNP carrying Cas9 mRNA and guide RNA targeting telomerase reverse transcriptase (TERT) or MYC have achieved significant tumor suppression, with minimal off-target effects and no overt toxicity[38]. Similarly, PLGA-based nanoparticles loaded with CRISPR plasmids targeting programmed cell death ligand 1 (PD-L1) or vascular endothelial growth factor (VEGF) have demonstrated robust anti-tumor effects in breast and colon cancer xenografts, often accompanied by increased immune cell infiltration and vascular normalization[39]. Dendrimer-based systems have shown efficient knockdown of oncogenes such as BCL2 and EZH2 in lymphoma and glioblastoma models, indicating their potential for targeting diverse tumor types[40].

Inorganic nanoparticles, particularly gold-based systems, have enabled tumor-specific delivery of CRISPR-Cas9 in xenograft models of ovarian and prostate cancer[41]. These platforms often incorporate tumor-targeting peptides or aptamers to enhance selectivity. Importantly, they enable real-time tracking via imaging modalities, providing a theranostic advantage[41]. Across these studies, non-viral carriers have consistently demonstrated favorable safety profiles, with low systemic toxicity, minimal immunogenicity, and efficient tumor accumulation, particularly when administered via localized routes or combined with tumor-penetrating agents[26]. Collectively, non-viral nanocarriers provide a flexible and safe

alternative for CRISPR-Cas9 delivery in oncolytic gene therapy. Non-viral nanocarriers' adaptability in surface chemistry, cargo loading, and release profiles enables precise tuning for specific tumor environments. Data from xenograft models support their efficacy and biocompatibility, underscoring their potential for clinical advancement in cancer genome editing[8-10].

4. Comparative assessment of viral and non-viral clustered regularly interspaced short palindromic repeats-Cas9 delivery in xenograft models

A critical aspect in the development of CRISPR-Cas9-based oncolytic gene therapies lies in the choice of an appropriate delivery vehicle[42]. Viral and non-viral nanocarriers differ markedly in their efficiency, safety, and translational potential[42]. A comparative analysis of these platforms, particularly in xenograft models, provides key insights into their respective advantages and limitations (Table 1).

In terms of delivery efficiency, viral vectors generally outperform non-viral systems[42]. Lentiviral and adenoviral vectors exhibit high transduction rates in both *in vitro* and *in vivo* settings, achieving genome editing efficiencies exceeding 70% in some tumor xenograft models[43]. Their innate ability to penetrate the nuclear envelope and integrate or transiently express CRISPR-Cas9 components contributes to their superior performance[44]. Adeno-associated viruses' vectors, despite their limited cargo capacity, have demonstrated efficient delivery in liver and muscle tissues, especially when using compact Cas9 variants or dual-vector systems[45]. In contrast, non-viral systems such as LNP and polymeric carriers typically indicate moderate delivery efficiency, often limited by endosomal entrapment or insufficient intracellular trafficking[46]. However, recent advances in ionizable lipid formulations, surface modifications, and tumor-targeting ligands have significantly improved the delivery performance of LNP, narrowing the gap with viral systems in certain contexts[47].

Table 1. Comparative summary of viral and non-viral clustered regularly interspaced short palindromic repeats-Cas9 delivery systems

Feature	Viral vectors (AAV, Lentivirus)	Non-viral nanocarriers (LNP, Polymers)
Delivery efficiency	High (especially in dividing cells)	Moderate to high (improving with newer systems)
Packaging capacity	Limited (especially AAV: ~4.7 kb)	Flexible (can accommodate large constructs)
Immunogenicity	Moderate to high	Typically, lower
Integration risk	Possible (lentivirus)	No genome integration
Scalability and GMP	Complex, costly	Easier to scale, more industrial-friendly
Targeting flexibility	Genetically engineered	Tunable via surface chemistry
Clinical maturity	Several in clinical trials	Gaining traction in recent years

AAV: Adeno-associated viruses; LNP: Lipid nanoparticles; GMP: Good manufacturing practice

Safety and biocompatibility represent a major strength of non-viral nanocarriers[48]. Viral vectors, especially adenovirus and lentivirus, are associated with immunogenicity, inflammation, and potential risks of insertional mutagenesis[49]. These concerns are particularly critical in therapeutic settings where long-term gene expression is not required or could be detrimental[49]. While AAV are generally considered safer due to their low immunogenic profile and episomal nature, they can still elicit neutralizing antibody responses, particularly upon repeated administration[50]. Non-viral nanocarriers, on the other hand, offer minimal immunogenicity and reduced systemic toxicity[7]. Lipid nanoparticles and polymer-based systems have demonstrated favorable safety profiles in both preclinical and clinical settings, with transient expression and rapid clearance reducing the risk of off-target or long-term effects[33,34,47]. Additionally, non-viral systems allow for greater control over dose and release kinetics, which can be tailored to the tumor microenvironment[49].

Comparative studies in xenograft models have highlighted the trade-offs between efficacy and safety in these two delivery strategies. For instance, in glioblastoma and hepatocellular carcinoma xenografts, lentiviral and adenoviral vectors achieved rapid tumor regression through efficient CRISPR-mediated knockdown of driver oncogenes[23,38]. However, these effects were often accompanied by vector-induced inflammation or off-target genome edits. In parallel models, non-viral carriers such as polyethylene glycolylated LNPs or PLGA-based nanoparticles induced more gradual tumor inhibition but showed lower levels of systemic cytokine release and organ toxicity[51]. Notably, in previous studies targeting the same gene, such as *Kirsten rat sarcoma viral oncogene homologue* (KRAS) or PD-L1, viral systems have produced higher editing rates, while non-viral platforms have provided better safety and repeatability[52].

The choice between the viral and non-viral delivery often depends on the therapeutic context[43]. For applications requiring high editing efficiency in a short timeframe, such as aggressive, rapidly progressing tumors, viral vectors may be preferable, provided immune responses are manageable[42]. In contrast, for chronic conditions, immunologically sensitive sites, or combination therapies involving repeated dosing, non-viral nanocarriers offer a more favorable safety profile[46,47]. Emerging hybrid systems that combine the strengths of both platforms, such as virus-like particles (VLP) or biomimetic nanocarriers, are currently under investigation and may bridge the gap between efficiency and biocompatibility[53]. Ultimately, data from xenograft models underscore that while viral vectors currently lead in raw efficiency, non-viral carriers hold strong potential for safe, modular, and clinically adaptable CRISPR-Cas9 delivery[54]. A rational, case-specific selection of delivery modality guided by tumor biology, therapeutic goals, and safety considerations is essential for advancing genome-editing-based cancer therapies toward clinical reality[54].

5. Clinical applications and emerging challenges in clustered regularly interspaced short palindromic repeats-based oncolytic therapy

The translation of CRISPR-Cas9 technology into clinical applications has marked a significant turning point in the field of oncology, especially within the context of precision and oncolytic gene therapies[55]. This gene-editing system offers a powerful platform for reprogramming cancer cells, modulating immune responses, and enhancing viral oncolytic strategies in a highly specific and controllable manner. While still in early stages of clinical development, CRISPR-Cas9 has already begun to redefine the boundaries of what is therapeutically possible in cancer treatment[55].

One of the most compelling roles of CRISPR-Cas9 in cancer therapy is its capacity to enable direct, sequence-specific targeting of oncogenic mutations[56]. Unlike conventional treatments that often affect both cancerous and healthy cells, CRISPR allows for the knockout or correction of driver mutations such as KRAS^G12D, TP53^mut, or BRAF^V600E, which are critical for tumor survival and proliferation[55]. In xenograft and organoid models, targeted disruption of these genes has led to significant tumor regression, providing proof-of-concept for precision genome editing as a viable therapeutic approach[8,9]. Additionally, CRISPR has been applied to disrupt genes responsible for drug resistance, such as ATP-binding cassette (ABC) transporters or DNA repair enzymes, thereby sensitizing tumors to chemotherapy or radiotherapy[57].

Beyond editing cancer cell genomes, CRISPR is being used to engineer immune effector cells for more potent anti-tumor responses[58]. In particular, CRISPR-modified T cells have been employed to knock out checkpoint inhibitory genes such as PD-1, Cytotoxic T lymphocyte antigen-4 (CTLA-4), or lymphocyte activation gene 3 (LAG-3), resulting in enhanced tumor cell killing and improved persistence *in vivo*[58]. These next-generation *Chimeric antigen receptor* (CAR-T) cells, often referred to as "armored" CAR-Ts, have demonstrated superior efficacy in preclinical models of solid tumors, which historically have been resistant to conventional T cell therapies[1,2,59]. In one notable clinical study, CRISPR was used to simultaneously knock out endogenous *T-cell receptor* (TCR) and PD-1 in patient-derived T cells, leading to safe expansion and detectable tumor infiltration post-infusion[60-63]. Such multiplex editing would be nearly impossible with earlier gene-editing tools, underscoring the versatility of the CRISPR system[62]. However, as CRISPR entered clinical pipelines, several safety and immunological concerns have surfaced that must be carefully addressed. Off-target effects remain one of the most frequently cited risks, wherein CRISPR induces unintended cuts at loci that resemble the target sequence[58,64-66]. Such edits can lead to genomic instability, disruption of tumor suppressors, or activation of proto-oncogenes, with potentially oncogenic consequences[58]. Although high-fidelity Cas9 variants (such as eSpCas9, SpCas9-HF1) and improved sgRNA design algorithms have significantly reduced these effects, the risk cannot be entirely eliminated[59]. Moreover, strategies such as delivery of Cas9 as a transient RNP complex and limiting exposure duration are being employed to further enhance genome editing precision[67-69].

The Cas9 is derived from bacterial species and can trigger both innate and adaptive immune responses in humans, particularly if administered systemically or repeatedly[70]. Pre-existing antibodies and T cell responses against Cas9 have already been detected in a significant proportion of the population[71]. This can lead to rapid clearance of CRISPR components or, worse, severe immune-mediated toxicity. To overcome this issue, efforts are underway to develop orthologous Cas nucleases from less common bacteria and to refine Cas9 proteins for improved compatibility with human systems, or encapsulate them within stealth nanocarriers that shield them from immune surveillance[71].

In the context of delivery, both viral and non-viral platforms face translational challenges. Viral vectors offer efficient gene delivery but raise concerns regarding immunogenicity, genome integration, and limited cargo capacity[72]. Non-viral systems, while safer and more flexible, often suffer from suboptimal delivery efficiency, especially in hard-to-reach solid tumors[6,7]. As a result, new strategies are being developed to combine the best aspects of both systems. These include virus-like particles (VLPs), cell membrane-coated nanoparticles, and extracellular vesicle-based delivery platforms capable of delivering CRISPR-Cas9 in a tumor-specific and immune-evasive manner[73].

The therapeutic landscape is also evolving through the integration of CRISPR with other modalities. Combination therapies that pair CRISPR-based genome editing with immune checkpoint inhibitors, oncolytic viruses, or epigenetic drugs are gaining traction[74,75]. For instance, knocking out PD-L1 in tumor cells using CRISPR has been shown to enhance the efficacy of anti-PD-1 antibodies in immunocompetent mouse models[62]. Similarly, CRISPR can be used to disable genes encoding immune-suppressive cytokines such as TGF-β in the tumor microenvironment, thereby amplifying the host immune response[2]. In an emerging approach, CRISPR is being employed to genetically engineer oncolytic viruses, enhancing their tumor selectivity and replication kinetics to develop more potent and programmable virotherapeutics[76].

Looking forward, several trends are likely to shape the future of CRISPR-based oncolytic therapies[77]. These therapies include the development of inducible or self-regulating CRISPR systems, which enable gene editing to be turned on or off in response to external stimuli, such as light, temperature, or small molecules[77]. Another exciting area in genome-editing technologies is the use of base editors and prime editing systems, which allow for precise nucleotide changes without inducing double-stranded DNA breaks, thus minimizing DNA damage and off-target mutations[77]. Patient-specific xenograft and organoid models are also being leveraged to tailor CRISPR treatments to individual tumors, advancing the promise of personalized medicine[2].

While considerable progress has been made, CRISPR-based cancer therapies are still at a formative stage, with only a handful of clinical trials completed and most others in

early phases[13]. Regulatory oversight, manufacturing scalability, ethical considerations (particularly for germline editing), and cost-effectiveness will all play crucial roles in determining how broadly these therapies are adopted in clinical practice[13]. Thus, CRISPR-Cas9 represents a paradigm shift in cancer therapy, offering unmatched precision, programmability, and potential for synergy with existing and emerging treatments. With careful optimization of delivery systems, rigorous preclinical validation, and robust safety profiling, CRISPR is poised to become a cornerstone technology in the next generation of oncolytic and personalized cancer therapies[56].

6. Regulatory and ethical considerations in clustered regularly interspaced short palindromic repeats-based oncolytic therapies

As CRISPR-Cas9 technology progresses from preclinical success toward clinical implementation in cancer therapy, regulatory and ethical considerations become increasingly critical[55]. Unlike traditional small-molecule drugs or biologics, genome editing introduces permanent changes to the genetic material of target cells, raising unique safety, legal, and moral questions[56]. These concerns are particularly relevant in oncolytic applications, where therapeutic interventions are intended to selectively destroy malignant cells but may interact unpredictably with host immunity, non-tumor tissues, or the broader tumor microenvironment[55].

From a regulatory standpoint, the delivery system used for CRISPR components plays a pivotal role in risk assessment and approval pathways[78]. Viral vectors such as AAV and lentivirus, despite their delivery efficiency, carry risks of insertional mutagenesis and long-term expression of foreign proteins, which must be rigorously evaluated through toxicology and biodistribution studies[72]. Non-viral nanocarriers, although perceived as safer, are not exempt from scrutiny, particularly when they incorporate synthetic polymers or metallic cores that may elicit immunogenic or off-target effects[6]. Regulatory bodies such as the U.S. food and drug administration (FDA) and European medicines agency (EMA) now require detailed characterization of nanocarrier properties, pharmacokinetics, and potential off-target editing before approving CRISPR-based therapies for clinical trials[79].

Another core issue is the long-term follow-up of patients treated with gene-editing interventions. Unlike conventional chemotherapies, CRISPR-based therapies may induce durable or even irreversible changes, necessitating decades-long monitoring for potential delayed effects such as secondary malignancies, genotoxicity, or unintended immune consequences[80]. This need for extended surveillance poses practical and financial challenges for clinical trial design and patient consent.

Ethically, one of the most debated topics surrounding CRISPR is the boundary between somatic and germline editing[81]. While current cancer therapies target only somatic cells, concerns remain about inadvertent germline modifications, particularly when CRISPR is delivered systemically or through viral vectors with broad tropism[82,83]. Even in somatic contexts, questions about patient autonomy, informed consent, and equitable access to emerging therapies remain unresolved[82]. Vulnerable populations, such as those with aggressive or rare cancers, may feel pressured to enroll in early-stage trials with uncertain outcomes, raising the risk of therapeutic misconception. Furthermore, disparities in global regulatory frameworks can create ethical gray zones. For example, some countries may fast-track CRISPR trials without robust oversight, potentially leading to premature clinical use or exploitation of patients[81]. Developing unified international guidelines that effectively balance innovation and patient safety remains a pressing priority. Organizations such as the international society for cell and gene therapy (ISCT) and the world health organization (WHO) have begun developing ethical governance models, but consistent enforcement remains a challenge[84]. In addition to regulatory reform, there is a growing call for broader societal dialogue around genome editing[80]. Public trust in CRISPR-based cancer therapies will depend not only on clinical efficacy but also on transparent communication, ethical oversight, and responsible innovation[80]. Incorporating patient perspectives, engaging bioethicists, and fostering interdisciplinary collaboration among scientists, clinicians, and policymakers is crucial to guiding CRISPR technologies toward safe and just clinical integration[78,79]. In conclusion, CRISPR-based oncolytic therapies offer great potential, but their safe and ethical implementation in clinical settings necessitates strong regulatory frameworks, long-term patient monitoring, and continuous ethical discussions. Proactively addressing these aspects is crucial to responsibly fulfill the promise of gene editing equitably.

7. Conclusion

CRISPR-Cas9-based cancer therapy has advanced significantly, with viral and non-viral nanocarriers improving targeted delivery. Preclinical xenograft models, particularly patient-derived xenografts, are crucial for evaluating efficacy and safety, bridging lab research and clinical trials. Key findings highlighted that the importance of delivery systems, optimal tumor penetration, immune evasion, and controlled cargo release are essential for therapeutic success, specifically, achieving efficient tumor regression while minimizing off-target effects and systemic toxicity. Next-generation strategies include ligand-functionalized nanoparticles for precise targeting, stimuli-responsive carriers for tumor-specific release, and RNP delivery to reduce immune reactions and genomic instability. Challenges remain, such as the immunogenicity of Cas proteins and delivery vectors. Solutions under exploration include less immunogenic Cas variants and exosome-based carriers. Additionally, tumor heterogeneity demands adaptable, patient-specific systems.

Future progress hinges on the integration of nanotechnology, synthetic biology, and personalized medicine. Multifunctional nanocarriers that co-deliver CRISPR with immunotherapies or diagnostics could enhance precision. Emerging tools such as base and prime

editors will require advanced delivery platforms. Ultimately, the clinical success of CRISPR-based cancer therapy depends on combining precise gene editing with safe, efficient delivery. By leveraging insights from xenograft models, particularly patient-derived xenografts and cell-line-derived xenografts, and innovations in nanocarriers, personalized genome-targeted treatments are approaching reality. Interdisciplinary collaboration will be essential for overcoming remaining challenges.

Declarations

Competing interests

The author had no conflict of interest.

Authors' contributions

Mehran Bahmani wrote the draft of the manuscript, editing and finalization the paper. The author has read and approved the final version of the manuscript before publication in the present journal.

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The author has reviewed all ethical problems, including plagiarism, consent to publish, data fabrication, and falsification.

Availability of data and materials

The data to support the present study finding is available upon reasonable request to the corresponding author.

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