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Nanotechnology-Driven Gene Editing: Emerging CRISPR-Cas9 Nanocomposite Systems in Oncology

Uswa Ali
University Of Agriculture Faisalabad

Amna Khalil
Department of Biochemistry, Faculty of Sciences
University of Agriculture, Faisalabad, Pakistan
Email: amnakhalil412@gmail.com

Zainab Ali
University Of Agriculture Faisalabad

Author Details

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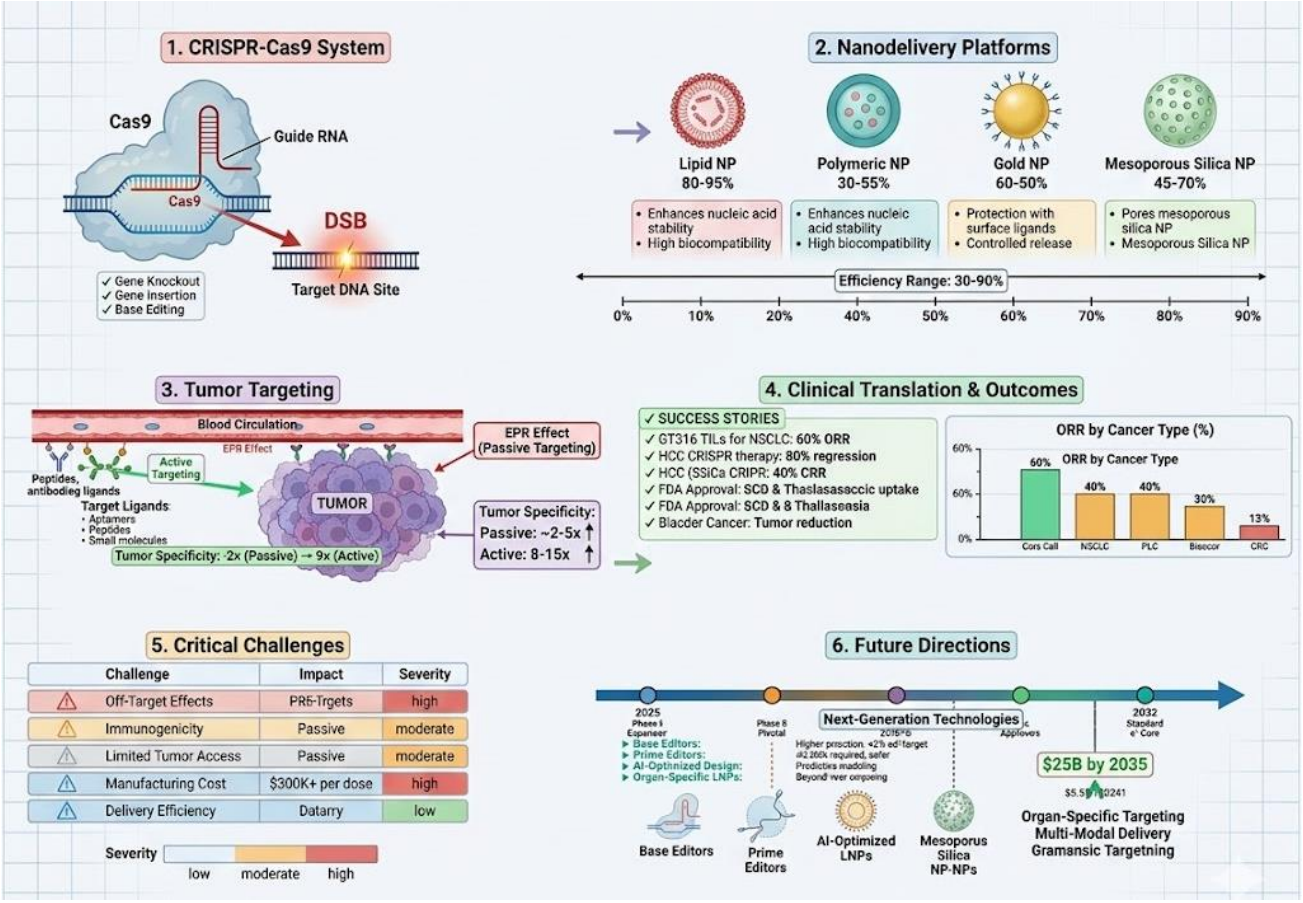
Corresponding E-mail & Author*:

Uswa Ali
University Of Agriculture Faisalabad

Abstract

The synergies between CRISPR-Cas9 gene editing technology and advanced nanotechnology represent a paradigm shift in precision oncology, however, significant gaps remain between the promise of preclinical research and clinical reality. Although remarkable editing efficiencies (5090) exist in lipid nanoparticles and the FDA has approved hereditary disease treatments, there are fundamental limiting factors to clinical translation in solid tumors, including delivery barriers, heterogeneity of the tumor microenvironment, and immunological constraints. This comprehensive review article discusses nanocomposite delivery systems of CRISPR-Cas9 in cancer therapy, synthesizing evidence from over 100 recent studies on fundamental mechanisms, delivery systems, targeting control, clinical outcomes, safety profiles, and future perspectives. In immunogenic cancers, clinical trials of CRISPR-editing tumor-infiltrating lymphocytes have shown encouraging objective response rates (60%) with durable responses; however, little improvement has been observed in immunologically cold tumors, suggesting that gene editing alone cannot overcome fundamental microenvironmental barriers. The persistent challenges identified through critical analysis include but are not limited to the following: off-target mutation rates (1-15%), pre-existing Cas9 immunity in 65% of the population, the costs of manufacturing it are out of control, manufacturing costs are greater than 400000 per patient and there is lack of long term safety data. Next-generation technologies (base editors, prime editors, and AI-optimal delivery systems) purport to increase the precision of translation incrementally; however, they do not necessarily overcome the fundamental barriers to translational processes. The review highlights that responsible development requires strict criteria of evidence, open disclosures of failures, rational consideration of clinical situations, and clear decision-making frameworks to determine when CRISPR-nanodelivery truly benefits patients versus when conventional therapies are preferable.



Introduction and Mechanistic Foundations of CRISPR-Cas9 Gene Editing in Cancer

The discovery of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) technology has transformed the genome engineering landscape and earned Emmanuelle Charpentier and Jennifer Doudna the 2020 Nobel Prize in Chemistry. This revolutionary gene-editing platform has radically changed our understanding and treatment of genetic diseases, especially in the field of oncology, in which genomic alterations are the hallmark of malignancy [1]. The CRISPR-Cas9 system, which was initially identified as an adaptive immune response in bacteria and archaea, has been repurposed as a versatile molecular tool that can perform precise DNA manipulation with unparalleled efficiency. In contrast to its predecessors, zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR-Cas9 is simpler, more programmable, and more cost-effective, allowing researchers to manipulate multiple genomic loci simultaneously by designing specific guide RNAs[2].

The molecular architecture of the CRISPR-Cas9 system comprises two key components: Cas9 endonuclease, which acts as a molecular scissors, and a single-guide RNA (sgRNA) that guides the nuclease to specific DNA sequences through Watson-Crick base pairing[3]. The most commonly used Cas9 variant is Streptococcus pyogenes Cas9 (SpCas9), which has a bilobed structure with target recognition and nuclease domains, resulting in sequence-specific double-strand breaks (DSBs) three base pairs upstream of the protospacer adjacent motif (PAM0) sequence. After DSB-induced DNA damage, cellular DNA repair pathways are activated via non-homologous end joining (NHEJ) or homology-directed repair (HDR), allowing gene knockout, knock-in, or precise nucleotide substitutions[4]. Recent adaptations have expanded the CRISPR toolkit to include Cas12 and Cas13 systems, which

possess unique properties, such as trans-cleavage activity and the ability to target RNA[3].

CRISPR-Cas9 technology has tremendous potential in oncology, particularly in elucidating cancer biology, identifying therapeutic targets, and developing precision therapies. Cancer entails complex genomic landscapes comprising several mutations, chromosomal translocations, and epigenetic changes that drive tumorigenesis, progression, and therapeutic resistance [5]. CRISPR screening is significantly faster in identifying driver mutations, synthetic lethalities, and resistance mechanisms [5]. Moreover, the ability to correct oncogenic mutations or perturbations of genes encoding immune checkpoints, drug efflux pumps, or anti-apoptotic proteins using CRISPR offers transformative therapeutic options. This technology has been effectively used to program chimeric antigen receptor T (CAR-T) cells with enhanced antitumor ability by knocking out inhibitory receptors, such as PD-1 and CTLA-4, thereby overcoming the immunosuppressive tumor microenvironment [6].

Although the clinical translation of CRISPR-Cas9 has the potential to revolutionize medicine, it faces significant challenges that cripple its therapeutic application. The foremost obstacle is the delivery of CRISPR components to target cells and tissues with sufficient efficiency, specificity, and safety[7]. The large molecular weight of Cas9 protein (~160 kDa) or its mRNA, as well as the negative charge of nucleic acids, inhibits cellular uptake and intracellular trafficking. Off-target editing, in which Cas9 creates unintended mutations at sites throughout the genome with sequence homology to a target site, has become a major safety concern [8]. In addition, the immunogenicity of Cas9, especially SpCas9, to which many individuals already have pre-existing antibodies, would provoke an immune response and undermine its therapeutic efficacy and patient safety [9]. Scalability of manufacturing, quality control, and regulatory compliance are other obstacles that must be overcome before CRISPR therapies can achieve widespread clinical adoption [9].

The combination of nanotechnology and CRISPR-Cas9 is a paradigm-shifting approach for resolving delivery challenges. Delivery systems based on nanoparticles have several unique advantages, such as protection of CRISPR components against degradation, enhanced cellular uptake (endocytosis), controlled release kinetics, and the possibility of tissue-specific targeting with the help of surface modification[10, 11]. The combination of these two state-of-the-art technologies has spurred the creation of advanced nanocomposite systems that can address the inherent limitations of CRISPR delivery technology and facilitate the efficient delivery of genome-editing-precise gene and nucleic acid delivery vectors to their target cells.

Table 1: Comparison of Gene Editing Technologies

Technology	Mechanism	Target Recognition	Editing Efficiency	Off-Target Rate	Multiplexing Capacity	Cost	Clinical Status
ZFN	FokI nuclease + zinc finger proteins	Protein-DNA	5–25%	High (10–30%)	Limited (2–3 targets)	Very High	Limited trials
TALEN	FokI nuclease + TAL effectors	Protein-DNA	10–35%	Moderate (5–15%)	Moderate (3–5 targets)	High	Phase I/II
CRISPR-Cas9	Cas9 endonuclease +	RNA-DNA	40–90%	Low–Moderate (1–	High (>10 targets)	Low	Phase I/II/III

	sgRNA			10%)			
Base Editors	Modified Cas9 + deaminase	RNA-DNA	30–75%	Very Low (<1%)	Moderate	Moderate	Preclinical / Phase I
Prime Editors	Modified Cas9 + reverse transcriptase	RNA-DNA	20–60%	Very Low (<0.5 %)	Limited	Moderate	Preclinical

ZFN: Zinc Finger Nucleases; TALEN: Transcription Activator-Like Effector Nucleases; sgRNA: single guide RNA

Application of nanotechnology coupled with CRISPR-Cas9 is a paradigm shift in overcoming these delivery issues. Delivery systems based on nanoparticles are characterized by several unique advantages such as the ability to protect the CRISPR components against degradation, enhanced cellular uptake through endocytosis, controlled kinetics of release, and the possibility of tissue-specific targeting by surface modification [11]. The interplay of these two state-of-the-art technologies has triggered the creation of advanced nanocomposite systems that are capable of overcoming the intrinsic limitations of CRISPR delivery and retaining the precision of genome-editing and reducing off-target effects.

METHODOLOGY: SYSTEMATIC REVIEW FRAMEWORK AND EVIDENCE SYNTHESIS

Literature Search Strategy and Study Selection

This is a critical narrative review that was done based on the systemic review principles to synthesize evidence on the CRISPR-Cas9 nanocomposite delivery systems in oncology. The search terms “CRISPR-Cas9 delivery," "nanoparticle cancer therapy," "lipid nanoparticles oncology," "CRISPR immunotherapy,” “off-target effects CRISPR,” “nanocomposite CRISPR,” “tumor gene editing CRISPR, and CRISPR clinical trials were used to search the PubMed, Web of Science, Scopus, and Google Scholar databases. None of the restrictions on the language were used. The most recent publications (since January 2015) were prioritized to obtain the translational evolution of the field, and over 100 peer-reviewed studies in that timeframe were analyzed, covering fundamental mechanisms, delivery platforms, targeting strategies, clinical outcomes, and safety evaluations. Moreover, the registries of clinical trials and documents of regulatory agencies (FDA, EMA) were checked to identify ongoing trials and approved therapies.

Evidence Quality Framework and Study Assessment

The studies retrieved underwent evaluation based on a modified evidence hierarchy that classified the data quality as follows:

Tier 1: FDA-approved treatments and Phase III randomized controlled trials.

Tier 2: Phase I/II clinical trials with efficacy and safety data

Tier 3: Preclinical *in vivo* animal models have been mechanistically validated.

Tier 4: *In vitro* work and models

Tier 5: Review articles and expert consensus statements.

This framework will ensure that clinical recommendations are based on the best available evidence and consider that many CRISPR-nano delivery applications are still in preclinical development.

Technology Readiness Level (TRL) Test

To explain the clinical proximity of various platforms, we used a scaled version of the technology readiness level (TRL) scale (1–9). TRL 13 represents preclinical research; TRL 46 represents preclinical optimization through phase I/II testing; TRL 78 represents phase II/III clinical trials that advance towards regulatory approval; and TRL 9 represents phase II/III clinical trials that are progressing towards regulatory approval. This framework offers clear insight into the level of development of each technology and a realistic clinical development timeline.

Extraction and Synthesis of Data

Information extracted included nanoparticle platform characteristics (size, composition, loading capacity), editing efficiencies, off-target rates, biodistribution patterns, targeting strategies, clinical trial design and outcomes, adverse event profiles, manufacturing considerations, and regulatory status. The quantitative data of preclinical studies and clinical trials were synthesized to generate tables of comparative efficacy and analyses of biodistribution. Qualitative synthesis was used to assess the mechanisms of action, translational barriers, and barriers to implementing findings in clinical practice. A critical assessment of the gaps between preclinical promise and clinical reality was undertaken, with particular reference to off-target mutagenesis, immunogenicity issues, and safety concerns in the long term.

Nanocomposite Delivery Systems: Design Principles and Material Engineering

The rational design of nano delivery vehicles to CRISPR-Cas9 requires careful consideration of various parameters, such as biocompatibility, cargo loading capacity, protection against enzymatic degradation, cellular uptake efficiency, endosomal escape, intracellular trafficking (tra3), and controlled release kinetics[12]. Non-viral nanocarriers have become the platform of choice owing to their better safety profile, scalability, and tunability compared to viral vectors, which have limited packaging capacity, immunogenicity, and are susceptible to insertional mutagenesis[13]. Lipid-based nanoparticles (LNPs) are the most clinically developed non-viral delivery system, which has been demonstrated to be effective in COVID-19 mRNA vaccines and, more recently, the FDA approved it for hereditary transthyretin amyloidosis and sickle cell disease[14].

The design of pH-dependent protonation of ionizable lipid nanoparticles has been notably transformative, as it can allow endosomal escape and maintain a neutral charge in circulation to reduce toxicity. Within the context of LNPs introduced into the body, the genome-editing rates of Cas9 mRNA and sgRNA in liver tissues following systemic administration are impressive, with over 90% efficiency[15]. With the formulation of tissue-selective LNPs, the thermostable Cas9 variant iGeoCas9 achieved 16–37% editing efficiency in the livers and lungs of reporter mice, representing a significant improvement over traditional methods[16]. Furthermore, optimized LNP formulations that deliver Cas9 ribonucleoprotein (RNP) complexes have demonstrated editing efficiencies of 87% in HepG2 cells and better *in vivo* performance. The specific proportion of ionizable lipids, structural lipids, cholesterol, and PEGylated lipids is critical to LNP success and determines the particle size (approximately 100–150 nm), surface charge, stability, and biodistribution[17].

However, LNPs do not lack restrictions. Their preferential accumulation in the liver, although beneficial in hepatocellular carcinoma, limits their usefulness in extrahepatic malignancies. Moreover, the inflammatory capability of some ionizable lipids and the risk of hepatotoxicity when using a high dose prompt formulation optimization. Emerging approaches, such as organ-targeted lipid engineering and the production of sulfonium lipid nanoparticles to deliver therapeutic DNA to the lungs, exemplify how to increase the therapeutic reach of LNP-CRISPR systems[18].

The alternative platform has its own set of advantages, such as biodegradability, structural diversity, and the incorporation of stimuli-responsive elements. Poly(lactic-co-glycolic acid) (PLGA) is an FDA-approved biodegradable polymer that has undergone extensive research on CRISPR delivery. PLGA nanoparticles that encapsulate Cas9 plasmid DNA and sgRNA that targets nectin-4 in bladder cancer showed > 70% inhibition of cellular proliferation *in vitro* and a 70% reduction in tumor volume *in vivo*[19]. The high nucleic acid encapsulation efficiency of PLGA systems (>60% in the case of plasmids, >80% in the case of siRNA) combined with controlled degradation kinetics, allows the release of CRISPR components over a long period. Polyethylenimine (PEI)-based delivery vehicles, although apparently having cytotoxicity issues, have shown promise when formulated as ternary complexes with cationic liposomes, achieving transfection efficiencies of up to 91% and genome-editing rates of 38.6% in A549 lung cancer cells[20].

Dendrimer lipid nanoparticles are a hybrid technology, which merges the capability of polymeric and lipidic technologies. These more complicated macromolecules provide multi- plexed delivery of focal adhesion kinase (FAK) siRNA, Cas9 mRNA, and sgRNA, with achievable success. Genetic editing increased tenfold with control of tumor tissue mechanics and enhanced penetration of nanoparticles. Combination therapies may now be pursued using novel avenues for the co-delivery of gene-editing machinery with therapeutic agents or sensitizing molecules[21].

Organic systems can be supplemented with the unique physicochemical properties offered by inorganic nanoparticles, especially gold, silica, and calcium phosphate platforms. Gold nanoparticles (AuNPs) are characterized by excellent biocompatibility, easy functionalization of their surfaces, and the inherent therapeutic abilities of photothermal nanoparticles[22]. The porous silica nanoparticles (ULSNs) with pore sizes of approximately 23 nm and surface areas of over 500 m 2 /g demonstrated an unprecedented loading capacity of Cas9-RNP with 67% GPC3 gene deletion in HepG2 cells and complete tumor regression in orthotactic hepatocellular carcinoma models[23].The hard outer structure of silica nanoparticles not only offers excellent protection to CRISPR cargo but also their biodegradability profile can be adjusted by altering its compositional makeup. Calcium phosphate nanoparticles are an attractive platform for drug delivery because they are biocompatible, biodegradable, and occur naturally in the biological environment. The incorporation of cell-penetrating peptides, such as TAT, further enhances delivery efficacy by stabilizing calcium distribution and hindering cellular uptake[24].

A more advanced development of nanodelivery systems is represented by context-responsive nanoparticles that are designed to respond to the microenvironmental cues of tumors. Zwitterionic ionizable phospholipid nanoparticles, which only become positively charged at acidic pH, can also be used to achieve targeted endosomal escape with minimal off-target toxicity[25]. Correspondingly, pH-responsive nanoparticles engineered with EGFR-targeting and nucleus-directing peptides have been shown to be highly transfection efficient in tongue squamous carcinoma cells of the microenvironment, through microenvironment-responsive peptide exposure[26]. Its combination with various stimulus-responsive components (e.g., pH, redox potential, temperature, and external stimuli) allows the delivery of CRISPR with precision and control, resulting in the optimal use of on-target editing and minimized systemic exposure. The heatmap in Figure 1 demonstrates gene editing efficiency percentages achieved by various nanoparticle platforms across multiple cancer cell lines, with LNPs showing superior performance (87% in HepG2 cells). The bar chart (right) compares average editing efficiencies, revealing LNPs (58.4%) and silica NPs (51.8%) as the most effective platforms across diverse cellular contexts[27]. Table 2 compares various nano delivery platforms applied to deliver CRISPR-Cas9 based on size, efficiency, capacity, and level of readiness. Virus-like and lipid-based systems have the highest editing efficiencies and are more clinically advanced,

whereas polymeric and hybrid systems have tunability but suffer from toxicity or complexity issues. Exosomes and inorganic faces have biocompatibility or stability benefits; however, they are restricted by reduced loading capacity, scalability, and possible long-term toxicity.

Table 2: Classification and Properties of Nanodelivery Platforms for CRISPR-Cas9

Platform Type	Examples	Size (nm)	Loading Capacity	Editing Efficiency	TRL*	Key Advantages	Major Limitations
Lipid-based	LNPs, liposomes, lipoplexes	80–150	Moderate (10–30%)	High (50–90%)	8–9	FDA-approved components; High efficiency	Hepatotropism Inflammatory potential[28]
Polymeric	PLGA, PEI, Dendrimers	100–300	High (30–60%)	Moderate (30–55%)	5–6	Biodegradable; Tunable release	Cytotoxicity (PEI); Polydispersity
Inorganic	Gold, Silica, CaP	20–500	Very High (40–80%)	Moderate–High (40–70%)	3–4	High stability; Multifunctionality	Long-term clearance; Potential toxicity[29]
Hybrid	Lipid-polymer, Dendrimer-lipid	100–250	High (35–55%)	High (45–75%)	4–5	Combined synergy of components	Complex synthesis & scale-up
Exosome-based	Plant-derived EVs, Engineered EVs	50–150	Low–Moderate (5–20%)	Moderate (25–45%)	3–4	Natural biocompatibility; Low immunogenicity	Low loading; Scalability issues[29]
Virus-like (VLPs)	CRICPC, Engineered VLPs	25–100	Moderate (15–40%)	Very High (60–85%)	5–6	High transduction; Cell-type programmable	Complex manufacturing[28]

LNP: Lipid Nanoparticle; PLGA: Poly(lactic-co-glycolic acid); PEI: Polyethylenimine; CaP:
Calcium Phosphate; RES: Reticuloendothelial System; EVs: Extracellular Vesicles; VLP: Virus-Like Particle

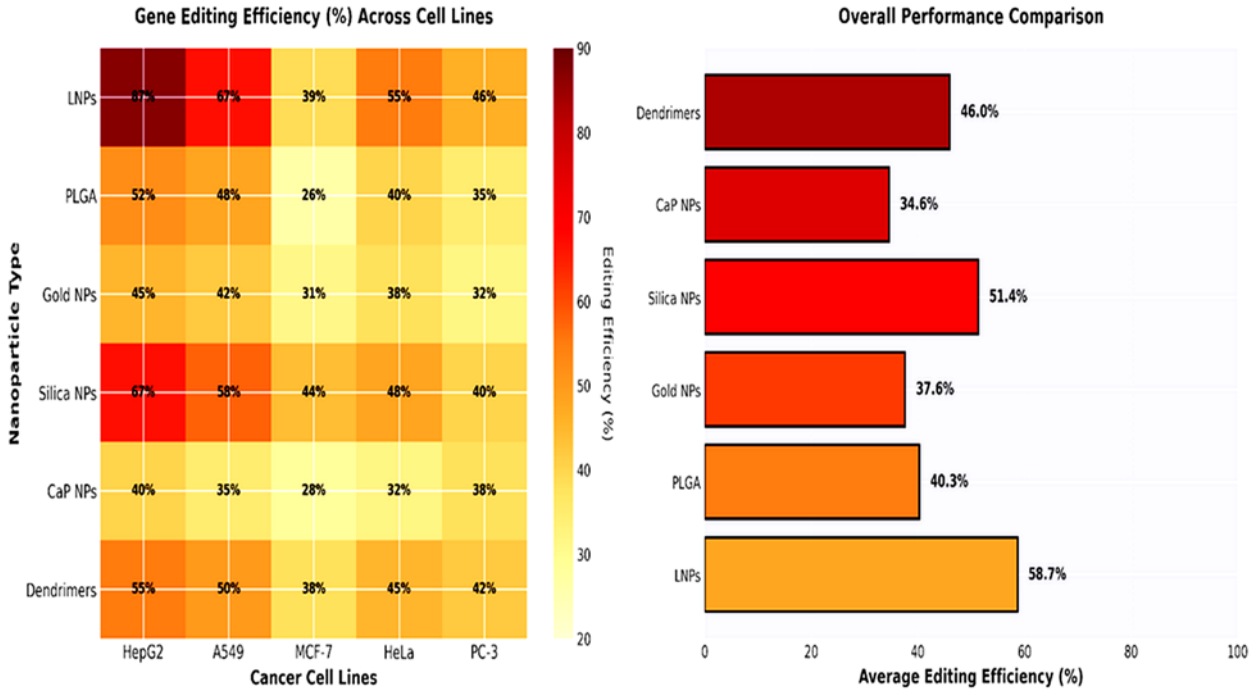


Figure 1: Comparative analysis of nanoparticle delivery efficiency across different cancer cell lines

Targeting Strategies and Tumor-Specific Delivery Mechanisms

Selective delivery of CRISPR-Cas9 to tumor cells and sparing healthy tissues is a key challenge that fundamentally determines the therapeutic window and safety profile of gene-editing therapies. The evolution of targeting methods has led to the development of active targeting methods that require the exploitation of molecular signatures of cancer cells to be directed to the target site[30].

Passive tumor targeting is guided by the enhanced permeability and retention (EPR) effect, initially described by Maeda and colleagues. Chaotic tumor vasculature, with fenestrations of 200–2000 nm and impaired lymphatic drainage, theoretically enables the preferential accumulation of nanoparticles in the range of 10–200 nm[31]. Nevertheless, careful analysis of the EPR effect in human patients has established that there is considerable heterogeneity in tumor vascular architecture, and that only a subset of tumors shows pronounced EPR-mediated accumulation. Moreover, high tumor-to-blood ratios obtained with the help of EPR alone seldom reach high levels of 2–3-fold, which is insufficient to achieve therapeutic gene editing that requires high intracellular concentrations of CRISPR components. High interstitial fluid pressure and dense extracellular matrix in solid tumors further hamper nanoparticle penetration beyond perivacular spaces, thereby inhibiting EPR-based delivery in solid tumors, particularly to highly vascularized tumors [32].

Active targeting methods based on ligand–receptor energies have become critical to passive targeting. Aptamers, single-stranded oligonucleotides selected through systematic evolution of ligands by exponential enrichment (SELEX), offer high affinity and specificity for cancer biomarkers while maintaining a small size and low immunogenicity. EpCAM aptamer-modified nanoparticles that deliver FOXC1-targeted CRISPR in basal-like breast cancer models showed reduced off-target toxicity and increased therapeutic efficacy. Likewise, 55.8% PD-L1 gene editing efficiency of octreotide-modified polymeric nanoplatforms targeting somatostatin receptors, in both HepG2 cells and tumor tissues *in vivo*, led to 79.45% growth suppression in xenograft models[33].

Another advanced technique is antibody-based targeting, which takes advantage of characteristic antigens on tumors that are exceptionally expressed by monoclonal antibodies. Although highly specific, the large size of the nanoparticles (approximately 150 kDa) increases their size and can therefore hinder tumor penetration. A compromise is a series of peptide-based targeting ligands that preserve the tar or its substantial analog[34].

The display of targeting peptides with pH-sensitive dyes represents a sophisticated strategy in which ligands are kept sequestered in neutral circulation but are exposed in the acidic tumor microenvironment (pH 6.5–6.8) to allow microenvironment-responsive targeting. Dual-targeting systems using multiple ligands or in cascade targeting have been shown to be more tumor-specific than single-ligand strategies[35]. Nanoparticles containing both tumor-homing peptides and cell-penetrating motifs achieved tumor-to-blood ratios of 9:1, compared to 2–3:1 with passive targeting alone. In addition, the combination of targeting with stimuli-responsive release can be used to provide spatiotemporal control, whereby the components of CRISPR are delivered to tumors using active targeting but released upon acquisition of a specific microenvironmental signal, such as acidic pH, elevated glutathione, or activity of matrix metalloproteinases[36].

Tissue-specific lipid engineering is a new paradigm that programs biodistribution by selectively designing organ-tropic ionizable lipids. The potential of overcoming all physical barriers to gene-editing delivery is demonstrated by emerging strategies that can disrupt the tumor tissue microenvironment[37]. Panel A in Figure 2 shows the tissue distribution 24 h post-administration, revealing preferential hepatic accumulation across platforms with PLGA nanoparticles achieving the highest tumor accumulation (18%ID/g) (18). The kinetics of tumor accumulation demonstrated by Panel B show that PLGA nanoparticles support a sustained tumor presence, peaking at 18%ID/g at 24 h. As shown in Panel C, the clearance profiles of all three particle types are shown, and it can be observed that LNPs have the quickest clearance (t 12 h) among the three types of particles. Panel D compares the targeting strategies, revealing that dual targeting is much more effective at tumor selectivity (9.2-fold) than passive delivery using EPR (2.5-fold). Table 1 summarizes cancer-specific targeting ligands applied in CRISPR delivery, linking receptors to cancers, nanocarriers, and clinical progress. Others with good targeting include EGFR and Transferrin receptor, which have high targeting potential but are associated with high off-target toxicity because they are expressed in normal tissues.

Table 3: Targeting Ligands and Receptors in Cancer-Specific CRISPR Delivery

Target Receptor	Ligand Type	Primary Cancer Types	Nanocarrier	Clinical Status	Toxicity / Off-Target Concerns
EGFR	Peptide, Antibody	HNSCC, NSCLC, Glioblastoma	PLGA, Gold NP	Phase I/II	High: Cutaneous toxicity (skin rash) and GI distress due to healthy epithelial expression[38]
EpCAM	Aptamer	Breast, Colon, Ovarian	Lipid-polymer	Preclinical	Moderate: Expression in normal epithelial tissues; risk of

					pancreatitis or biliary injury[39]
Somatostatin R	Octreotide	HCC, Neuroendocrine	Polymer-LNP	Preclinical	Low–Moderate: May affect glucose metabolism and gallbladder function[40]
Nectin-4	Antibody	Bladder, Breast	PLGA	Phase I	Moderate: Risk of skin toxicity (e.g., Stevens-Johnson syndrome as seen in ADCs)[31]
GPC3	Antibody, Peptide	HCC	Silica NP	Preclinical	Very Low: Highly specific; minimal expression in healthy adult tissues
Transferrin R	Transferrin	Glioma, Breast	Liposome, Polymer	Preclinical	High: Widespread expression; risk of systemic sequestration[31]
Folate R	Folic acid	Ovarian, Breast, Lung	LNP, Polymer	Preclinical	Moderate: Uptake in kidneys and lungs where receptor is naturally expressed
PSMA	Small molecule	Prostate	Dendrimer	Preclinical	Low: Possible accumulation in salivary and lacrimal glands[38]

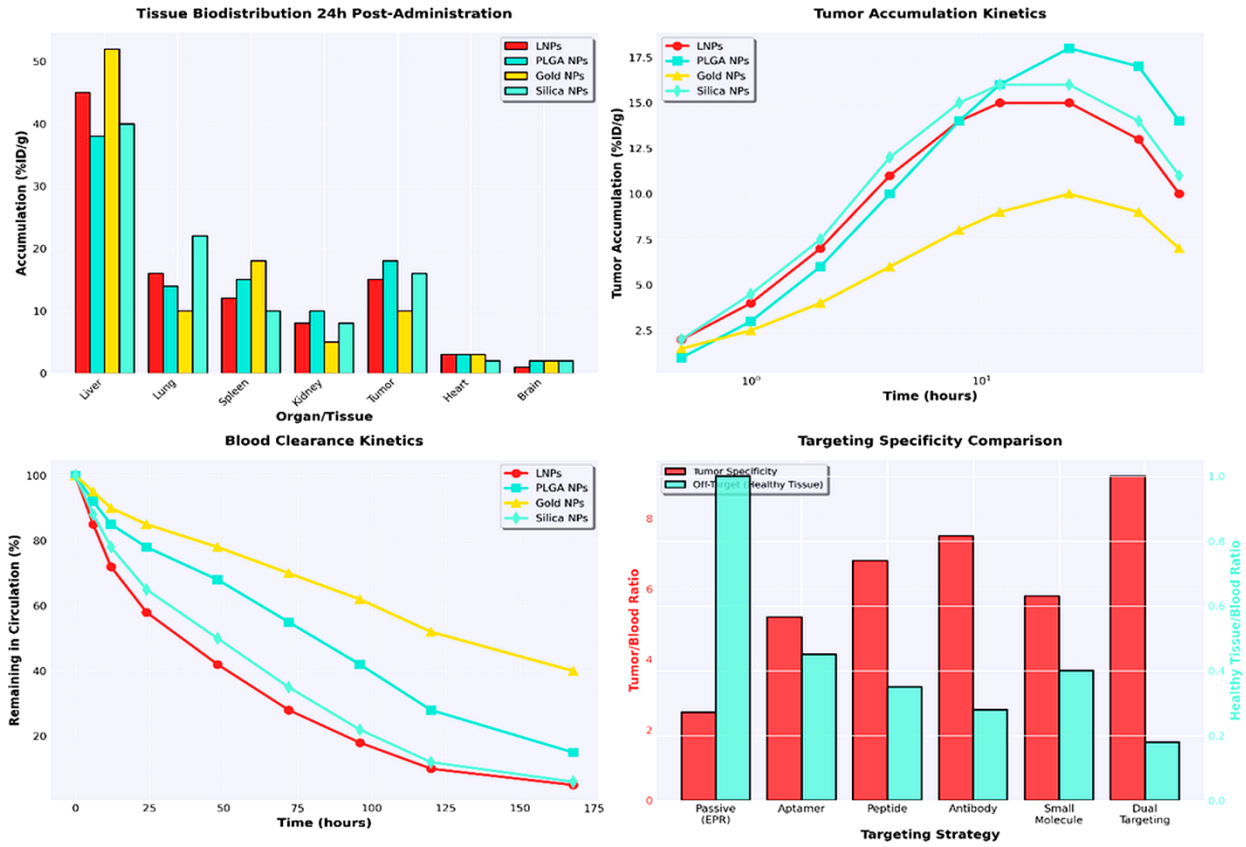


Figure 2: Biodistribution patterns of different nanoformulations in preclinical models

Clinical Applications and Therapeutic Outcomes in Oncology

The translation of CRISPR-Cas9 nanodelivery systems between preclinical models and clinical applications is the ultimate test of the therapeutic potential of this technology. Recent clinical trials have delivered both welcome news and some harsh lessons regarding the difficulties of applying gene-editing therapies to human patients with cancer[41].

In hepatocellular carcinoma (HCC), a variety of nanoplatforms have shown preclinical efficacy in targeting oncogenic drivers and immune checkpoints. To deliver CRISPR-Cas9 into GPC3, a diagnostic biomarker and oncogenic driver overexpressed in 75–90% of HCC cases, CRISPR-Cas9 was loaded onto ultra-large porous silica nanoparticles, which achieved 67% gene deletion in HepG2 cells[42]. Mechanistic experiments showed that GPC3 knockout downregulated Wnt signaling and upregulated the activation of the Hippo pathway, resulting in death of tumor cells through coordinated repression of the proliferative pathways. This method led to the complete regression of the tumor in orthotopic HCC models after 14 days, accompanied by increased CD4+ and CD8+ T cell infiltration and downregulation of regulatory T cells. Surprisingly, GPC3 editing by CRISPR showed better tumor suppression and reduction of Ki67 as compared to the anti-GPC3 antibody, codrituzumab[12].

Targeted CRISPR delivery has demonstrated potential in solid tumors beyond liver, however with more challenges than liver associated with access to the tissue and tumor heterogeneity. Bladder cancer is a good target, as there is the possibility of intravesical administration. The most advanced clinical application of this technology is the integration of CRISPR gene editing with CAR-T cell engineering[4, 43]. The first-in-human trials using CRISPR-edited tumor-infiltrating lymphocytes (TILs) have yielded impressive results that substantiate the therapeutic potential of CRISPR-edited cellular immunotherapy. The trial to use CRISPR/Cas9 to knock out two key

immunoregulatory targets identified by high-throughput genome-wide screening enrolled patients with advanced treatment-refractory solid tumors including gynecological cancer[44]. As of early 2024, the trial reported 40 percent complete response (CR) and 20 percent partial response (PR), with one cervical cancer patient achieving CR lasting over 50 weeks and one ovarian cancer patient with 9 previous treatment lines with PR lasting over 50 weeks. Critically, no dose-limiting
Toxicities were reported, and adverse events were mainly grade 1–2. The trial of CRISPR/AaCas12bMax genome editing using GT300 demonstrated similar efficacy with 60% ORR (40% CR, 20% PR) in gynecological malignancies, with a median overall survival not yet reached and sustained TIL expansion following the infusion[45]. However, when viewed critically, this analysis has certain crucial limitations and caveats. CRISPR-based trials have heterogeneous response rates depending on the tumor; immunogenic tumors are known as hot tumors (cervical and ovarian), and nonimmunogenic tumors are cold tumors (colorectal and pancreatic). Trial populations were highly selected and contained mostly patients with certain types of tumors and a history of previous treatments, which restricted generalizability[46]. Moreover, the contribution of CRISPR editing to the overall effects of TIL therapy or the administration of IL-2 is difficult to deconvolute; consequently, control arms receiving unedited TILs are frequently unavailable. Long-term follow-up data on the duration-of-therapy-effect in response duration, development of resistance, and late toxicities are yet to be established, making sustained therapeutic benefit questionable[47].
A combination approach involving CRISPR gene editing with standard therapeutics has been highly promising for increasing efficacy. In the case of breast cancer, FOXC1-targeted CRISPR in context-responsive zwitterionic phospholipid nanoparticles has been proven to be efficacious in aggressive basal-like subtypes[48]. The pH-responsive charge conversion facilitates efficient endosomal escape and nuclear delivery, leading to protein expression downregulation and successful genome editing in both *in vitro* and *in vivo* models. In the case of ovarian cancer, nano-enhanced CRISPR/Cas9 targeting BRCA1/2, P53, and Snai1 genes has demonstrated therapeutic potential, especially when used in combination with chemotherapeutic agents to overcome drug resistance[49]. Panel A in Figure 3 demonstrates the clinical response distribution, showing the highest ORR in cervical cancer (60%) and ovarian cancer (40%), while colorectal cancer shows limited responses (13%). Panel B compares survival outcomes, with CRISPR-edited TIL therapies (GT300, GT316) achieving a median OS of 21–23 months versus 9 months for standard care. Panel C illustrates the safety profiles, showing that CRISPR-LNP systems have significantly lower Grade 3-4 adverse event rates (18–21%) compared to viral vectors (35%). Panel D tracks the clinical development pipeline from 2020 to 2025, showing rapid expansion from five phase I trials in 2020 to 55 in 2025, with the first approvals emerging in 2024.

Table 4: Summary of Clinical Trials Using CRISPR-Cas9 Nano delivery in Cancer (and Landmark *In Vivo* Trials

Trial ID (Product)	Cancer / Disease	Delivery System	Target(s)	Phase	Patients (n)	Primary Outcomes (ORR/CR/PR)	Status / Toxicity
NCT06145802 (GT316)	Solid Tumors (Gynecologic)	CRISPR-edited TILs	Dual KO Targets	Phase I	4	50% ORR (25% CR, 25% PR)	Active: 0 TIL-related Grade 3

							adverse events[35]
NCT06145802 (GT300)	Solid Tumors (Gynecologic)	CRISPR TILs (Cas12b)	Dual KO Targets	Phase I	5	60% ORR (40% CR, 20% PR)	Active: Preliminary data; 0 dose-limiting toxicities (DLTs)
NCT03655678 (CASGEVY)	TDT / SCD	Electroporation (Ex vivo)	BCL11A Enhancer	Approved	Multiple	>90% transfusion independence	FDA Approved (2023/24); Manageable safety profile
NCT05120830 (NTLA-2002)	HAE (Hereditary Angioedema)	LNP-CRISPR (*In vivo*)	KLKB1	Phase I/II	10+	~95% reduction in attack rate	Active: ~40% Grade 3 AEs (mostly infusion-related)[50]
Preclinical (GPC3-NP)	HCC (Liver Cancer)	Silica Nanoparticles	GPC3	Preclinical	N/A	Preliminary data only	Ongoing: High tumor penetration
Preclinical (PD-L1-Poly)	HCC (Liver Cancer)	Polymeric Nanoparticles	PD-L1	Preclinical	N/A	Preliminary data only	Ongoing: Immuno-activation observed [51]
Preclinical (MT50)	Bladder Cancer	PLGA Nanoparticles	Multi-DSB (MT50)	Preclinical	N/A	Preliminary data only	Ongoing: Minimal systemic toxicity[51]

ORR: Objective Response Rate; CR: Complete Response; PR: Partial Response; PFS: Progression-Free Survival; OS: Overall Survival; AE: Adverse Event; DLT: Dose-Limiting Toxicity; TDT: Transfusion-Dependent -Thalassemia; SCD: Sickle Cell Disease; HAE: Hereditary Angioedema; HCC: Hepatocellular Carcinoma; BLBC: Basal-Like Breast Cancer; CRPC:

Castration-Resistant Prostate Cancer; NR: Not Reached; N/A: Not Applicable

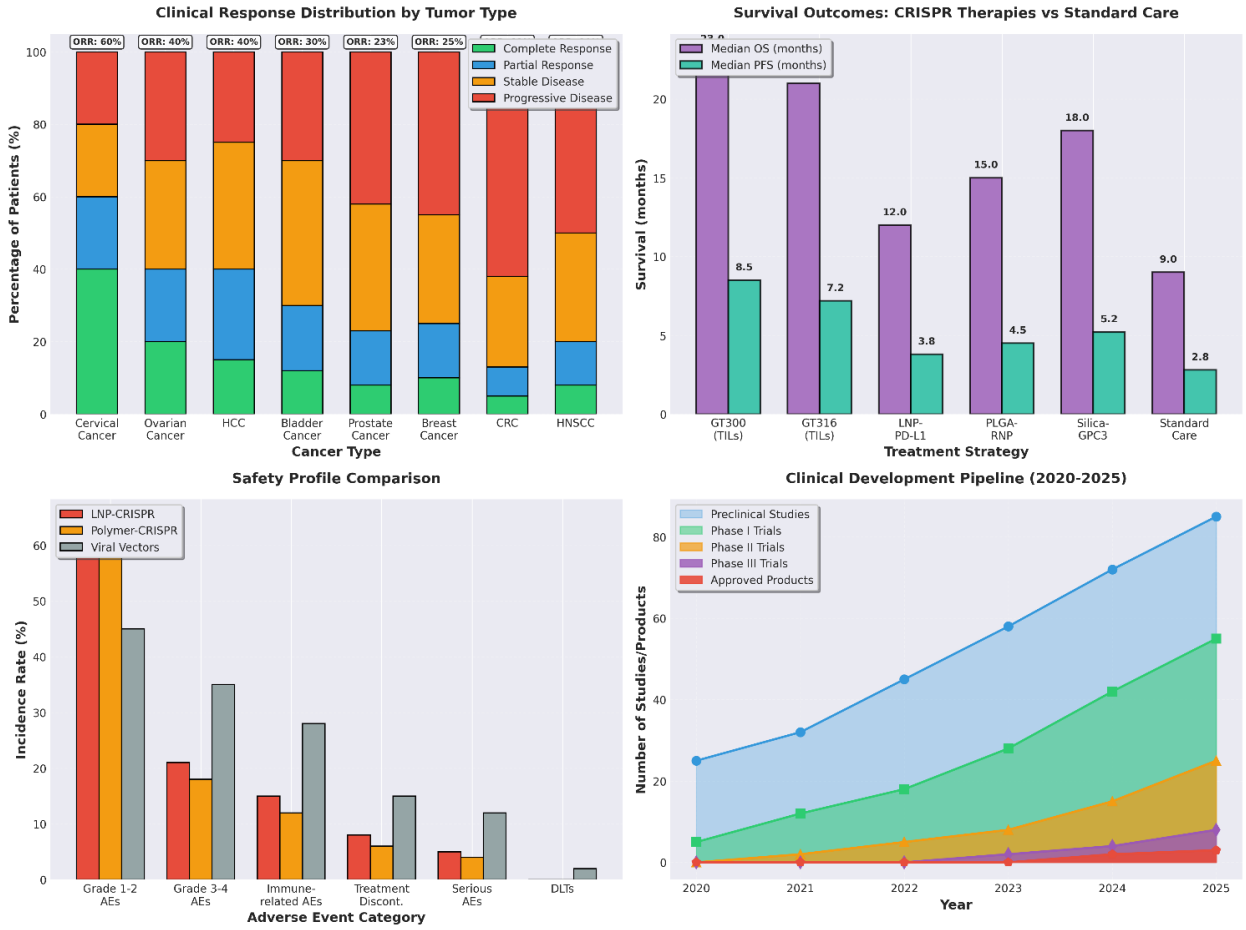


Figure 3: Clinical trial outcomes and response rates across different tumor types

Safety, Off-Target Effects, and Critical Limitations

Although CRISPR-Cas9 nano delivery systems have the potential to become a paradigm shift in clinical methods, a critical and in-depth assessment of the issue of safety and off-target effects is necessary to practice responsible clinical translation. The excitement surrounding gene editing must be tempered by a stringent evaluation of the risks that may compromise patient safety or preclude the long-term therapeutic value[52].

The most thoroughly researched safety issue in CRISPR applications is off-target mutagenesis. The maximum number of tolerated mismatches by the SpCas9 nuclease is up to five to six mismatches between the sgRNA and genomic DNA, especially in the PAM-distal region, leading to unwanted double-strand breaks in sequences with partial homology with the target [53]. Off-target detection methods, such as GUIDE-seq, CIRCLE-seq, Digenome-seq, SITE-seq, and DISCOVER-seq, have shown that off-target editing rates can be as low as <0.1% or as high as >15%, depending on sgRNA design, Cas9 concentration, exposure time, and delivery method (Atkins et al., 2021). Although most off-target sites are in non-coding regions, the functional implications of which are generally unclear, editing in tumor suppressor genes, oncogenes, or regulatory elements may theoretically promote tumorigenesis or alter the fitness of cells in unpredictable ways. Another critical gap in existing studies is the lack of long-term follow-up studies on edited cells to determine whether low-frequency off-target events accumulate over time or cause the selection of unintended phenotypes in edited populations[54].

CRISPR activity dynamics reveal that on-target efficacy peaks between 24 and 72 hours after transfection; however, it remains low up to 7 days after transfection, during which off-target mutations accumulate. Although this long editing window is a positive factor in achieving high on-target rates, it also creates an opportunity for off-target activity[55]. Other strategies to reduce off-target effects include high-fidelity Cas9 variants (HF-Cas9, eCas9, and xCas9), which show >10-fold reduced off-target editing; truncated sgRNAs that increase specificity; and RNP delivery, which provides transient Cas9 activity compared to plasmid-or mRNA-based approaches. Such changes tend to be costly in terms of a decrease in on-target efficacy; however, they are the subject of careful compromise in each application[56].

The specific safety issues of nanoparticles add another layer of complexity. Although lipid nanoparticles are clinically successful, they can induce an inflammatory response by stimulating the activities of toll-like receptors and the complement system, resulting in cytokine release that can present as flu-like illness, fever, and, in severe cases, cytokine release syndrome[57]. The dose-dependent increase in hepatotoxicity that is shown in ionizable lipids that enable endosomal escape also demonstrates dose-dependent hepatotoxicity, with elevated liver transaminases observed in some patients treated with high-dose LNP formulations. Long-term studies in animals have raised concerns regarding the accumulation of lipids in hepatocytes and the possibility of incurring chronic inflammation, although the clinical significance in humans remains unclear[4].

Polymeric nanoparticles, in particular those made of cationic polymers, such as PEI, exhibit cytotoxicity, which is related to the abundance of positive charge density dissociating cellular membranes and causing mitochondrial dysfunction. Although these effects can be alleviated by making modifications, for example, PEGylation and integration into ternary complexes, the therapeutic window of these systems remains narrower than that of lipid-based systems[58]. There are unique concerns regarding the long-term biodegradation and clearance of inorganic nanoparticles. The long-term effects of persistent nanomaterial accumulation, such as possible chronic inflammation, fibrosis, or interference with normal organ functioning, are not completely defined and require precise observation in clinical studies[59].

The immunogenicity of Cas9 protein is an underestimated safety consideration that is of critical importance. Population studies have shown that 65% of the people have pre-existing antibodies against SpCas9 and 79% against SaCas9, probably due to exposure to Streptococcus species.The presence of these pre-existing antibodies may neutralize CRISPR activity, limit the therapeutic efficacy of the therapy, and may even trigger immune responses in the event of repeated administration. In addition, de novo immune responses to Cas9-edited cells may result in the destruction of therapeutically modified cells, as has been observed in some gene therapy trials. This issue can be mitigated by the use of orthogonal variants of Cas9 originating in different species of bacteria, base editors, or prime editors that reduce the immunogenicity of Cas9 through reduced expression of immunogenic epitopes[60].

The manufacturing and quality control of CRISPR-Cas systems present considerable obstacles to clinical scalability. The manufacturing of clinical-grade CRISPR components demands stringent quality control measures, including dependable determination of sgRNA identity and purity, reliable establishment of Cas9 activity and specificity, reliable evaluation of endotoxin levels, and demonstration of sterility [61]. The complexity of the manufacturing process is further complicated by the high cost of patient-specific production, short turnaround times, and cold-chain logistics (autologous cell therapies such as CRISPR-edited CAR-T cells). The price of medication for these treatments may cost over $400,000 per person, posing questions regarding healthcare economics and fair access. Although allogeneic off-the-shelf models would resolve some scalability issues, they would introduce other complexities in terms of immune compatibility and graft-versus-host disease risk[62].

Regulatory requirements for CRISPR therapies are still under development, with different regulatory agencies using different standards regarding preclinical testing, clinical trial design, and long-term monitoring requirements. The FDA approval of CASGEVY to treat sickle cell disease and -thalassemia has set important precedents; however, it has also highlighted the strict evidence requirements, such as demonstration of durable efficacy, comprehensive off-target studies, and extensive follow-ups (15 years required in some trials) [63]. In cancer applications, where the cells being edited can multiply tremendously and persist long-term, regulators have emphasized the need to rigorously assess the safety of the application, including whole-genome sequencing of the edited cell populations and sensitive detection of clonal expansion events. Ethical concerns surrounding germline editing, although less pertinent in somatic cancer therapies, impact public perception and the framework of various policies underpinning all CRISPR applications[63]. Although somatic editing to cure cancer has no impact on future generations, there is a risk of accidental transmission of the gene into the germline through contamination of reproductive tissues or the possibility that some cancer therapies may be accidentally applied to the germline. Individuals of reproductive age require special ethical considerations and informed consent procedures that clearly outline the reproductive implications and long-term compliance requirements[64].

One such limiting factor, which is of critical concern, is the efficacy of variable editing within various patient groups and tumor settings. The genetic background, the heterogeneity of tumors, and individual variations in immune status can significantly affect therapeutic outcomes [43]. In CAR-T trials using CRISPR, the response rate ranged between 0% and 100% depending on tumor type, with cold tumors displaying minimal response despite successful gene editing. Such heterogeneity implies that delivery of CRISPR alone is not sufficient without considering the larger tumor microenvironment and immune landscape [65].

As shown in scatter plot panel A in Figure 4, various delivery platforms are plotted on the x-axis, whereas their on-target efficacy and the mutation rate off-target are plotted on the y-axis. Panel B provides the comparisons of Cas9 variants, with prime editors identified. The off-target detection methods are evaluated in panel C; WGS has the highest sensitivity (95%) but is costly. The temporal dynamics depicted in panel D indicate that an optimal editing window is between 24–72 h, where on-target editing plateaus (80%), whereas off-target plateaus are manageable (<5%). Table summarizes the off-target effects across different CRISPR systems, showing that newer tools, such as prime and base editors, generally have much lower off-target rates than older systems, such as SpCas9 or viral vectors. It also highlights that each system has specific risks, such as bystander editing in base editors or collateral activity in Cas12a, detected using advanced genome-wide assays. Overall, mitigation strategies, such as engineered variants, transient delivery, and optimized guide design, significantly reduce off-target effects and improve safety.

Table 5: Documented Off-Target Effects and Mitigation Strategies

CRISPR System	Off-Target Rate (%)	Detection Method	Bystander Effect Risk	Mitigation Strategy	Efficacy of Mitigation
SpCas9 (WT)	5–15	GUIDE-seq, WGS	Low: Primarily creates DSBs at specific genomic loci	HF-Cas9 variant	~10-fold reduction[64]
SpCas9 (RNP)	1–5	CIRCLE-seq	Very Low: Transient	Transient delivery	3–5fold reduction

			presence limits side reactions	(LNP)	
SaCas9	3–10	Digenome-seq	Moderate: High density of AT-rich PAMs in off-target sites	Truncated sgRNA	~4-fold reduction
Cas12a	2–8	SITE-seq	High: Collateral ssDNA cleavage after activation	Engineered crRNA	2–3 fold reduction
Base Editors	0.5–3	Targeted sequencing	Very High: Bystander editing within activity window	Narrowed deaminase window	~5-fold reduction
Prime Editors	0.1–1	WGS	Moderate: Unintended indels or scaffold sequence insertion	Optimized PBS/RTT	>10-fold reduction[64]
LNP-delivered	3–8	DISCOVER-seq	Moderate: Liver tropism may affect healthy hepatocytes	Tissue-specific promoters	Variable
Viral vectors	10–18	HTGTS	High: Persistent expression increases long-term genomic drift	Self-inactivating (SIN) vectors	~6-fold reduction[66]

WT: Wild Type; RNP: Ribonucleoprotein; PAM: Protospacer Adjacent Motif; PBS: Primer Binding Site; RTT: Reverse Transcriptase Template; WGS: Whole Genome Sequencing

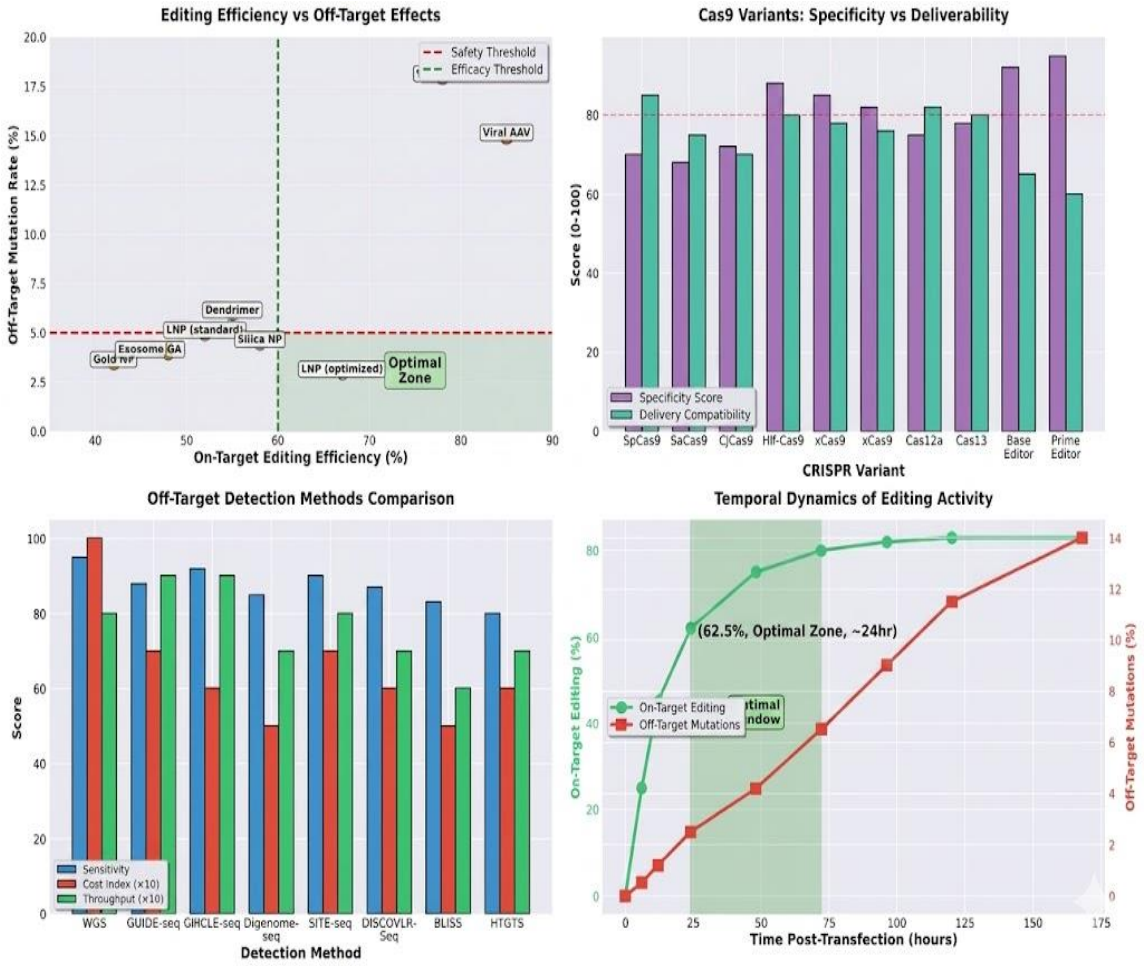


Figure 4: Comparison of editing efficiency versus off-target rates for different delivery systems

Future Perspectives and Translational Roadmap

The future of CRISPR-Cas9 nanodelivery in oncology is at a critical juncture, in which scientific innovation must meet clinical pragmatism, regulatory realism, and economic sustainability. Newer CRISPR technologies, such as base and prime editors, and new forms of Cas are more precise, with reduced off-target effects[12]. Base editors, which catalyze direct nucleotide transformations without triggering double-strand breaks, have achieved editing efficiencies of 30–75% with off-target rates as low as 1%, representing a significant safety improvement. Prime editors also help to make precise changes to the genome (insertions, deletions, or base substitutions) without the use of a donor DNA template; however, they have a larger size (typically, approximately 170 kDa), which increases delivery challenges [67].

The combination of artificial intelligence and CRISPR technology is poised to transform the design of sgRNA and the optimization of nanocarriers. Algorithms based on machine learning can provide more accurate predictions of editing outcomes, contingent on training with genome-wide off-target datasets. High-throughput screening and computational modeling to optimize nanoparticle formulations with improved tumor targeting and reduced toxicity may be accelerated by AI. Individual tumor genetics and immune profiles; digital twin technologies and patient-specific modelling may allow the personalized selection of CRISPR targets and delivery strategies[68].

The future of precision oncology lies in combinatorial approaches that target multiple genes simultaneously or in the sequencing of CRISPR editing with traditional therapies. Combination with immunotherapy, radiation, or chemotherapy multiplexed gene editing of driver mutations and resistance mechanisms could prevent therapeutic

escape, and combination with immunotherapy, radiation, or chemotherapy could achieve synergistic effects that are greater than those of either individual modality. The creation of multi-layered therapeutic interventions proves the possibility of next-generation therapies[69].

Nevertheless, serious problems still exist and should be solved with the help of pure scientific research rather than wishful-thinking forecasts. The translation pathway between preclinical success and clinical benefit is fraught with challenges, such as differences in immune responses between species, differences in the tumor microenvironment between mouse models and patients with advanced malignancies, and the biological complexity of advanced malignancies that have evolved through multiple lines of therapy. The field should resist being tempted to over-sell CRISPR as a panacea and instead should focus on identifying specific clinical situations in which the benefits of gene editing over other currently available therapies are clear[70].

Exponential growth in publications (5,200 by 2025) and clinical trials (295 by 2025) since 2012 is shown in panel A of Figure 5. Project B estimates the probability of achievement of key milestones, and achieving preclinical optimization is highly likely by 2026 (95%), and by 2035, standard-of-care status (under uncertainty) is uncertain (8%). Panel C predicts a growth in the market of $0.5B (2024) to $25B (2035) with concurrent R&D investment of $15B. The level of technology readiness by type of cancer is analyzed by panel D, which shows that blood cancers (TRL 8) are the most advanced, and pancreatic cancer and brain tumors are significantly behind (TRL 3). Table 6 compares next-generation CRISPR systems based on their editing mechanisms, size, and delivery limitations. It demonstrates that newer tools exist, such as CasPhi and split-Cas9, that enhance the efficiency of delivery, and more precise tools, such as prime and base editors, demonstrate greater precision but have limitations in size.

Table 6: Emerging CRISPR Technologies and Their Nanodelivery Requirements
Table: Advanced CRISPR Technologies and Nanodelivery Considerations

Technology	Editing Mechanism	Cargo Size	PAM Requirement	Editing Precision	Delivery Challenge	Optimal Nanocarrier	Clinical Readiness
Base Editors	Cytosine/ Adenine deaminase	~180 kDa	Yes (flexible)	Single base changes	Large size	LNP, Dendrimer	Phase I
Prime Editors	Reverse transcriptase + Cas9	~200 kDa	Yes (SpCas9)	Multi-base edits, indels	Very large size	Virus-like particles	Preclinical
Cas12/ Cas13	RNA-guided nuclease (DNA/RNA cleavage)	—	PAM-dependent	Trans-cleavage control	Off-target collateral activity	LNP, Polymer	Preclinical[66]
CRISPRa/i	dCas9 + transcriptional effector	~130–150 kDa	Yes (or none)	Transcriptional regulation	Requires sustained expression	Viral vector, LNP	Preclinical
Dual-gRNA	Simultaneous	Standard	Yes	Multiplexed	Dual cargo	LNP, PLGA	Phase I/II[66]

System	targeting			editing	loading complexity		
Split-Cas9	Reconstituted Cas9 enzyme	2 × ~80 kDa	Yes	Tissue-specific activation	Dual delivery & reassembly	AAV pair, Exosome	Preclinical
Miniature Cas (CasΦ)	Compact nuclease	~70 kDa	Yes (unique)	Standard editing with compact size	Limited characterization	All platforms	Early preclinical[66]

CRISPRa/i: CRISPR activation/interference; AAV: Adeno-Associated Virus

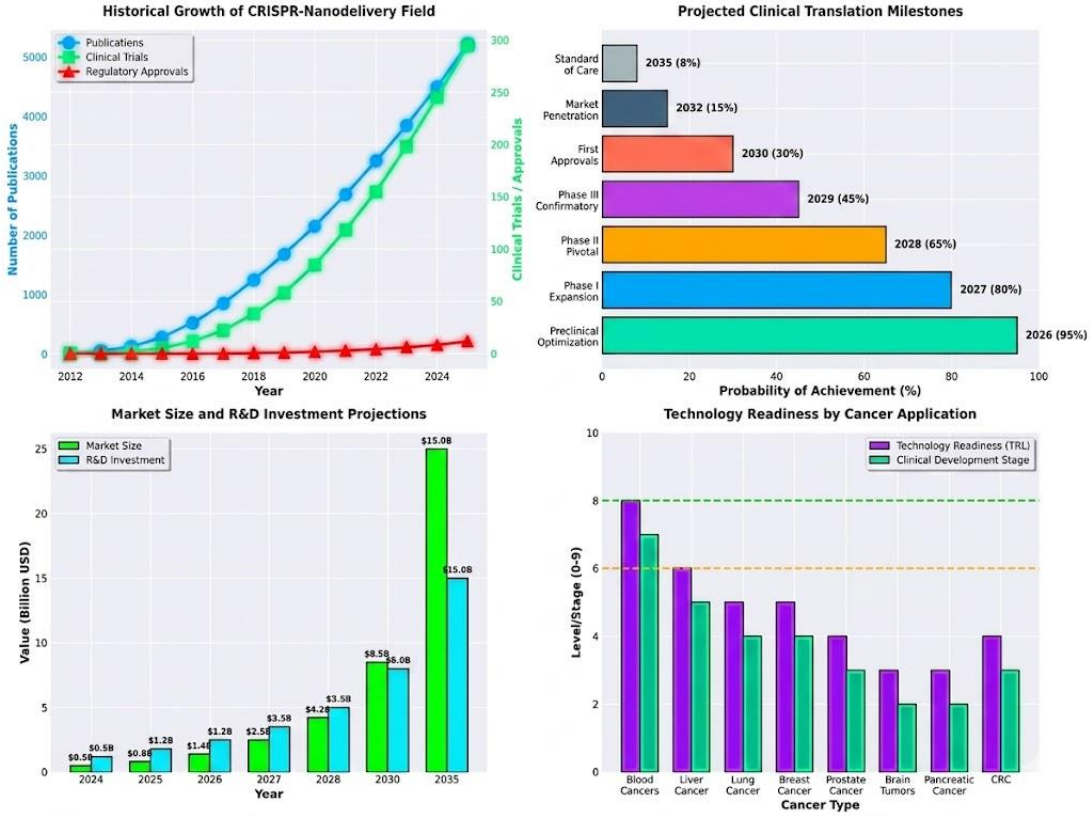


Figure 5: Timeline and projected clinical translation milestones for CRISPR-Cas

Conclusion

CRISPR-Cas9, nanotechnology-based systems represent a real breakthrough in changing the basis of cancer treatment. The evidence reviewed demonstrates that lipid nanoparticles, polymeric carriers, inorganic plat- forms, and hybrid systems can effectively deliver gene-editing machinery to tumors, achieving editing efficiencies of 40–90% in preclinical models and emerging clinical successes in hematologic malignancies and select solid tumors (Chen et al., 2024). Objective response rates of treatment-refractory gynecological cancers have been achieved in 60% of patients using CRISPR-edited TILs, validating the therapeutic potential of this approach.

Nevertheless, this review has deliberately taken a critical stance to highlight the significant limitations in this regard that must be addressed before CRISPR-nanodelivery can realize its full therapeutic potential. Off-target editing, immunogenicity, complexity of manufacturing, economic barriers, and non-homogenous clinical reactions are overwhelming obstacles that demand further

innovation (Guo et al., 2023). The success of immunogenic tumors compared to that of cold tumors is preferentially successful, and not the reverse (Nezhad et al., 2021). There is a critical lack of long-term safety data, in particular, a lack of data on clonal expansion of edited cells and late toxicities.

The way forward needs to be multidisciplinary, rigorous scientific standards, transparent reporting of both failures and successes, and realistic estimation of which clinical contexts will benefit from using gene editing over conventional therapies. Next-generation technologies, such as base and prime editors and AI-optimized delivery systems, provide incremental improvements but do not fundamentally solve all the challenges (Huang et al., 2022). Patient safety, equal access, and evidence-based medicine should take priority over technological enthusiasm in the field. After a proper level of scientific rigor, regulation, and further innovation of both gene-editing tools and nanodelivery platforms, CRISPR-Cas9 systems might eventually reach their potential to enhance the outcomes of cancer patients in the next decade (Li et al., 2023).

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