

CRISPR-Loaded Nanoparticles For Gene Silencing in Solid Tumors: a systematic review

¹Trupti Cholera, ²Rushikesh Desale

Department of pharmaceuticals, SMES Mahavir institute of pharmacy, Nashik, Maharashtra, India.

Abstract:

Solid malignancies represent a formidable global health burden, accounting for the vast majority of cancer-related mortality. While traditional therapeutic modalities such as surgical resection, systemic chemotherapy, and radiation form the foundation of clinical oncology, their long-term success is frequently compromised by acquired drug resistance, severe off-target toxicities, and disease recurrence. The advent of CRISPR-Cas genome editing has introduced a transformative avenue for precision oncology, enabling sequence-specific, permanent knockouts of oncogenic drivers. However, translating CRISPR systems into clinical practice remains bottlenecked by systemic delivery hurdles, rapid clearance, and safety concerns. To circumvent these limitations, non-viral nanocarriers particularly functionalized lipid, polymeric, and inorganic nanoparticles have gained widespread traction. Recent preclinical investigations demonstrate that CRISPR-loaded nanoparticle formulations can achieve robust gene silencing of critical oncogenes (KRAS, EGFR, HER2, and PLK1) across diverse solid tumor models, including breast, non-small cell lung, hepatocellular, pancreatic, ovarian, and colorectal carcinomas. This systematic review provides a comprehensive synthesis of recent breakthroughs in CRISPR-nanoparticle platforms for targeted gene disruption in solid tumors. We critically evaluate the underlying gene-editing mechanisms, nanocarrier engineering strategies, therapeutic efficacy, and toxicity profiles. Additionally, major translational barriers such as off-target cleavage, physiological transport obstacles, host immunogenicity, and regulatory hurdles are discussed alongside future clinical translation strategies. Overall, CRISPR-loaded nanocarriers represent a compelling paradigm in targeted therapeutics, offering new pathways for managing refractory solid tumors.

Keywords: CRISPR-Cas9; Gene Silencing; Nanoparticles; Solid Tumors; Precision Oncology; Cancer Nanomedicine; Genome Editing; Non-Viral Delivery.

1. Introduction

Cancer remains one of the leading causes of mortality worldwide, with the global burden reaching an estimated 20 million new cases and nearly 9.7 million deaths annually (1). Among all malignancies, solid tumors including breast, lung, colorectal, prostate, hepatocellular, and pancreatic carcinomas account for the vast majority of cancer diagnoses and represent a major public health challenge (2). While conventional therapeutic modalities, such as surgical resection, systemic chemotherapy, radiotherapy, and small-molecule targeted therapies, have substantially improved patient survival over recent decades, their long-term efficacy is frequently limited (2,3). Major clinical bottlenecks include non-specific tissue toxicity, intrinsic or acquired drug resistance, high rates of local recurrence, and metastatic dissemination. Consequently, there is an urgent need to develop highly specific, targeted therapeutic strategies that can selectively disrupt driver oncogenes while minimizing systemic adverse effects (1–3).

1.1 The Evolution of Gene Silencing and Precision Genome Editing

Among modern therapeutic approaches in precision oncology, gene silencing strategies have gained significant momentum by directly targeting key molecular pathways that govern tumor proliferation, angiogenesis, metastatic dissemination, and acquired treatment resistance (4). Initial gene-silencing modalities relied heavily on RNA interference (RNAi) pathways—primarily small interfering RNAs (siRNAs) and microRNAs (miRNAs)—to transiently downregulate disease-causing messenger RNAs (mRNAs) at the post-transcriptional level (4,5). While RNAi demonstrated early clinical promise, its therapeutic potential is often constrained by incomplete gene knockdown, transient duration of action, and off-target post-transcriptional effects. The advent of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas system has revolutionized the field of therapeutic gene modulation by providing a highly specific, programmable, and permanent genome-editing platform (6). Unlike RNAi-mediated strategies that require repeated administration to maintain target suppression, CRISPR-Cas technology targets double-stranded DNA directly, inducing site-specific double-strand breaks (DSBs) or precise nucleotide edits that lead to irreversible gene disruption. This capacity for permanent oncogenic driver knockout renders CRISPR-based platforms uniquely suited for durable cancer therapeutics (4–6).

1.2 Mechanisms of CRISPR-Cas Systems and the Imperative for Non-Viral Delivery

The CRISPR-Cas platform operates as a two-component system: a single-guide RNA (sgRNA) that provides sequence specificity by binding to a complementary genomic target, and a Cas endonuclease (such as Cas9) that introduces a targeted double-strand break (DSB) at the specified loci (7). Following cleavage, the cell repairs the DSB primarily through one of two pathways: error-prone Non-Homologous End Joining (NHEJ), which induces insertions or deletions (indels) that disrupt oncogenic reading frames, or Homology-Directed Repair (HDR), which enables precise donor-templated sequence repair (7,8). In oncology research, CRISPR technology has been harnessed to systematically disrupt oncogenes, repair tumor suppressor mutations, and engineer chimeric antigen receptor (CAR)

T-cells to augment anti-tumor immunity (8,9). Despite its transformative potential, the translational utility of CRISPR-based therapies hinges on the development of safe and efficient delivery systems capable of traversing complex physiological barriers (9). Historically, engineered viral vectors (e.g., adeno-associated viruses, lentiviruses) served as the primary vehicles for gene transfer due to their high transduction efficiency (10). However, clinical translation of viral vectors is restricted by significant drawbacks, including host immunogenicity, risk of insertional mutagenesis, limited cargo capacity, and substantial manufacturing scalability bottlenecks (10, 11).

To address these limitations, non-viral delivery platforms—specifically functionalized nanocarriers—have emerged as a safer, versatile alternative (11). Nanoparticles constructed from lipids, synthetic or natural polymers, gold, and mesoporous silica can encapsulate diverse CRISPR payloads (plasmid DNA, Cas9 mRNA, or ribonucleoprotein complexes), protecting them from enzymatic degradation by serum nucleases (11, 12). Furthermore, these nanocarriers enhance cellular internalization, facilitate endosomal escape, and can be surface-modified with targeting ligands to deliver CRISPR components directly to solid tumor microenvironments (12).

1.3 Objectives of this Review

This systematic review critically evaluates the current landscape of nanoparticle-mediated CRISPR delivery systems for targeted gene silencing in solid tumors. Specifically, we examine:

- Design strategies and surface-engineering techniques for non-viral nanocarriers.
- Therapeutic efficacy and oncogene-silencing outcomes across major solid tumor models.
- Physiological delivery barriers, off-target editing risks, and host toxicity profiles
- Translational perspectives and regulatory frameworks governing clinical deployment

2. Methodology

2.1 Review Design and Protocol

This systematic review was designed and conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) statement guidelines (13, 14). The protocol was established prior to data extraction to ensure transparent, reproducible reporting across all stages of literature identification, screening, eligibility assessment, and inclusion.

2.2 Information Sources and Search Strategy

A comprehensive, systematic literature search was executed across four primary electronic databases: PubMed/MEDLINE, Web of Science, Embase, and Scopus (15, 16). The search focused on peer-reviewed preclinical and clinical studies evaluating nanoparticle-mediated delivery of CRISPR components for targeted gene silencing in solid tumour models (17).

To maximize search sensitivity and specificity, Boolean logic was applied using combinations of Medical Subject Headings (MeSH) terms and text keywords, including:

- ("CRISPR" OR "Cas9" OR "CRISPR-Cas")
- AND ("nanoparticle" OR "lipid nanoparticle" OR "polymeric nanoparticle" OR "non-viral vector")
- AND ("gene silencing" OR "gene knockout" OR "oncogene targeting")
- AND ("solid tumor" OR "carcinoma" OR "neoplasm")

2.3 Eligibility Criteria

Study selection was guided by predefined inclusion and exclusion criteria based on the PICOS (Population, Intervention, Comparator, Outcome, and Study Design) framework to ensure systematic screening and data consistency (20, 21).

Table 1: Eligibility Criteria for Study Selection (PICOS Framework)

Category	Inclusion Criteria	Exclusion Criteria
Population / Disease	Preclinical (in vitro / in vivo) or clinical studies evaluating solid tumors (e.g., breast, lung, liver, pancreatic, colorectal).	Haematological malignancies (e.g., leukaemia, lymphoma, myeloma) or non-oncological disease models.
Intervention	Non-viral nanoparticle platforms (lipid, polymeric, inorganic, or hybrid) delivering CRISPR components (Plasmid DNA, Cas9 mRNA, or RNPs) for target gene silencing.	Non-CRISPR gene silencing modalities (e.g., standalone siRNA, shRNA, antisense oligonucleotides) or viral vector-only delivery (e.g., AAV, lentivirus).
Comparators	Untreated controls, mock-transfected cells, empty nanoparticles, or non-targeting sgRNA controls.	Studies lacking appropriate negative or non-targeting vehicle controls.

Outcomes	Quantifiable gene knockdown/editing efficiency, oncogene disruption, cellular apoptosis, tumor growth inhibition, or toxicity evaluation.	Studies lacking quantitative editing efficiency, target expression metrics, or therapeutic outcomes.
Study Design & Type	Original peer-reviewed primary research articles published in English with full-text accessibility.	Review articles, editorials, commentaries, book chapters, conference abstracts, and non-English publications.

2.4 Study Selection and Screening Process

All identified citations were imported into reference management software (EndNote 20), where automated and manual duplicate removal was performed (22). Two reviewers independently conducted a two-stage screening process:

- **Title and Abstract Screening:** Records were evaluated against eligibility criteria to exclude non-relevant articles.
- **Full-Text Assessment:** Potential full-text articles were thoroughly evaluated for final inclusion. Disagreements between reviewers were resolved through consensus or consultation with a third senior reviewer.

2.5 Data Extraction

Data were extracted independently by two investigators using a standardized, pre-tested data extraction form (23, 24). Extracted parameters included:

- **Study Identification:** First author, year of publication, and target tumor type.
- **CRISPR Components & Cargo:** Cas enzyme variant (e.g., Cas9, Cas12a), guide RNA target gene, and payload format (plasmid DNA, Cas9 mRNA, or RNP).
- **Nanoparticle Engineering:** Nano carrier classification (LNP, polymer, inorganic, biomimetic), surface functionalization/targeting ligands, size, zeta potential, and encapsulation efficiency.
- **Therapeutic Efficacy:** Target gene silencing/knockdown efficiency, cellular uptake mechanisms, in vitro cytotoxicity, and in vivo tumor growth suppression.
- **Safety & Biocompatibility:** Off-target editing frequency, endosomal escape efficiency, systemic immunogenicity, and histopathological organ safety profiles.

2.6 Methodological Quality and Risk of Bias Assessment

To evaluate the internal validity and methodological quality of included studies, two calibrated instruments were utilized based on study design (25,26): Preclinical In Vivo Studies: Evaluated using the SYRCLE (Systematic Review Centre for Laboratory animal Experimentation) Risk of Bias Tool, assessing sequence generation, baseline characteristics, allocation concealment, random housing, investigator blinding, random outcome assessment, incomplete outcome data, selective reporting, and other biases (25). Clinical Trials (where applicable): Evaluated using the Cochrane Risk of Bias 2 (RoB 2) Tool across five domains: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result (26).

Table 2: Methodological Risk of Bias Evaluation Framework

Risk Domain	Evaluation Criteria	Judgment Categories
Selection Bias	Random sequence generation and allocation concealment protocols	Low / High / Unclear
Performance Bias	Random housing conditions and investigator/handler blinding	Low / High / Unclear
Detection Bias	Random outcome selection and outcome assessor blinding	Low / High / Unclear
Attrition Bias	Completeness of outcome data and handling of withdrawals/exclusions	Low / High / Unclear
Reporting Bias	Selective outcome reporting matching pre-specified endpoints	Low / High / Unclear
Other Sources of Bias	Validation of controls, toxicity reporting, and funding conflicts	Low / High / Unclear

3. CRISPR-Mediated Gene Silencing

3.1 Molecular Mechanisms of CRISPR-Cas Systems

Derived from the adaptive immune defence mechanisms of bacteria and archaea, the CRISPR-Cas system has emerged as a revolutionary tool for targeted genome engineering (27). Among the various CRISPR classes, the Type II CRISPR-Cas9 platform is the most widely utilized for sequence-specific gene editing and transcriptional regulation. The core editing complex comprises two primary elements: a synthetic single-guide RNA (sgRNA) that provides target specificity, and a Cas9 endonuclease that executes double-stranded DNA cleavage (27, 28).

The editing cascade initiates when the sgRNA directs the Cas9 enzyme to a complementary 20-nucleotide target sequence in the genomic DNA. Target recognition strictly requires the presence of a short, adjacent sequence known as the Protospacer Adjacent

Motif (PAM) typically 5'-NGG-3' for *Streptococcus pyogenes* Cas9 (SpCas9) located immediately downstream of the target site (28). Upon PAM binding and local DNA unwinding, Cas9 introduces a site-specific double-strand break (DSB) approximately three base pairs upstream of the PAM motif.

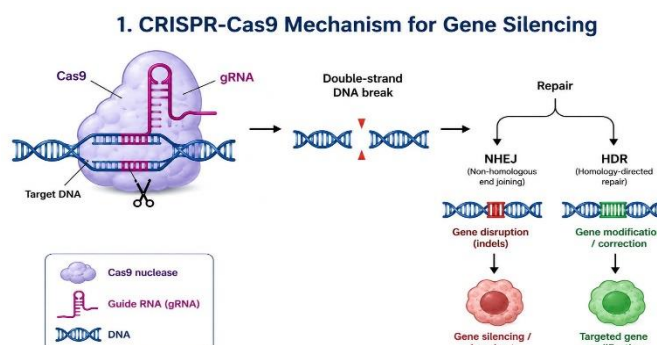


Figure 4: CRISPR-Cas9 Mechanism for Gene Silencing

Following DSB generation, the host cell activates one of two primary intrinsic DNA repair pathways:

- **Non-Homologous End Joining (NHEJ):** An error-prone, template-independent repair pathway active throughout the cell cycle. NHEJ frequently introduces frameshift insertions or deletions (indels) at the cleavage site, leading to premature stop codons and permanent knockouts of target oncogenes (27,29).
- **Homology-Directed Repair (HDR):** A high-fidelity, template-dependent pathway active primarily during the S/G2 phases of the cell cycle. When an exogenous donor DNA template is co-delivered, HDR enables precise nucleotide substitutions, knock-ins, or targeted gene corrections (28, 30).

3.2 Therapeutic Rationales for CRISPR in Solid Tumors

The application of CRISPR-Cas9 technology to selectively target disease-associated genes has transformed preclinical cancer management (29). In solid tumor oncology, CRISPR-mediated gene silencing is predominantly employed to knock out driving oncogenes, disrupt aberrant signal transduction cascades, restore tumor suppressor pathways, and overcome chemo- or radio-resistance (29, 30).

Compared to conventional post-transcriptional gene silencing modalities such as RNA interference (RNAi) using small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) CRISPR-Cas9 offers distinct therapeutic advantages:

Table 1: Comparative Evaluation of RNA Interference (RNAi) and CRISPR-Cas9 Gene Knockout

Feature / Parameter	RNA Interference (RNAi / siRNA)	CRISPR-Cas9 Knockout Platform
Target Molecule	Messenger RNA (mRNA) in cytoplasm	Genomic double-stranded DNA in nucleus
Mechanism of Action	Post-transcriptional mRNA degradation / translational repression	Targeted double-strand break (DSB) & genomic indel disruption
Duration of Effect	Transient (requires repeated administration)	Permanent and hereditary across cell divisions
Target Suppression Level	Incomplete gene knockdown (residual expression remains)	Complete genomic gene knockout (null phenotype)
Cell Cycle Dependence	Active across all cell cycle phases	NHEJ active all phases; HDR restricted to S/G2 phases
Off-Target Risk Profile	High (microRNA-like seed sequence off-target binding)	Moderate-Low (mitigated by optimized sgRNA design & high-fidelity Cas9)

Table 2: Key Characteristics of Cellular DNA Repair Pathways Following CRISPR-Cas Cleavage

Repair Pathway	Template Requirement	Cell Cycle Phase	Primary Outcome & Applications
Non-Homologous End Joining (NHEJ)	None (Template-Independent)	Active throughout all cell cycle phases (G1, S, G2, M)	Error-prone insertion/deletion (indel) formation; leads to frameshift mutations, premature stop codons, and permanent oncogene knockout.
Homology-Directed Repair (HDR)	Requires exogenous donor DNA template	Restricted to S and G2 phases (high sister chromatid presence)	High-fidelity sequence repair; allows precise single-nucleotide substitutions, targeted gene insertions, and correction of tumor suppressor mutations.

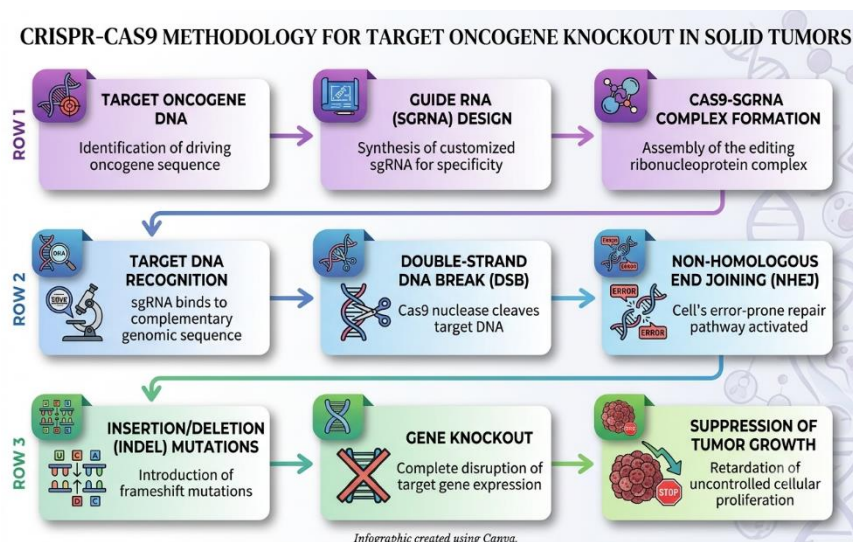


Figure 3: CRISPR Gene Knockout Process

3.2 Oncogene Silencing in Solid Tumors

Targeted oncogene disruption represents one of the most promising applications of CRISPR-mediated precision oncology. Driver oncogenes—frequently activated through gain-of-function mutations, gene amplifications, or chromosomal translocations—promote unscheduled cell proliferation, tissue invasion, metastatic progression, and therapeutic resistance (31). Preclinical studies have demonstrated that CRISPR-Cas9 can efficiently knock out key oncogenes, including KRAS, MYC, EGFR, PLK1, and BCL2, across diverse solid tumor types (31, 32). Genomic disruption of these targets systematically impairs tumor growth, restores intrinsic apoptotic pathways, and sensitizes cancer cells to conventional chemotherapeutics and targeted therapies (32)

Table 3: Representative Oncogenes Targeted by CRISPR-Cas9 in Solid Malignancies

Target Oncogene	Primary Tumor Types	Primary Biological Function	Observed Effect of CRISPR Knockout
KRAS	Pancreatic, Colorectal, NSCLC	GTPase signal transducer; drives cell proliferation and survival	Suppressed Ras-ERK signalling, induced cell cycle arrest, inhibited tumor growth in vivo
EGFR	Non-Small Cell Lung (NSCLC), Glioblastoma	Receptor tyrosine kinase; regulates cell growth and survival	Reduced downstream Akt/MAPK signalling, restored sensitivity to EGFR-TKIs
PLK1	Pancreatic, Ovarian, Triple-Negative Breast	Serine/threonine kinase; regulates mitotic entry and spindle assembly	Induced mitotic arrest (G2/M blockage), triggered massive apoptosis in cancer cells
BCL2	Ovarian, Prostate, Renal	Anti-apoptotic protein; blocks mitochondrial death pathways	Restored intrinsic apoptosis, sensitized chemoresistant cells to cisplatin/paclitaxel
MYC	Triple-Negative Breast, Hepatocellular	Master transcription factor; regulates cell metabolism, growth, and stemness	Impaired tumor cell metabolism, reduced self-renewal capacity of cancer stem cells

Specific deletion of oncogenic mutant KRAS (e.g., KRAS G12D or G12V variants) via CRISPR platforms has demonstrated robust anti-tumor efficacy in pancreatic adenocarcinoma, colorectal cancer, and non-small cell lung cancer (NSCLC) models where KRAS mutations drive tumorigenesis (33). Furthermore, delivering CRISPR components via functionalized nanoparticles to target PLK1 or anti-apoptotic genes such as surviving (BIRC5) has resulted in marked reductions in cancer cell proliferation and significant increases in tumor cell death in vivo (33,34). These findings underscore the potential of CRISPR-based oncogene knockout as a cornerstone of precision oncology, enabling the selective targeting of tumor-specific driver mutations.

3.3 Off-Target Effects and Engineering Strategies for Specificity

Despite the high target site selectivity of the CRISPR-Cas9 system, potential off-target cleavage remains a primary challenge for clinical translation (35). Off-target editing occurs when the Cas9 endonuclease binds to and cleaves non-targeted genomic loci that display sequence homology with the 20-nucleotide sgRNA sequence (35, 36). These unintended double-strand breaks can induce

point mutations, large genomic deletions, chromosomal translocations, unintended tumor suppressor disruption, or proto-oncogene activation—raising significant safety concerns regarding secondary malignancies and genotoxicity (36).

To mitigate off-target cleavage while enhancing editing fidelity, several protein engineering and rational sgRNA design strategies have been developed (37):

- **High-Fidelity Cas9 Variants:** Engineered Cas9 enzymes (e.g., SpCas9-HF1, eSpCas9, HypaCas9) incorporate structure-guided mutations that weaken non-specific protein-DNA interactions, requiring stringent sequence complementarity for cleavage (37).
- **Paired Cas9 Nickases (Cas9n):** Utilizing a mutated Cas9 variant that introduces single-stranded nicks rather than double-strand breaks. Requiring two adjacent sgRNAs to generate a functional DSB drastically reduces off-target mutagenesis (37, 38).
- **Base and Prime Editors:** Catalytically impaired Cas9 proteins fused to deaminase or reverse transcriptase domains enable precise single-nucleotide conversions without creating double-strand DNA breaks, avoiding error-prone NHEJ processing (38).
- **Bioinformatics and Machine Learning Algorithms:** Advanced computational tools (e.g., COSMID, CRISPR-P, Off-Finder) evaluate sgRNA binding kinetics and secondary structures to predict off-target loci in silico prior to therapeutic deployment (37, 38).
- **Continued advancements in Cas endonuclease engineering,** coupled with rigorous computational design, continue to improve the safety profile of CRISPR therapeutics, paving the way for safer clinical implementation (35–38).

3.4 Translational Challenges and the Rationale for Nanoparticle Delivery

Despite the transformative therapeutic potential of CRISPR-mediated gene silencing, several critical physiological, biological, and clinical hurdles must be overcome to enable successful translational implementation in solid oncology (39).

1. Physiological and Extracellular Barriers

Systemic administration of unformulated or "naked" CRISPR components—whether delivered as plasmid DNA, messenger RNA (mRNA), or ribonucleoprotein (RNP) complexes—is severely limited by poor bio-stability. Unprotected nucleic acids and proteins undergo rapid enzymatic cleavage by circulating serum nucleases, renal filtration, and rapid clearance by the mononuclear phagocyte system (MPS) (39). Furthermore, solid tumors exhibit a complex, dense extracellular matrix (ECM), elevated interstitial fluid pressure (IFP), and aberrant tumor vasculature, which together form a formidable physical barrier that restricts the deep tissue penetration and cellular uptake of therapeutic payloads (39).

2. Immunogenicity and Genotoxicity

Host immune recognition poses a major safety concern. Because common CAS endonucleases (e.g., SpCas9 and SaCas9) originate from human pathogens (*Streptococcus pyogenes* and *Staphylococcus aureus*), pre-existing humoral and cell-mediated adaptive immune responses against Cas proteins can trigger neutralizing antibody production, acute systemic inflammation, and rapid clearance of transduced cells. Additionally, potential long-term genotoxicity—stemming from persistent Cas9 expression, off-target double-strand breaks, chromosomal translocations, and p53-mediated DNA damage responses—raises substantial safety concerns regarding therapeutic durability and secondary oncogenesis.

3. Translational Solutions via Nanotechnology

Engineered nanoparticle delivery platforms offer a robust strategy to overcome these biological obstacles. Advanced nanocarriers enhance the therapeutic window of CRISPR systems through several mechanisms (39)

- **Enzymatic Protection:** Encapsulation within lipidic, polymeric, or inorganic matrices shields CRISPR cargoes from enzymatic degradation in the bloodstream.
- **Prolonged Circulation:** Surface modification (e.g., PEGylation) reduces opsonisation, extending circulation half-life and reducing systemic clearance.
- **Tumor-Targeted Accumulation:** Nanoparticles exploit the Enhanced Permeability and Retention (EPR) effect for passive targeting, or incorporate surface targeting ligands (e.g., antibodies, aptamers, and peptides) for receptor-mediated active internalization into tumor cells.

- Facilitated Endosomal Escape: Functionalized materials promote rapid endosomal destabilization, releasing active CRISPR components directly into the cytosol before lysosomal degradation occurs.
- Continued interdisciplinary convergence between nanotechnology, protein engineering, and precision oncology is expected to refine the clinical safety, editing efficiency, and targeted delivery of CRISPR therapeutics for refractory solid tumors 39

4. NANOPARTICLE PLATFORMS FOR CRISPR DELIVERY:

4.1 Lipid Nanoparticles (LNPs)

Lipid nanoparticles (LNPs) represent the most extensively investigated non-viral delivery system for CRISPR-mediated gene therapies. Structurally, LNPs self-assemble from four core functional components: ionisable cationic lipids, helper phospholipids, cholesterol, and polyethylene glycol (PEGylated) lipids. This lipid shell efficiently encapsulates negative CRISPR payloads—including Cas9 mRNA, single-guide RNA (sgRNA), or pre-assembled ribonucleoprotein (RNP) complexes—protecting them from extracellular enzymatic degradation.

The ionisable lipid component plays a dual physiological role: it maintains a neutral net charge at systemic blood pH (approx. 7.4), mitigating non-specific membrane toxicity, but becomes protonated in the acidic microenvironment of early endosomes (pH 5.5 - 6.5). Protonation triggers endosomal membrane destabilization, facilitating cytosolic release of the functional CRISPR cargo. Following the clinical success of LNP-based mRNA vaccines, LNPs have gained significant traction as scalable, bio-compatible vectors for target oncogene knockout in solid malignancies.

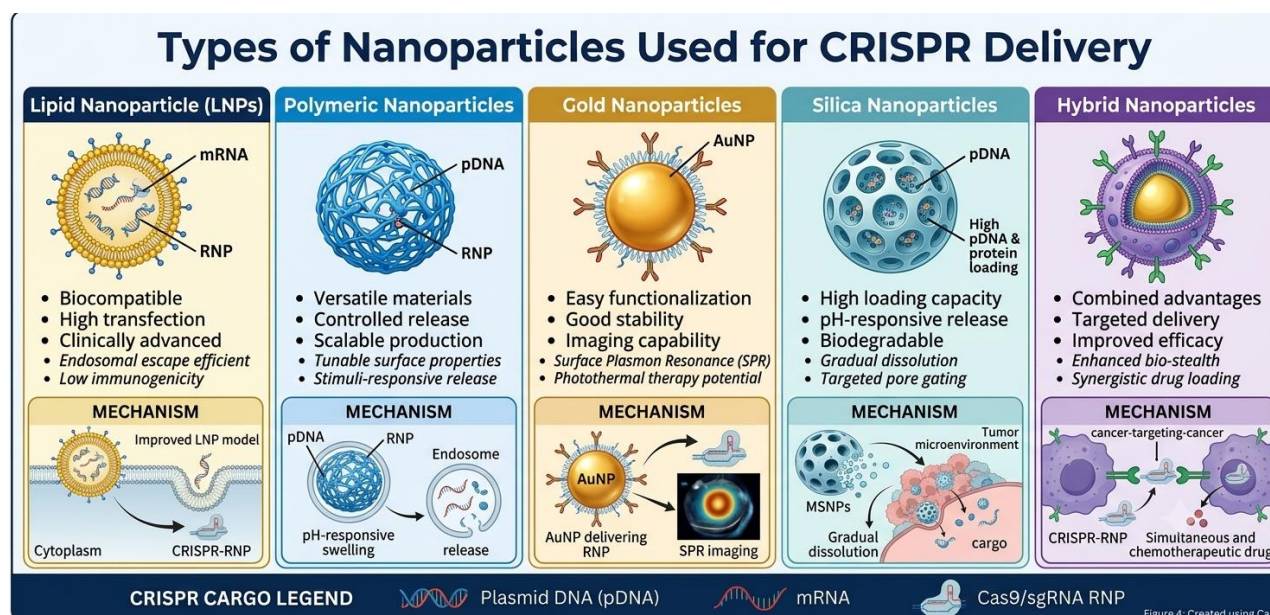
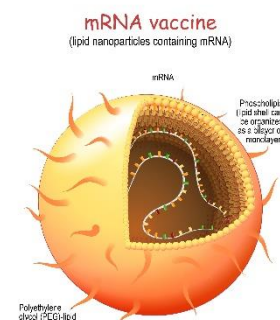


Figure 5 : Types of Nanoparticles

4.2 Polymeric Nanoparticles

Polymeric nanoparticles fabricated from natural or synthetic materials such as poly (lactic-co-glycolic acid) (PLGA), linear/branched polyethylene imine (PEI), chitosan, and poly (β -amino esters) (PBAEs) offer tunable structural frameworks for genome-editing components. These platforms provide high mechanical stability, controlled degradation kinetics, and adaptable surface chemistry, making them versatile vehicles for plasmid DNA (pDNA) and RNP delivery into tumor cell. Polymeric platforms can be functionalized with targeting moieties (e.g., cell-penetrating peptides, aptamers), imaging agents, or stimuli-responsive linkages (such as pH-, redox-, or matrix metalloproteinase-sensitive bonds) to restrict cargo release to the tumor microenvironment. However, cationic polymers like high-molecular-weight PEI can induce membrane destabilization and intrinsic cytotoxicity; thus, rational formulation optimization such as PEGylation, lipid grafting, or co-polymer blending is essential to balance transfection efficiency with systemic biocompatibility.

4.3 Gold Nanoparticles

Gold nanoparticles (AuNPs) possess distinctive physicochemical properties including high surface area, ease of functionalization, excellent biocompatibility, and optical responsiveness. These features make them attractive carriers for CRISPR-Cas systems. Surface

alteration with nucleic acids, peptides, or polymers enables efficient loading and delivery of genome-editing components into target cells. Gold nanoparticle-mediated CRISPR delivery has shown promising results in achieving precise gene editing with minimal toxicity. Furthermore, AuNPs can be combined with photo thermal treatment, providing synergistic anticancer impact by simultaneously inducing tumor cell death and gene silencing .

4.4 Silica Nanoparticles

Among the reasons for choosing mesoporous silica nanopaters (MSNs) as delivery vehicles is their widely accessible and highly stable nature, with wide surface area, variable pore size, high loading capacity, and stability. The nanoparticles serve as a protective layer for Cas9 proteins, guideRNAs, and plasmid DNA, preventing their damage during circulatory processes. Targeting ligands through surface functionalisation of MSNs increases tumor-specific collection and cellular uptake. A number of studies have demonstrated that CRISPR-based oncogene silencing, the use of silica-containing nanocarriers in models of solid tumour improvement, improves treatment effectiveness and reduces systemic toxicity.

6. Therapeutic Outcomes of Nanoparticle-Mediated CRISPR Delivery

The integration of engineered nanoparticle platforms with CRISPR-mediated gene editing has yielded substantial preclinical success across diverse solid tumor models. By overcoming systemic delivery barriers and enabling precise genomic disruption, CRISPR-loaded nanocarriers drive multi-faceted anti-tumor responses that significantly enhance therapeutic efficacy in precision oncology.

Key Therapeutic Mechanisms & Outcomes

1. Oncogene Knockout and Proliferation Arrest

Targeted silencing of driver oncogenes (e.g., KRAS, MYC, EGFR, and PLK1) via functionalized nanoparticles directly suppresses downstream proliferative signaling pathways (such as Ras-ERK and PI3K/Akt). This genomic disruption induces cell cycle arrest at the G1/S or G2/M checkpoints, leading to marked retardation of primary tumor growth in vivo (45).

2. Apoptosis Induction and Metastatic Suppression

CRISPR-mediated inactivation of anti-apoptotic factors—such as BCL2 or survivin (BIRC5)—restores intrinsic mitochondrial apoptotic cascades in malignant cells. Concurrently, knocking out key metastatic drivers (e.g., MMP-9, TWIST1, or SNAI1) impairs focal adhesion dynamics and epithelial-to-mesenchymal transition (EMT), thereby suppressing local tissue invasion and distant metastatic dissemination.

3. Reversal of Chemoresistance and Therapeutic Sensitization

Solid tumors frequently acquire resistance to conventional chemotherapeutics (e.g., cisplatin, paclitaxel, gemcitabine) through enhanced drug efflux, DNA repair upregulation, or anti-apoptotic buffering. Nanoparticle-mediated CRISPR delivery can selectively knockout resistance-associated genes (e.g., MDR1/ABCB1, MGMT), thereby re-sensitizing refractory tumor cells and producing synergistic anti-tumor effects when co-administered with standard-of-care chemotherapies.

4. Potentiation of Anti-Tumor Immunity

Disrupting immune checkpoint ligands (e.g., PD-L1/CD274) or immunosuppressive factors within the tumor microenvironment (TME) via CRISPR nanoparticles reverses T-cell exhaustion. This genomic reprogramming enhances cytotoxic T lymphocyte (CTL) infiltration, promotes antigen presentation, and converts immunologically "cold" solid tumors into "hot," immune-reactive phenotypes.

Table 7: Challenges and Limitations of CRISPR-Loaded Nanoparticles for Gene Silencing in Solid Tumors

Challenge	Description	Impact on Therapy
Delivery Barriers	Poor diffusion through tumor microenvironment	Reduced gene-editing efficiency
Off-Target Effects	Unplanned modification of non-target genes	Safety concerns and genomic instability
Immune Response	Recognition of Cas proteins or nanoparticles by the immune system	Inflammation and reduced efficacy
Nanoparticle Toxicity	Potential toxicity of carrier materials	Damage to healthy tissues
Low Editing Efficiency	Incomplete gene knockout or silencing	Reduced therapeutic benefit

Tumor Heterogeneity	Variability among tumor cells	Inconsistent treatment outcomes
Stability Issues	Degradation of CRISPR cargo during circulation	Reduced delivery efficiency
Manufacturing Challenges	Complexity in large-scale production	Improved production cost
Regulatory Challenges	Strict clinical approval requirements	Delayed clinical translation
Ethical Concerns	Risks associated with genome editing	Public and regulatory concerns (46) (47) (48)

7. Future Prospects and Emerging Horizons

The convergence of next-generation genome engineering and functionalized nanomedicine presents unprecedented opportunities for cancer therapeutics. While CRISPR-Cas9 has laid the groundwork for targeted gene disruption, overcoming translational bottlenecks requires continuous innovation across endonuclease engineering, smart drug delivery systems, precision oncology, and combination treatment strategies.

7.1 Next-Generation CRISPR Systems and Precision Editors

Beyond conventional SpCas9 platforms, novel Cas orthologs and engineered precision editors offer enhanced targeting specificity and reduced off-target risks: Cas12a (Cpf1) and Cas13: Unlike Cas9, Cas12a recognizes T-rich protospacer adjacent motifs (PAMs) and introduces staggered double-strand breaks, facilitating efficient homology-directed repair (HDR). Cas13 systems target single-stranded RNA (ssRNA) directly, enabling transient, non-genotoxic post-transcriptional gene silencing without permanently altering host genomic DNA.

Base and Prime Editors: Fusing catalytically impaired Cas endonucleases (dCas9 or Cas9 nickase) with nucleoside deaminases or engineered reverse transcriptases enables precise single-nucleotide conversions or small insertions/deletions without generating double-strand DNA breaks (DSBs). This dramatically reduces the risk of chromosomal translocations and large-scale genotoxicity in solid tumors.

7.2 Stimuli-Responsive and Biomimetic Nanocarriers

Future translational success relies heavily on "smart" nanomedicines capable of traversing complex physiological barriers within solid tumors:

Microenvironment-Responsive Delivery: Next-generation nanoparticles are designed to undergo structural or chemical transformations in response to tumor-specific triggers—such as low intratumoral pH, hypoxia, elevated matrix metalloproteinases (MMPs), or high glutathione (GSH) concentrations—ensuring targeted, site-specific payload release. Biomimetic and Cell-Membrane Coating: Encapsulating synthetic nanoparticle cores within cell-derived membranes (e.g., cancer cell membranes, macrophage membranes, or extracellular vesicles) imparts natural bio-stealth characteristics. This strategy enables long systemic circulation times, immune evasion, and enhanced homologous tumor-homing capacity.

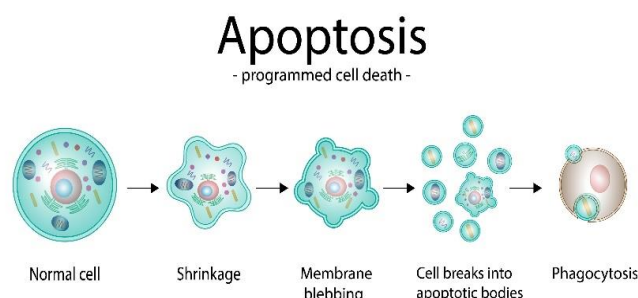
7.3 Integration with Precision Oncology and Combination Therapies

As high-throughput multi-omics profiling advances, CRISPR-based nanomedicines will become central to personalized cancer care: Patient-Specific Target Discovery: Genomic sequencing of patient-derived organoids or tumor biopsies will allow researchers to design custom multi-plexed sgRNA formulations tailored to an individual's unique driver mutation profile. Synergistic Combination Regimens: CRISPR-loaded nanoparticles are increasingly co-formulated or co-administered with existing clinical modalities: Immuno-Oncology: Combining CRISPR checkpoint gene disruption (PD-L1, TIGIT) with immune checkpoint inhibitors or CAR-T cell therapies to reverse immunosuppression in "cold" solid tumors. Chemotherapy and Radiotherapy: Co-delivering CRISPR components to silence resistance-inducing DNA repair enzymes (e.g., MGMT, PARP1) alongside standard chemotherapeutics to restore drug sensitivity and lower required toxic dosages. Despite remaining regulatory, manufacturing, and safety hurdles, ongoing preclinical validation and expanding clinical trials are expected to accelerate the transition of CRISPR-based nanomedicines from experimental platforms to mainstream clinical oncology

8. Conclusion

The integration of CRISPR-Cas genome engineering with functionalized nanoparticle delivery platforms represents a transformative frontier in non-viral precision oncology. By combining the target selectivity of CRISPR-mediated gene silencing with the protective, organ-homing, and microenvironment-responsive capabilities of nanotechnology, these platforms provide a robust framework to selectively disrupt driver oncogenes, halt tumor cell proliferation, trigger intrinsic apoptosis, and overcome therapeutic resistance in solid malignancies. Preclinical evaluations across diverse solid tumor models underscore the therapeutic versatility of non-viral carriers—most notably lipid, polymeric, gold, silica, and hybrid nanostructures. Despite these remarkable advancements, significant translational hurdles persist prior to widespread clinical implementation. Issues regarding systemic delivery efficiency, deep tissue

penetration in dense tumor stroma, potential off-target cleavage, immunogenicity, long-term genotoxicity, and scalable Good Manufacturing Practice (GMP) production remain critical barriers. Nevertheless, rapid advancements in high-fidelity Cas endonucleases, precision base/prime editing, computational sgRNA design, and biomimetic nanocarrier engineering are actively overcoming these limitations. With continued interdisciplinary research, rigorous safety profiling, and clinical validation, nanoparticle-mediated CRISPR therapeutics are poised to become a cornerstone of personalized cancer management, offering novel, highly targeted treatment paradigms for patients with refractory solid tumors.



References

1. Bramer, W. M., de Jonge, G. B., Rethlefsen, M. L., Mast, F., & Kleijnen, J. (2018). A systematic approach to searching: An efficient and complete method to develop literature searches. *Journal of the Medical Library Association*, 106(4), 531–541. <https://doi.org/10.5195/jmla.2018.283>
2. Bramer, W. M., Rethlefsen, M. L., Kleijnen, J., & Franco, O. H. (2017). Optimal database combinations for literature searches in systematic reviews: A prospective exploratory study. *Systematic Reviews*, 6(1), 245. <https://doi.org/10.1186/s13643-017-0644-y>
3. Booth, A., Sutton, A., & Papaioannou, D. (2016). *Systematic approaches to a successful literature review* (2nd ed.). SAGE Publications.
4. Büchter, R. B., Weise, A., & Pieper, D. (2020). Development, testing and use of data extraction forms in systematic reviews: A scoping review. *BMC Medical Research Methodology*, 20(1), 259. <https://doi.org/10.1186/s12874-020-01143-3>
5. Chen, X., Gonçalves, M. A. F. V., & colleagues. (2022). Engineered CRISPR systems for cancer therapy. *Nature Reviews Cancer*, 22(11), 643–660. <https://doi.org/10.1038/s41568-022-00511-x>
6. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346(6213), Article 1258096. <https://doi.org/10.1126/science.1258096>
7. Falagas, M. E., Pitsouni, E. I., Malietzis, G. A., & Pappas, G. (2008). Comparison of PubMed, Scopus, Web of Science, and Google Scholar: Strengths and weaknesses. *The FASEB Journal*, 22(2), 338–342. <https://doi.org/10.1096/fj.07-9492LSF>
8. Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*, 391(6669), 806–811. <https://doi.org/10.1038/35888>
9. Fu, Y., Foden, J. A., Khayter, C., Maeder, M. L., Reyon, D., Joung, J. K., & Sander, J. D. (2013). High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nature Biotechnology*, 31(9), 822–826. <https://doi.org/10.1038/nbt.2623>
10. Hanahan, D. (2022). Hallmarks of cancer: New dimensions. *Cancer Discovery*, 12(1), 31–46. <https://doi.org/10.1158/2159-8290.CD-21-1059>
11. He, Q., Gao, Y., Zhang, L., Zhang, Z., Gao, F., Ji, X., & Li, Y. (2019). Mesoporous silica nanoparticles for cancer-targeted delivery of CRISPR/Cas9 systems. *Acta Biomaterialia*, 93, 1–15. <https://doi.org/10.1016/j.actbio.2019.04.018>
12. Higgins, J. P. T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M. J., & Welch, V. A. (Eds.). (2023). *Cochrane handbook for systematic reviews of interventions* (Version 6.4). Cochrane.
13. Hooijmans, C. R., Rovers, M. M., de Vries, R. B. M., Leenaars, M., Ritskes-Hoitinga, M., & Langendam, M. W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Medical Research Methodology*, 14(1), Article 43. <https://doi.org/10.1186/1471-2288-14-43>
14. Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, 157(6), 1262–1278. <https://doi.org/10.1016/j.cell.2014.05.010>
15. 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(6096), 816–821. <https://doi.org/10.1126/science.1225829>
16. Kleinstiver, B. P., Pattanayak, V., Prew, M. S., Tsai, S. Q., Nguyen, N. T., Zheng, Z., & Joung, J. K. (2016). High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*, 529(7587), 490–495. <https://doi.org/10.1038/nature16526>
17. Lee, K., Conboy, M., Park, H. M., Jiang, F., Kim, H. J., Dewitt, M. A., Mackley, V. A., Chang, K., Rao, A., Skinner, C., Irwin, D. A., Liu, B., Huang, W. C., Yu, F., Murthy, N., & Conboy, I. M. (2017). Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair. *Nature Biomedical Engineering*, 1(11), 889–901. <https://doi.org/10.1038/s41551-017-0137-2>

18. Li, L., Hu, S., & Chen, X. (2020). Non-viral delivery systems for CRISPR/Cas9-based genome editing. *Frontiers in Bioengineering and Biotechnology*, 8, Article 583223. <https://doi.org/10.3389/fbioe.2020.583223>
19. Li, T., Vedula, S. S., Hadar, N., Parkin, C., Lau, J., Dickersin, K., & AHRQ Methods Working Group. (2015). Innovations in data extraction, management, and archiving for systematic reviews. *Annals of Internal Medicine*, 162(4), 287–294. <https://doi.org/10.7326/M14-1603>
20. Lino, C. A., Harper, J. C., Carney, J. P., & Timlin, J. A. (2018). Delivering CRISPR: A review of the challenges and approaches. *Drug Delivery*, 25(1), 1234–1257. <https://doi.org/10.1080/10717544.2018.1474964>
21. Liu, C., Zhang, L., Liu, H., & Cheng, K. (2017). Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. *Journal of Controlled Release*, 266, 17–26. <https://doi.org/10.1016/j.jconrel.2017.09.012>
22. Martín-Martín, A., Thelwall, M., Orduna-Malea, E., & Delgado López-Cózar, E. (2021). Google Scholar, Microsoft Academic, Scopus, Dimensions, Web of Science, and OpenCitations' COCI: A multidisciplinary comparison. *Scientometrics*, 126(1), 871–906. <https://doi.org/10.1007/s11192-020-03690-4>
23. Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Medicine*, 6(7), Article e1000097. <https://doi.org/10.1371/journal.pmed.1000097>
24. Mout, R., Ray, M., Lee, Y. W., Scaletti, F., & Rotello, V. M. (2017). In vivo delivery of CRISPR/Cas9 for therapeutic gene editing: Progress and challenges. *Bioconjugate Chemistry*, 28(4), 880–884. <https://doi.org/10.1021/acs.bioconjchem.7b00057>
25. Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., ... Moher, D. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*, 372, Article n71. <https://doi.org/10.1136/bmj.n71>
26. Page, M. J., Moher, D., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., ... McKenzie, J. E. (2021). PRISMA 2020 explanation and elaboration: Updated guidance and exemplars for reporting systematic reviews. *BMJ*, 372, Article n160. <https://doi.org/10.1136/bmj.n160>
27. Pickar-Oliver, A., & Gersbach, C. A. (2019). The next generation of CRISPR-Cas technologies and applications. *Nature Reviews Molecular Cell Biology*, 20(8), 490–507. <https://doi.org/10.1038/s41580-019-0131-5>
28. Qiu, M., Glass, Z., Chen, J., Haas, M., Jin, X., Zhao, X., Yang, X., & Xu, Q. (2022). Lipid nanoparticle-mediated codelivery of CRISPR/Cas9 for cancer therapy. *Nature Nanotechnology*, 17(5), 522–532. <https://doi.org/10.1038/s41565-022-01106-x>
29. Rosenblum, D., Gutkin, A., Dammes, N., & Peer, D. (2020). Progress and challenges towards CRISPR/Cas clinical translation. *Advanced Drug Delivery Reviews*, 154–155, 176–186. <https://doi.org/10.1016/j.addr.2020.07.004>
30. Setten, R. L., Rossi, J. J., & Han, S. P. (2019). The current state and future directions of RNAi-based therapeutics. *Nature Reviews Drug Discovery*, 18(6), 421–446. <https://doi.org/10.1038/s41573-019-0017-4>
31. Sterne, J. A. C., Savović, J., Page, M. J., Elbers, R. G., Blencowe, N. S., Boutron, I., Cates, C. J., Cheng, H. Y., Corbett, M. S., Eldridge, S. M., Emberson, J. R., Hernán, M. A., Hopewell, S., Hróbjartsson, A., Junqueira, D. R., Jüni, P., Kirkham, J. J., Lasserson, T., Li, T., ... Higgins, J. P. T. (2019). RoB 2: A revised tool for assessing risk of bias in randomized trials. *BMJ*, 366, Article l4898. <https://doi.org/10.1136/bmj.l4898>
32. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2024). Global cancer statistics 2024: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 74(3), 229–263. <https://doi.org/10.3322/caac.21834>
33. Tsai, S. Q., & Joung, J. K. (2016). Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. *Nature Reviews Genetics*, 17(5), 300–312. <https://doi.org/10.1038/nrg.2016.28>
34. Vakulskas, C. A., Dever, D. P., Rettig, G. R., Turk, R., Jacobi, A. M., Collingwood, M. A., Bode, N. M., McNeill, M. S., Yan, S., Camarena, J., Lee, C. M., Park, S. H., Behlke, M. A., Porteus, M. H., & Bao, G. (2018). A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enhances genome-editing specificity. *Nature Medicine*, 24(8), 1216–1224. <https://doi.org/10.1038/s41591-018-0137-0>
35. Wang, P., Zhang, L., Xie, Y., Wang, N., Tang, R., Zheng, W., & Jiang, X. (2021). Genome editing for cancer therapy: Delivery of CRISPR/Cas9 by nanoparticles. *Advanced Drug Delivery Reviews*, 168, 1–20. <https://doi.org/10.1016/j.addr.2020.05.003>
36. World Health Organization. (2025). *Cancer*. World Health Organization. <https://www.who.int/news-room/fact-sheets/detail/cancer>
37. Xu, X., Wan, T., Xin, H., Li, D., Pan, H., Wu, J., & Ping, Y. (2023). CRISPR/Cas9-based therapeutic genome editing for cancer treatment: Advances and challenges. *Advanced Drug Delivery Reviews*, 194, Article 114728. <https://doi.org/10.1016/j.addr.2023.114728>

38. Yin, H., Kauffman, K. J., & Anderson, D. G. (2017). Delivery technologies for genome editing. *Nature Reviews Drug Discovery*, 16(6), 387–399. <https://doi.org/10.1038/nrd.2016.280>
39. Zuris, J. A., Thompson, D. B., Shu, Y., Guilinger, J. P., Bessen, J. L., Hu, J. H., Maeder, M. L., Joung, J. K., Chen, Z. Y., & Liu, D. R. (2015). Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. *Nature Biotechnology*, 33(1), 73–80. <https://doi.org/10.1038/nbt.3081>

Copyright & License:

© Authors retain the copyright of this article. This work is published under the Creative Commons Attribution 4.0 International License (CC BY 4.0), permitting unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.