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Review

Nanotechnology Enhanced RNAi Therapies in Cancer: A Systematic Review

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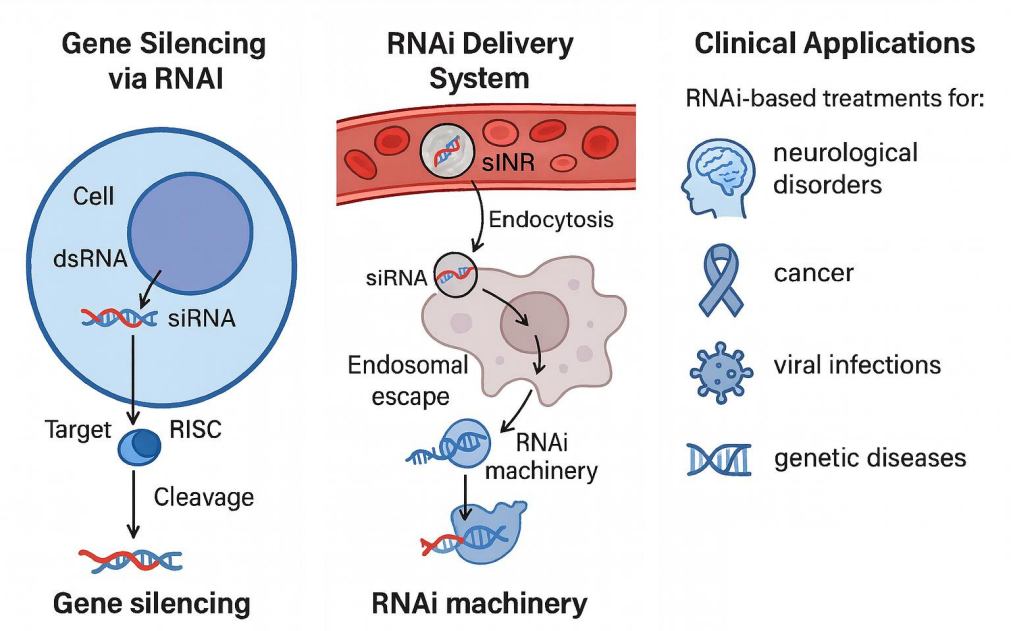
Abstract

Cancer remains a major cause of illness and death worldwide, with conventional treatments often hindered by systemic toxicity, lack of specificity, and the development of resistance. RNA interference (RNAi) provides a highly targeted approach to silencing cancer-causing genes and disrupted signalling pathways, laying the groundwork for precision cancer treatment. Although it holds great promise, clinical use of RNAi is currently limited by poor stability, rapid breakdown in the blood, limited cell uptake, entrapment in endosomes, and barriers within the tumour microenvironment. Nanotechnology-based delivery systems have become a key strategy for overcoming these challenges, enhancing RNAi stability, promoting targeted tumour accumulation, facilitating efficient cell internalisation, and enabling controlled release within cells. This review provides a comprehensive assessment of the biological basis of RNAi, the obstacles to its effective use, and the various types of nanocarriers developed to improve therapeutic outcomes. Combination approaches combining RNAi with chemotherapy, immunotherapy, and gene editing are also explored, alongside advances in passive and active tumour targeting. A summary of preclinical and clinical data proving the pharmacokinetic benefits, safety, and effectiveness of nanocarrier-assisted RNA interference is provided. Finally, new developments, including patient-specific RNAi treatments, biodegradable and intelligent nanomaterials, and integration with biomarker-driven tactics, are emphasised. This review highlights the revolutionary potential of RNA interference (RNAi) enhanced by nanotechnology for safe, accurate, and efficient cancer treatment, while also pointing to directions for further translational research.

Graphical Abstract



1. Introduction

Cancer continues to represent a major global health burden, exerting profound impacts on morbidity, mortality, and healthcare expenditure. Recent epidemiological estimates indicate that in 2022 there were nearly 20 million newly diagnosed cases worldwide, accompanied by approximately 9.7 million cancer-related deaths [1]. Projections indicate a substantial escalation in the global cancer burden, with annual incidence expected to approach 35 million cases by 2050. This upward trend is primarily linked to demographic transitions, notably population aging and growth, in addition to modifiable lifestyle and environmental determinants such as tobacco use, obesity, and exposure to pollutants [2].

Conventional modalities for cancer management, such as surgery, radiotherapy, and chemotherapy, have significantly improved patient outcomes but remain constrained by notable shortcomings. Chemotherapeutic regimens, in particular, are associated with systemic toxicity due to their inability to distinguish malignant from healthy tissues. Furthermore, the emergence of acquired resistance mechanisms frequently undermines therapeutic efficacy, predisposing patients to treatment failure and disease recurrence. A further limitation lies in the lack of selectivity, as these interventions often inflict collateral damage on normal cells, thereby restricting their overall clinical success [3,4].

RNA interference (RNAi) is an evolutionarily conserved mechanism of post-transcriptional gene silencing, orchestrated by short double-stranded RNA molecules such as small interfering RNAs (siRNAs) and microRNAs (miRNAs). Since its discovery, RNAi has gained prominence as a therapeutic approach for diseases characterized by dysregulated gene expression, with cancer representing one of the most intensively explored applications [5]. In oncology, RNAi can selectively suppress oncogenes, interfere with dysregulated signaling cascades, and reshape the tumor microenvironment. Unlike conventional chemotherapy, which often lacks specificity and damages both malignant and healthy tissues, RNAi operates through precise sequence complementarity, allowing targeted silencing of tumor-promoting genes. This specificity makes it a strong candidate for the development of patient-tailored treatment strategies [6]. Several RNAi-based agents have advanced from experimental work into clinical testing. For example, siRNA constructs targeting VEGF and KRAS mutations have shown efficacy in reducing angiogenesis and tumor proliferation in preclinical models. Importantly, the FDA approval of patisiran (Onpattro®) for hereditary transthyretin amyloidosis provided clinical proof-of-concept for RNAi therapeutics in humans, establishing a foundation for translation into oncology [7]. Despite substantial advances, the clinical translation of RNAi remains hindered by several obstacles, including rapid degradation in circulation, inefficient cellular internalization, off-target gene silencing, and sequestration within endosomal compartments. Addressing these challenges necessitates the development of effective delivery platforms, among which nanotechnology-based carriers are particularly promising. Such systems safeguard RNA molecules from enzymatic degradation, facilitate deeper tumor penetration, and optimize pharmacokinetic behavior [6,7].

The clinical utility of RNA interference is constrained by the inherent instability and suboptimal pharmacokinetic characteristics of unmodified RNA molecules. Small interfering RNAs (siRNAs) and microRNA mimics are particularly vulnerable to rapid nuclease-mediated degradation, resulting in markedly reduced circulation half-lives. Moreover, their polyanionic nature and pronounced hydrophilicity impede passive membrane translocation, thereby limiting efficient intracellular delivery [6]. Nanotechnology offers tailored delivery platforms that can overcome these limitations. Lipid nanoparticles (LNPs), polymeric systems, and inorganic nanomaterials encapsulate RNAi molecules, protecting them from enzymatic degradation and prolonging systemic circulation [8]. By shielding RNA molecules from premature degradation, nanocarriers prolong their stability and preserve biological activity. A further advantage lies in their targeting capacity: nanocarriers can accumulate within tumor tissues through the enhanced permeability and retention (EPR) effect and can be engineered for active targeting using ligands such as antibodies, aptamers, or peptides. These strategies enhance delivery precision and limit off-target interactions. Consequently, targeted nanocarrier systems not only improve therapeutic efficacy but also mitigate systemic toxicity relative to conventional treatment modalities [9]. Equally critical, nanocarriers enhance cellular internalization and facilitate endosomal escape, thereby ensuring that siRNAs and miRNAs are delivered to the cytoplasmic RNA-induced silencing complex (RISC), the site of gene silencing. The emergence of stimuli-responsive delivery systems-triggered by pH variations, redox gradients, or tumor-associated enzymes-further augments release efficiency and confers greater tumor selectivity [10].

This review examines the transformative role of nanotechnology in enhancing the therapeutic potential of RNA interference (RNAi) for cancer treatment. RNAi offers a highly specific means of silencing oncogenic pathways; however, its clinical translation has been impeded by rapid degradation in circulation, immune activation, limited tumor penetration, and inefficient intracellular delivery. Advances in nanocarrier design have addressed many of these barriers by improving molecular stability, enabling tumor-targeted delivery, facilitating cellular uptake, and promoting cytoplasmic release. Within this context, the review outlines the biological underpinnings of RNAi and its relevance in oncology, discusses the principal obstacles to its direct application, and evaluates diverse nanocarrier platforms-including lipid-based systems, polymers, inorganic nanoparticles, and hybrid constructs-developed to overcome these limitations. It also synthesizes recent progress from preclinical investigations and clinical trials, while addressing safety, regulatory, and scalability considerations. Ultimately, the aim is to provide a critical assessment of the current landscape of nanotechnology-

enabled RNAi therapeutics, identify persisting knowledge gaps, and highlight future directions toward more effective, safe, and personalized cancer interventions.

2. Methodology

This study was conducted over a three-month period, from June 2 to September 5, 2025, following ethical approval from the Ethics Committee for Research at the University of Biological & Applied Sciences (UBAS), Lahore, Pakistan (Reference No. ERB-PHRMD-DP/2093). The review process was performed in accordance with the PRISMA guidelines, with primary literature retrieved from PubMed and the Cochrane Library. Search strategies incorporated a combination of relevant keywords, including nanotechnology, nanomedicine, nanoparticles, drug delivery systems, gene therapy, RNA interference (RNAi), siRNA, miRNA, shRNA, cancer therapy, oncogene silencing, targeted therapy, tumor targeting, precision medicine, and nucleic acid therapeutics, covering studies published between 2010 and 2025. To ensure comprehensiveness, supplementary searches were also conducted using additional databases, such as Google Scholar. Only peer-reviewed articles published in English within the defined time frame were considered eligible for inclusion. The PRISMA flow diagram summarises the systematic screening and selection process (Figure 1).

2.1 Exclusion Criteria

- Other related non-cancer studies, non-therapeutic/basic biology only, non-RNAi-based nanomedicine, inappropriate article types, and animal-free / in silico-only studies, etc.
- Research articles published in languages other than English
- Studies published before 2010

2.2 Data Extraction

The extracted data included author details, the year of the study, study type, nanotechnology details, RNAi characteristics, cancer context, therapeutic outcomes, Safety and pharmacokinetics, Key Findings and limitations, and Future Perspectives.

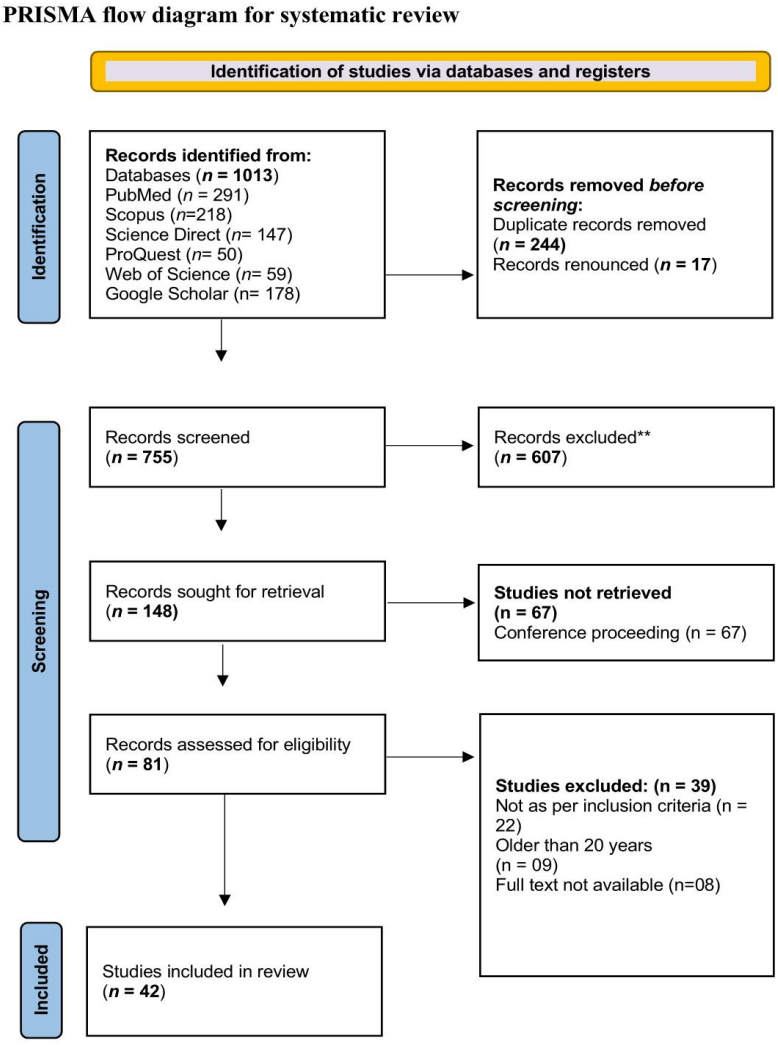


Figure 1. PRISMA flow diagram for systematic review.

The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. For more information, visit: http://www.prisma-statement.org/

3. Basics of RNAi in Cancer Therapy

3.1 Mechanisms of RNAi: siRNA, shRNA, miRNA Mimics/Inhibitors

RNA interference (RNAi) regulates gene expression by preventing the translation of specific messenger RNAs (mRNAs) through small RNA molecules. Three major approaches are commonly applied in cancer therapeutics: small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), and microRNA (miRNA) mimics or inhibitors. siRNAs are short double-stranded RNA fragments that, once delivered into the cytoplasm, are incorporated into the RNA-induced silencing complex (RISC). Guided by one strand of the siRNA, the complex identifies complementary mRNA and promotes its cleavage, effectively blocking protein production. This makes siRNAs highly suitable for silencing oncogenes or drug-resistance genes. shRNAs are expressed inside cells from plasmid or viral vectors. They form hairpin-shaped transcripts that are processed by Dicer into siRNA-like molecules, which then enter RISC to suppress target mRNAs. Unlike siRNAs, which act transiently, shRNAs provide prolonged gene silencing, although vector-based expression raises safety considerations such as immune stimulation or insertional effects. miRNA mimics and inhibitors build upon the natural role of endogenous miRNAs in fine-tuning gene expression. In cancer, many tumor-suppressive miRNAs are downregulated, while oncogenic miRNAs are upregulated. Synthetic miRNA mimics can restore the activity of suppressed miRNAs, whereas miRNA inhibitors (antagomirs or antisense oligonucleotides) block the function of overexpressed oncogenic miRNAs. These strategies aim to correct the imbalanced regulatory networks that drive cancer initiation, progression, and therapy resistance [11].

3.2 Key Cancer-Related Targets (Oncogenes, Signaling Pathways Like PI3K/AKT, KRAS, MYC)

Cancer development often hinges on the dysregulation of signaling pathways and oncogenes that drive malignant behaviors like uncontrolled proliferation, survival, and metastasis. PI3K/AKT is a critical survival pathway frequently activated in tumors, promoting growth, evading apoptosis, and contributing to resistance against chemotherapy and targeted treatments. RNAi approaches targeting components of this axis offer potential to restore treatment sensitivity and curb tumor growth. KRAS mutations are among the most common oncogenic drivers in cancers such as pancreatic, colorectal, and lung malignancies. Because KRAS has long been challenging to target with conventional drugs, RNAi offers a promising alternative. For example, nanocarriers delivering siRNA against KRAS G12D mutations have shown remarkable efficacy-achieving up to 90% suppression of KRAS expression and dramatic tumor inhibition, including complete regression in a subset of mice [12]. MYC, a key transcription factor, controls genes involved in cell growth, metabolism, and survival. Overexpression of MYC is associated with poor prognosis across various cancer types. Recent innovations in RNAi technology include chimeric RNAi molecules that simultaneously silence both KRAS and MYC, achieving synergistic tumor suppression through dual targeting [13].

3.3 Clinical Promise and Challenges of Naked RNAi Therapeutics

Naked RNAi molecules, such as siRNAs and miRNA agents, administered without a delivery vehicle, offer the significant advantage of precise gene silencing, allowing targeted knockdown of oncogenes or resistance-related transcripts. Their specificity also reduces off-target interactions relative to less targeted modalities, an appealing feature in oncology [14]. However, the direct use of naked RNAi in clinical oncology is undermined by several critical limitations. These molecules are highly susceptible to nuclease degradation, resulting in extremely short circulatory half-lives of just a few minutes (e.g., 5-10 minutes) [15]. Their negative charge and hydrophilic nature greatly restrict cell membrane penetration, leading to poor intracellular access [16]. A rapid renal and reticuloendothelial system (RES) clearance diminishes systemic exposure and therapeutic potential [15]. Naked RNAi therapies frequently trigger off-target gene modulation and immune stimulation, complicating safety profiles in translational settings [17]. Even after cellular uptake, RNAi molecules often become trapped in endosomes, failing to reach the cytosol where silencing mechanisms operate [18]. While chemical modifications (e.g., 2’-O-methyl groups, phosphorothioate backbones, GalNAc conjugates) have somewhat improved RNA stability and immunogenicity, they do not fully overcome delivery hurdles especially for tissues beyond the liver. As such, despite early clinical successes in other indications (e.g., liver-targeted siRNA drugs), systemic administration of naked RNAi agents remains largely impractical in oncology without delivery enhancement [16].

4. Barriers to Effective RNAi Delivery

4.1 Enzymatic Degradation in Circulation

Therapeutic nucleic acids and protein-based agents undergo rapid enzymatic degradation once in the bloodstream, markedly reducing their circulation half-life. Serum nucleases (e.g., RNase A, DNase I) cleave unmodified oligonucleotides within minutes - for example, naked siRNAs often have plasma half-lives of 5-10 minutes due to nuclease action and rapid renal clearance [19]. Chemical modifications such as 2′-O-methyl, 2′-fluoro, phosphorothioate backbones, or locked nucleic acids, as well as encapsulation in lipid- or polymer-based nanoparticles, can extend stability to several hours or more [20]. In vitro serum-stability assays and in vivo pharmacokinetic profiling consistently demonstrate that protective formulations slow enzymatic attack and improve systemic exposure [21]. Key enzymes in circulation and their impact on therapeutic stability are listed in Table 1.

4.2 Poor Cellular Uptake

A major barrier to effective RNAi therapeutics is their inherently poor cellular uptake. Naked siRNAs and miRNAs are hydrophilic, negatively charged, and relatively large (~13-15 kDa), which prevents efficient passage across the lipophilic plasma membrane [22,23]. As a result, only a negligible fraction of administered oligonucleotides enters the cytoplasm where the RNA-induced silencing complex (RISC) operates, with most molecules trapped in endosomes or degraded in the extracellular space [24]. To overcome this limitation, various strategies have been employed, including lipid nanoparticles, polymeric carriers, cell-penetrating peptides, and ligand conjugation (e.g., N-acetylgalactosamine for hepatocyte targeting). These systems enhance endocytosis, promote endosomal escape, and significantly improve cytosolic bioavailability of RNAi agents [25]. A summary of the key mechanisms and their impact on RNAi therapeutics is presented in Table 1.

4.3 Endosomal Entrapment

Even when RNAi therapeutics are successfully internalized, most remain confined within endosomal compartments and are subsequently trafficked to lysosomes for degradation. This sequestration drastically reduces the fraction of siRNA or miRNA that reaches the cytoplasm to engage the RNA-induced silencing complex (RISC). Endosomal escape is therefore considered one of the most critical bottlenecks for RNAi efficacy, with estimates suggesting that typically <2% of internalized oligonucleotides escape into the cytosol [26]. To overcome this barrier, delivery systems have been engineered with fusogenic lipids, pH-responsive polymers, endosomolytic peptides, and ionizable lipids that destabilize the endosomal membrane upon acidification. Such strategies improve cytosolic release, thereby enhancing target gene knockdown efficiency [25]. A summary of the key mechanisms and their impact on RNAi therapeutics is presented in Table 1.

4.4 Off-Target Effects and Immune Activation

Off-target effects and immune activation are major challenges in RNAi therapeutics. siRNAs and miRNAs may silence unintended genes through partial sequence complementarity, while double-stranded RNAs can trigger innate immune sensors such as Toll-like receptors and RIG-I, leading to cytokine release and inflammation. To address these limitations, chemical modifications (e.g., 2′-O-methyl, 2′-fluoro) and optimized delivery carriers have been employed to minimize unintended interactions and reduce immunogenicity [27]. A summary of the key mechanisms and their impact on RNAi therapeutics is presented in Table 1.

4.5 Tumor Microenvironment Barriers

Table 1. Major barriers to RNAi delivery.

Barrier	Mechanism	Examples	Impact on RNAi Therapeutics
Enzymatic degradation in circulation	Serum enzymes rapidly cleave unmodified oligonucleotides, reducing stability.	RNase A, DNase I, esterases, proteases	Naked siRNAs have plasma half-lives of only 5-10 min; leads to rapid clearance and negligible systemic exposure. Requires protective chemical modifications or encapsulation.
Poor cellular uptake	RNAi molecules are negatively charged, hydrophilic, and relatively large, limiting passive membrane diffusion.	siRNA (~13-15 kDa); miRNA mimics	<5% of administered RNAi reaches the cytoplasm; poor uptake prevents engagement with the RNA-induced silencing complex (RISC).
Endosomal entrapment	Internalized RNAi is sequestered in endosomes and trafficked to lysosomes, preventing cytosolic release.	Lysosomal hydrolases; acidic pH trapping	<2% of siRNAs escape into cytoplasm; insufficient release reduces gene silencing efficiency.
Off-target effects & immune activation	Partial complementarity causes unintended silencing; dsRNA activates innate immune sensors.	Toll-like receptors (TLR3, TLR7, TLR8), RIG-I, MDA5; cytokine pathways (IL-6, TNF-α, IFN-α/β)	Off-target gene modulation, cytokine storms, inflammation, and toxicity reduce therapeutic safety and precision.
Tumor microenvironment (TME) barriers	Abnormal vasculature, dense ECM, high IFP, and hostile conditions limit nanoparticle penetration.	Collagen-rich ECM, fibroblasts, hypoxia, acidic pH, VEGF-mediated angiogenesis	Uneven intratumoral distribution, poor penetration, resistance to therapy; reduces uniformity of response and overall efficacy.
Rapid renal clearance and RES uptake	Small RNAs and nanoparticles below renal threshold are cleared rapidly; larger carriers are sequestered by mononuclear phagocyte system.	Renal filtration (<40 kDa), Kupffer cells, spleen macrophages	Short half-life, liver/spleen accumulation, reduced tumor bioavailability.

The tumor microenvironment (TME) presents substantial barriers to the effective delivery of RNAi therapeutics. Abnormal vasculature and high interstitial fluid pressure limit nanoparticle penetration, while dense extracellular matrix components impede diffusion. In addition, hypoxia, acidic pH, and immunosuppressive stromal cells create a hostile milieu that promotes drug resistance and restricts therapeutic efficacy. Strategies such as matrix-degrading enzymes, pH-responsive carriers, and immune-modulating nanoparticles have been developed

to overcome these barriers and improve RNAi delivery. Tumor heterogeneity-arising from differences in blood vessel architecture, stromal composition, and underlying genetic or epigenetic alterations creates major barriers to uniform RNAi delivery. Such diversity often results in uneven nanoparticle distribution and inconsistent therapeutic responses among patients. To counter these obstacles, researchers have designed specialized nanocarriers. Examples include matrix-penetrating systems that degrade dense extracellular stroma and tumour-microenvironment-responsive carriers that trigger siRNA release in acidic, hypoxic, or enzyme-rich niches. In addition, multifunctional platforms capable of co-delivering RNAi molecules together with stromal modifiers or immune checkpoint inhibitors enhance tissue penetration and help achieve more consistent effects across different tumor regions. Collectively, these strategies are aimed at mitigating both spatial and molecular variations within solid tumors, thereby improving the reliability of RNAi-based interventions [9]. A summary of the key mechanisms and their impact on RNAi therapeutics is presented in Table 1.

5. Nanotechnology Solutions for RNAi Delivery

5.1 Lipid-Based Nanocarriers: Liposomes, Lipid Nanoparticles (LNPs - e.g., Onpattro® Approved for siRNA)

Lipid-based nanocarriers remain the most established platform for RNAi delivery due to their high biocompatibility, protection against enzymatic degradation, and efficient cellular uptake. Conventional liposomes can encapsulate siRNAs within aqueous cores or intercalate them into lipid bilayers, while ionizable lipid nanoparticles (LNPs) enable endosomal escape through pH-triggered membrane destabilization. The clinical success of Onpattro® (patisiran), the first FDA-approved siRNA drug delivered via LNPs, highlights the translational potential of this approach and has spurred development of next-generation formulations with improved stability, tissue selectivity, and reduced immunogenicity [28]. The graphical representation of polymeric nanoparticles is shown in Figure 2.

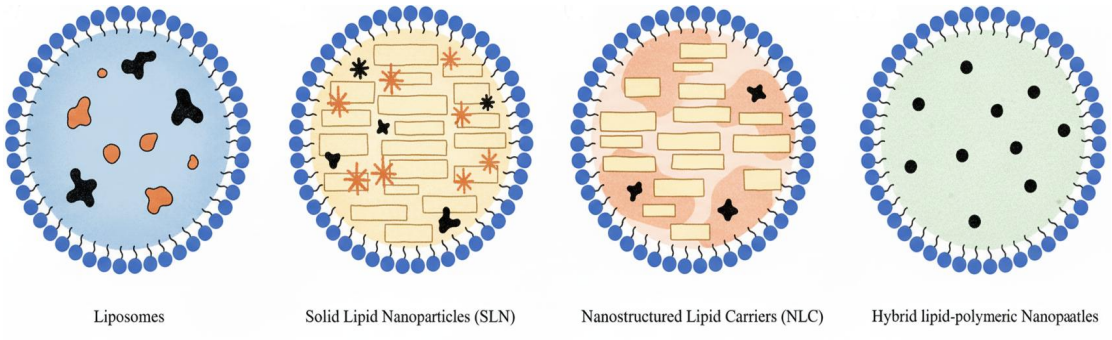


Figure 2. Graphical representation of lipid-based nanocarriers.

5.2 Polymeric Nanoparticles: PLGA, Chitosan, Dendrimers

Polymeric nanoparticles represent a versatile class of carriers for RNAi delivery, offering tunable physicochemical properties and controlled release profiles. Biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA) provide protection against nuclease degradation and enable sustained release. Chitosan, a naturally derived cationic polysaccharide, facilitates electrostatic complexation with negatively charged siRNAs, improving stability and cellular uptake. Dendrimers, with their highly branched architecture and multivalent surface groups, allow efficient nucleic acid binding and promote endosomal escape. Collectively, these polymer-based systems enhance RNAi stability, bioavailability, and intracellular delivery, while ongoing research focuses on minimizing cytotoxicity and improving tissue selectivity [29]. The graphical representation of polymeric nanoparticles is shown in Figure 3.

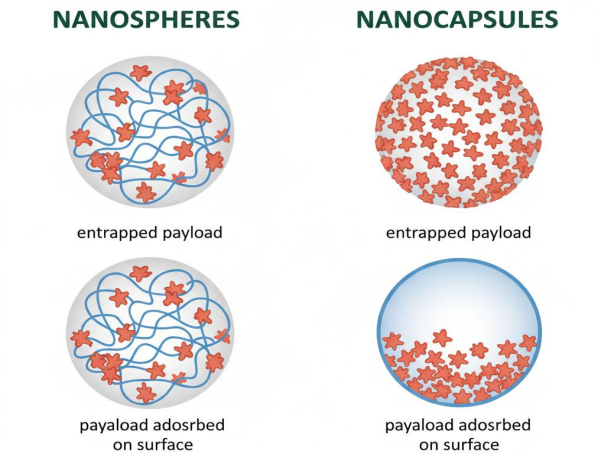


Figure 3. Graphical representation of polymeric nanoparticles.

5.3 Inorganic Nanomaterials: Gold Nanoparticles, Silica, Quantum Dots

Inorganic nanomaterials provide unique physicochemical properties that make them attractive carriers for RNAi therapeutics. Gold nanoparticles (AuNPs) offer high surface-area-to-volume ratios and facile surface modification, enabling efficient conjugation of siRNAs and ligands for targeted delivery. Silica nanoparticles, particularly mesoporous silica, provide tunable pore sizes and high loading capacity, facilitating controlled release of nucleic acids. Quantum dots (QDs), while primarily applied as imaging agents, can also serve as multifunctional platforms combining RNAi delivery with real-time tracking. Despite these advantages, concerns regarding long-term toxicity and clearance remain key challenges that require further investigation [30]. The graphical representation of Inorganic nanomaterials is shown in Figure 4.

Figure 4. Graphical representation of inorganic nanomaterials. 1. Mesoporous silica nanoparticles, 2. Quantum dots, 3. silver nanoparticles, 4. gold nanoparticles, 5. carbon nanotubes.

5.4 Hybrid Nanocarriers: Lipid-Polymer Hybrids, Exosome-Nanoparticle Hybrids

Hybrid nanocarriers combine the complementary features of different delivery systems to enhance RNAi therapeutic performance. Lipid-polymer hybrids integrate the structural stability of biodegradable polymers (e.g., PLGA) with the biocompatibility and efficient endosomal escape properties of lipids, resulting in prolonged circulation, enhanced cellular uptake, and controlled release. Exosome–nanoparticle hybrids leverage the innate targeting ability and immune-evasive properties of natural exosomes, while incorporating synthetic nanoparticle cores for high RNAi loading and tunable functionality. Such hybrid systems represent a promising next-generation strategy to overcome biological barriers and improve precision in RNAi delivery [31]. The graphical representation of hybrid nanocarriers is shown in Figure 5.

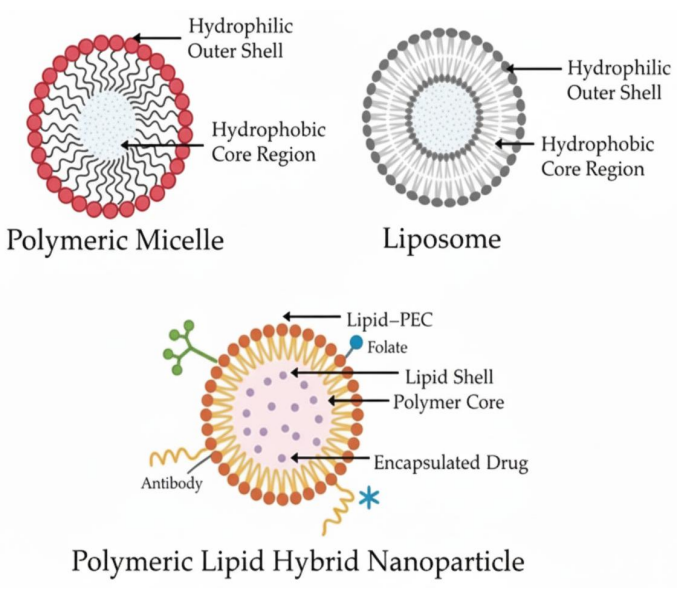


Figure 5. Graphical representation of hybrid nanocarriers.

5.5 Stimuli-Responsive Nanocarriers: pH, Redox, Enzyme-Triggered, Magnetic or Light-Activated

Stimuli-responsive nanocarriers offer spatiotemporal control over RNAi release by responding to intrinsic or extrinsic triggers. pH-sensitive systems exploit the acidic tumor microenvironment or endosomal compartments to enhance drug release. Redox-responsive carriers utilize the elevated intracellular glutathione levels in cancer cells to trigger disulfide bond cleavage and siRNA liberation. Enzyme-sensitive platforms incorporate substrates degraded by overexpressed tumor-associated enzymes (e.g., matrix metalloproteinases), enabling site-specific release. Additionally, external stimuli such as magnetic fields, ultrasound, or light can be applied to activate drug release with high precision. These “smart” carriers significantly improve therapeutic specificity and reduce off-target toxicity, making them a powerful direction in RNAi nanomedicine [32]. The graphical representation of stimuli-responsive nanocarriers is shown in Figure 6.

Figure 6. Graphical representation of Stimuli-responsive nanocarriers.

5.6 Comparative Discussion of Pros and Cons

Different nanocarriers address RNAi delivery barriers with various strengths and limitations. Lipid-based systems (liposomes, LNPs) are the most advanced, offering biocompatibility and clinical validation (e.g., Onpattro®), but they are limited by stability and immune concerns. Polymeric carriers (PLGA, chitosan, dendrimers) allow controlled release and chemical flexibility, although issues like toxicity and variability remain. Inorganic nanoparticles (gold, silica, quantum dots) offer multifunctionality and imaging potential, but poor biodegradability restricts clinical application. Hybrid platforms (lipid-polymer or exosome–nanoparticle) combine benefits of natural and synthetic systems, though scalability is challenging. Stimuli-responsive designs (pH, redox, enzyme, light, magnetic) enable site-specific release with greater precision, even though their complexity hinders practical translation. Combining these approaches could lead to more effective RNAi therapeutics. A summarized comparison of these delivery systems is provided in Table 2.

Table 2. Comparative overview of nanocarrier systems for RNAi delivery.

Nanocarrier Type	Advantages	Limitations	Clinical Status/Examples
Lipid-based (Liposomes, LNPs)	High biocompatibility, protect RNAi from degradation, efficient uptake, clinically validated	Stability issues, rapid clearance, potential immune activation	Onpattro® (patisiran, FDA-approved)
Polymeric (PLGA, chitosan, dendrimers)	Tunable degradation, sustained release, versatile modification	Possible cytotoxicity, variability in synthesis, limited FDA approval	PLGA-based systems in trials
Inorganic (AuNPs, silica, QDs)	High stability, surface modification, imaging and theranostics	Poor biodegradability, long-term toxicity, clearance issues	Preclinical studies
Hybrid (lipid-polymer, exosome-nanoparticle hybrids)	Combine biocompatibility with stability, enhanced targeting, immune evasion	Manufacturing complexity, scalability issues	Early clinical/preclinical evaluation
Stimuli-responsive (pH, redox, enzyme, light, magnetic)	Controlled and site-specific release, reduced off-target effects	Complex design, regulatory challenges, reproducibility concerns	Mostly preclinical, proof-of-concept

6. Combination of Therapeutic Strategies

6.1 Chemotherapy (siRNA Against MDR1 and Doxorubicin)

Combination therapy using RNAi with chemotherapy has shown promising results in overcoming drug resistance. Delivering siRNAs that silence resistance-associated genes such as *MDR1* or *BCL2* together with cytotoxic agents like doxorubicin can increase the intracellular retention of drugs by suppressing efflux transporters. The combination strategies of siRNA with doxorubicin, its mechanisms and efficacy signals are described in Table 3. Nanocarrier-based co-delivery systems have been developed to encapsulate both siRNA and doxorubicin, enabling synchronized release and improved therapeutic outcomes compared to single-agent treatment [33]. The graphical representation is shown in Figure 7.

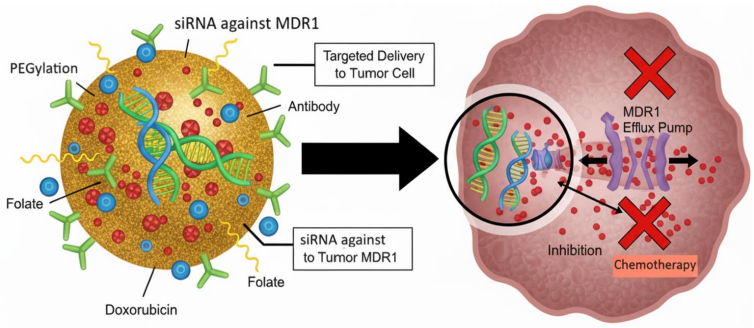


Figure 7. Graphical representation of chemotherapy (siRNA against MDR1 and doxorubicin).

6.2 Immunotherapy (siRNA and Checkpoint Inhibitors)

RNAi-based strategies are increasingly being integrated with immune checkpoint inhibitors (ICIs) to potentiate antitumor immunity. Small interfering RNAs (siRNAs) directed against targets such as PD-1, PD-L1, or CTLA-4 suppress inhibitory signaling pathways within tumor or immune cells, thereby restoring T-cell functionality. When siRNAs are designed to block immune checkpoint molecules such as *PD-1* or *PD-L1*, they reduce inhibitory signaling within the tumor microenvironment. Used alongside checkpoint-blocking antibodies, this approach prolongs T-cell activation. Nanocarrier platforms make it possible to coordinate the delivery of both agents, reducing immune fatigue and strengthening anti-tumor immunity. The Combination Strategies of siRNA with immunoantibodies, its mechanisms and efficacy signals are described in Table 3.The use of nanocarrier-mediated delivery further improves the precision of gene silencing within the tumor microenvironment, reducing systemic immune-related toxicities while amplifying therapeutic efficacy [34]. The graphical representation is shown in Figure 8.

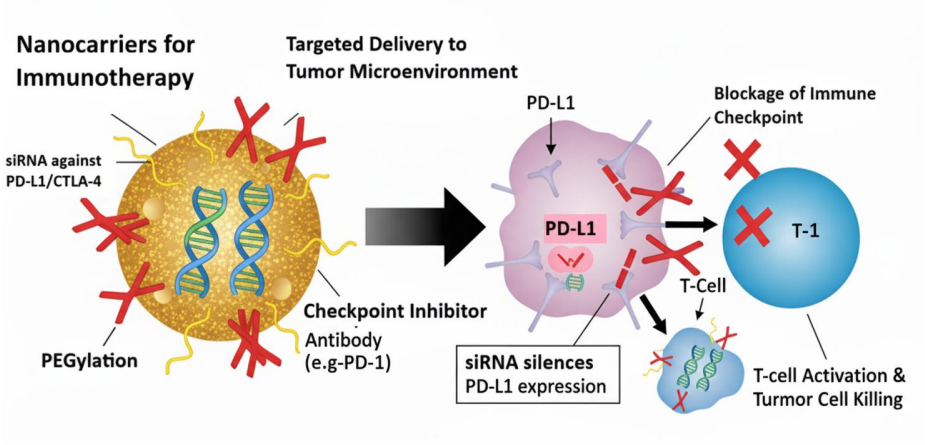


Figure 8. Graphical representation of immunotherapy (siRNA and checkpoint inhibitors).

6.3 Gene Editing Tools (CRISPR and RNAi)

RNA interference (RNAi) and CRISPR-Cas systems represent complementary platforms for gene silencing and therapeutic modulation. RNAi utilizes siRNAs or shRNAs to promote post-transcriptional degradation of messenger RNA through the RNA-induced silencing complex (RISC), resulting in transient and reversible suppression of gene expression. By contrast, CRISPR–Cas technology operates at the genomic level, enabling targeted knockouts, knock-ins, or precise sequence editing. RNAi is generally considered safer due to its reversibility and reduced risk of permanent off-target alterations in DNA, although it may be limited by incomplete knockdown and short-lived effects. The combination strategies of siRNA with CRISPR, its mechanisms and efficacy signals are described in Table 3. Conversely, CRISPR provides durable genetic modifications but raises concerns regarding off-target mutations, delivery challenges, and immunogenicity. Integrating the two approaches offers strategic advantages, whereby RNAi can be applied to validate candidate targets prior to irreversible CRISPR-mediated editing, thus improving translational outcomes [35]. The graphical representation is shown in Figure 9.

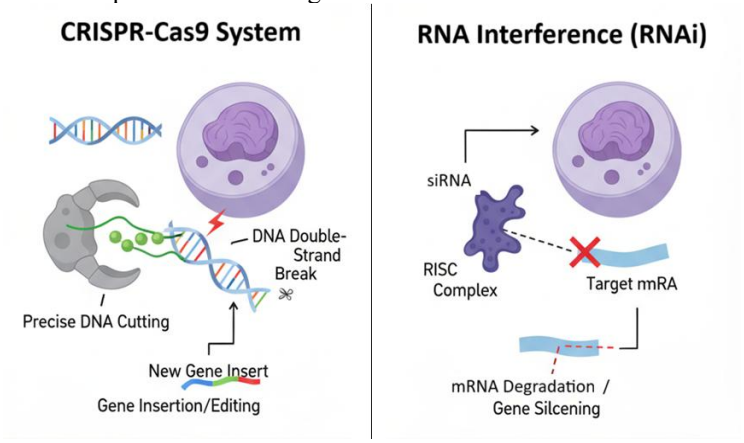


Figure 9. Graphical representation of Gene editing tools (CRISPR and RNAi).

6.4 Synergistic Nanocarrier Designs for Multi-Modal Treatment

Synergistic nanocarrier platforms are being developed to integrate multiple therapeutic modalities within a single system, thereby overcoming the limitations of monotherapies. These multifunctional carriers can co-deliver RNAi agents with chemotherapeutics, immunomodulators, or gene-editing tools, enabling simultaneous suppression of resistance pathways, immune activation, and cytotoxicity. For example, lipid–

polymer hybrids have been engineered to encapsulate siRNA against MDR1 alongside doxorubicin, restoring chemosensitivity in resistant tumors. Similarly, exosome-mimetic carriers decorated with checkpoint-blocking ligands can enhance immunotherapy when combined with RNAi silencing of PD-L1. The modular nature of these designs allows controlled release, targeted delivery, and potential integration of imaging agents, supporting precision medicine approaches in oncology [36].

6.5 Combination Nanocarrier Strategies Against Tumor Resistance

Nanocarrier systems are increasingly recognized as valuable tools in addressing tumor drug resistance. One effective strategy involves co-encapsulation of siRNAs that suppress resistance-associated genes such as MDR1, BCL2, or KRAS together with conventional chemotherapeutics, thereby restoring drug sensitivity in resistant cancer cells. Hybrid platforms, including lipid–polymer constructs and exosome-inspired carriers, have also been employed to deliver RNAi molecules in combination with immune checkpoint inhibitors, countering both immune evasion and acquired resistance simultaneously. The ability of these multifunctional carriers to co-package and release multiple therapeutic agents in a controlled manner enables precise targeting of resistance pathways while minimizing systemic toxicity. Recent reports have confirmed that such co-delivery strategies improve therapeutic response and reduce relapse in preclinical tumor models [37].

Table 3. Combination strategies: mechanisms & efficacy signals.

Combination	Mechanistic intent	Example nanocarrier	Model/setting	Outcome signal
siRNA(MDR1/BCL2) + Doxorubicin	Block efflux/apoptosis evasion → ↑intracellular drug	Polymeric or lipid NP co-load	Preclinical solid tumors	Restored chemosensitivity; lower chemo dose; reduced toxicity [33]
siRNA(PD-1/PD-L1) + ICI	Relieve checkpoint signaling & sustain T-cell activation	LNP / exosome-mimetic	Immuno-oncology models	↑T-cell function; amplified anti-tumor response [34]
RNAi + CRISPR	RNAi primes/validates; CRISPR gives durable edit	Hybrid NP	Preclinical	Complementary, staged efficacy; attention to dosing/schedule [35,36]

7. Advances in Tumor Targeting

7.1 Passive Targeting via the EPR Effect

Leaky tumor vasculature and inadequate lymphatic drainage permit passive enrichment of nanocarriers, a phenomenon described as the enhanced permeability and retention (EPR) effect. Despite its utility, inter-tumor variability often restricts its clinical predictability [38].

7.2 Active Targeting with Ligands

Functionalization of delivery systems with antibodies, aptamers, or small peptides facilitates receptor-mediated uptake, improving tumor selectivity and minimizing distribution to healthy tissues [39].

7.3 Carriers Adapted to the Tumor Microenvironment

Nanoplatforms sensitive to intratumoral conditions such as acidity, hypoxia, redox status, or protease activity can achieve controlled payload release and deeper tumor penetration, thereby enhancing RNAi therapeutic outcomes [40].

8. Preclinical and Clinical Progress

8.1 Preclinical Studies (Animal Models)

Extensive preclinical investigations in murine and xenograft models have demonstrated that RNAi therapeutics, when encapsulated in nanocarriers, achieve potent tumor growth inhibition and prolonged survival. For example, siRNA-loaded lipid nanoparticles targeting KRAS or VEGF have shown significant tumor regression with minimal systemic toxicity, validating RNAi as a powerful approach in oncology [41]. The Preclinical RNAi-Nanocarrier Outcomes are described in Table 4.

8.2 Ongoing Clinical Trials of RNAi–Nanoparticle Therapies in Cancer

Several RNAi–nanoparticle formulations have entered clinical testing. For instance, siRNA therapies targeting VEGF, KRAS, and MYC are under evaluation for solid tumors, often using lipid or polymer-based carriers. These trials aim to optimize dosing, assess safety, and monitor antitumor efficacy, moving RNAi strategies closer to clinical translation [42]. A detailed summary of ongoing clinical trials of RNAi nanocarrier therapy is provided in Table 5.

8.3 Case Study: Success of Lipid Nanoparticles in mRNA COVID-19 Vaccines as a Model for RNAi in Oncology

The rapid global deployment of lipid nanoparticle (LNP)-formulated mRNA vaccines during the COVID-19 pandemic demonstrated the scalability, safety, and regulatory approval pathway of this technology. These successes provide a strong precedent for the adoption of LNPs in RNAi-based cancer therapy, highlighting their ability to protect nucleic acids, enable efficient cytosolic delivery, and meet manufacturing standards [43].

Table 4. Preclinical RNAi-nanocarrier outcomes (selected exemplars).

Study (Year)	Cancer model / n	Target	Nanocarrier & route	Dosing (repr.)	Primary outcomes	Safety
Huang et al., 2023 (Acta Biomater.)	Pancreatic Ca (KRAS^G12D^) murine	KRAS^G12D^ siRNA	Targeted polymeric NP (i.v.)	Multi-dose q3-4d	~90% KRAS knockdown; marked tumor inhibition; complete regression in a subset	Well-tolerated in study window
Zhao et al., 2023 (ACS AMI)	NSCLC (KRAS-mut) orthotopic	KRAS siRNA	Inhalable NP (pulmonary)	Scheduled nebulization	Enhanced tumor targeting; significant tumor growth suppression	No major pulmonary toxicity reported
Chimeric RNAi (JCI 2025)	KRAS-driven cancers (mouse)	Dual MYC+KRAS RNAi	Systemic NP (i.v.)	Multi-dose	Synergistic co-silencing; superior tumor control vs single-target	Acceptable acute tolerability
LNP–VEGF siRNA (preclinical)	Solid tumor xenografts	VEGF siRNA	LNP (i.v.)	Multi-dose	Tumor regression with low systemic toxicity	Favorable lab safety profile

Table 5. Clinical trials of nanocarrier-based rnai therapeutics in oncology.

Drug / Sponsor	Target	Nanocarrier	Indication	Phase / NCT	Key outcomes / status
siG12D-LODER (Silenseed)	KRAS^G12D^	Local polymer implant	Pancreatic ductal adenocarcinoma	Ph II NCT01676259	Feasible and safe; local disease control; signal for benefit with chemo
TKM-080301 (Tekmira)	PLK1	LNP (i.v.)	GI/Liver cancers	Ph I/II NCT01262235, NCT01437007	On-target knockdown; DLTs included cytopenias/cytokines
Atu027 (Silence Tx)	PKN3	Lipid formulation	Solid tumors; prostate; pancreatic	Ph I NCT00938574; Ph II NCT01808638	Acceptable safety; preliminary antitumor activity
siRNA-EphA2-DOPC	EphA2	Neutral DOPC liposome	Ovarian cancer	Ph I NCT01591356	Safe; clinical activity in heavily pretreated pts
CALAA-01 (Calando)	RRM2	Cyclodextrin polymer NP	Solid tumors	Ph I NCT00689065	First human siRNA-NP with mRNA suppression; program halted for CMC/formulation reasons
LNP-siRNA (VEGF/KRAS/MYC)	VEGF/ KRAS/MYC	LNP	Solid tumors	Ongoing NCT04675996	Recruiting/ongoing; early PK/safety favorable

9. Safety, Immunogenicity, and Pharmacokinetics

The therapeutic promise of RNA interference (RNAi) has been markedly strengthened by innovations in nanomaterial-based delivery platforms. Nevertheless, successful clinical translation requires rigorous assessment of their safety, the implementation of strategies to minimize immunogenicity, and the fine-tuning of pharmacokinetic behavior.

9.1 Toxicological Considerations of Nanomaterials

Nanomaterials employed in RNAi delivery possess distinct physicochemical characteristics that critically shape their toxicity profiles. Parameters such as particle size, surface charge, and chemical composition have been shown to exert significant influence on nanocarrier biocompatibility. For example, cationic nanoparticles, although efficient in encapsulating siRNAs, can elicit cytotoxic responses through mechanisms including oxidative stress and membrane destabilization. In contrast, lipid-based nanoparticles generally display superior safety and compatibility, positioning them as promising candidates for RNAi therapeutics [44].

9.2 Strategies to Reduce Immunogenicity

The immunogenicity of RNAi therapeutics remains a major challenge, as unintended activation of the host immune system can compromise both safety and efficacy. To address this, recent efforts have emphasized chemical modification of RNA molecules and optimization of their delivery platforms to attenuate immune recognition. Notably, incorporation of 2′-O-methyl groups into siRNA sequences has been shown to improve molecular stability while reducing recognition by pattern recognition receptors, thereby limiting immune activation. In addition, lipid nanoparticles incorporating ionizable lipids have demonstrated reduced immunogenicity, as these components enable efficient endosomal escape while minimizing activation of immune pathways [45].

9.3 Pharmacokinetic Optimization of RNAi Delivery Systems

The pharmacokinetic behavior of RNAi therapeutics is a critical determinant of their clinical performance. Multiple strategies have been investigated to enhance molecular stability, optimize biodistribution, and

improve cellular uptake. Among these, conjugation with targeting ligands such as N-acetylgalactosamine (GalNAc) has proven particularly effective, facilitating selective hepatic uptake while extending circulation half-life [46]. The design of delivery systems capable of protecting RNA molecules from nuclease-mediated degradation while promoting efficient endosomal escape is essential for maximizing therapeutic efficacy [47]. Table 6 shows key summaries of safety, immunogenicity and pharmacokinetics of nanocarrier RNAi therapies

Table 6. Safety, immunogenicity & pharmacokinetics (cross-study).

Domain	Key issue	Evidence-backed mitigation
Toxicology	Cationic surfaces/ROS; inorganic persistence	Prefer ionizable lipids/biodegradables; surface shielding (PEG) [43,44]
Immunogenicity	Innate sensing (TLR/RIG-I), cytokines	2′-O-Me/2′-F mods; optimized ionizable lipids; careful infusion mgmt [44]
PK/BD	Rapid nuclease clearance; hepatic bias	Encapsulation; GalNAc (liver) for specific targets; design for endosomal escape [25,26,45]

10. Emerging Trends and Future Perspectives

Recent advances in nanotechnology, coupled with computational methodologies, are reshaping the development and application of RNA interference (RNAi) therapeutics. Emerging directions emphasize the integration of artificial intelligence (AI) and machine learning (ML) to guide nanocarrier design, the personalization of RNAi-based interventions, the engineering of smart nanomaterials, and the incorporation of liquid biopsy platforms to support precision medicine approaches.

10.1 AI/ML in Designing Nanocarriers

Artificial intelligence (AI) and machine learning (ML) are increasingly being applied to accelerate the design of RNAi therapeutics and their nanocarrier systems. These tools can analyze large datasets to identify potent siRNA sequences with minimal off-target activity, while also predicting the physicochemical properties of lipid or polymer-based nanoparticles such as size, stability, and encapsulation efficiency. By integrating computational modeling with experimental data, AI/ML approaches allow researchers to optimize carrier composition and delivery performance more efficiently than traditional trial-and-error methods. Such strategies not only reduce development timelines but also improve the likelihood of successful translation into clinical applications. A recent review by Zhang et al. (2025) highlights how ML-guided optimization is being used to fine-tune nanoparticle parameters-including charge, surface chemistry, and ligand density-for more effective tumor-specific RNAi delivery [48]. AI-driven platforms enable the high-throughput screening of extensive nanomaterial libraries, thereby accelerating the identification of optimal formulations tailored to specific therapeutic applications.

10.2 Personalized RNAi Therapy

Personalized medicine is an emerging cornerstone of modern therapeutic strategies, and RNAi-based approaches exemplify this trend. By integrating patient-specific genetic profiles, RNAi therapeutics can be customized to selectively silence disease-associated genes with high precision. Nanocarriers further enable the targeted delivery of siRNAs identified through genomic analyses, aligning treatments with individual molecular signatures. This strategy not only enhances therapeutic efficacy but also reduces off-target effects and adverse reactions [49].

10.3 Smart Nanomaterials: Self-Assembling and Biodegradable Systems

The development of smart nanomaterials has enabled dynamic and responsive systems for RNAi delivery. Self-assembling nanostructures, including micelles and hydrogels, can spontaneously form upon administration to encapsulate and release RNAi agents efficiently. These materials may be engineered to respond to specific stimuli, such as pH, redox state, or temperature changes, thereby enabling site-specific delivery to diseased tissues and improving therapeutic precision. Biodegradable nanomaterials degrade into non-toxic byproducts, thereby minimizing long-term accumulation and associated toxicity, ultimately improving the safety profile of RNAi therapeutics [50].

10.4 Integration with Liquid Biopsy for Biomarker-Driven RNAi Therapy

Liquid biopsy technologies, which analyze biomarkers from non-invasive samples such as blood, are increasingly being integrated with RNAi-based therapeutics to enable real-time monitoring and personalized treatment adjustments. The detection of circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and other tumor-derived biomarkers provides valuable insights into patient-specific genetic alterations. This information facilitates the tailoring of RNAi therapies to individual molecular profiles. Moreover, such integration supports dynamic treatment strategies, allowing therapeutic regimens to be adapted in response to evolving tumor landscapes, thereby improving clinical outcomes while minimizing unnecessary interventions [51]. The summary of the emerging trend and future prospective is presented in Table 7.

Table 7. Emerging trends and future perspectives.

Trend	Description	Key Advantages / Applications
AI/ML in Nanocarrier Design	Utilization of artificial intelligence and machine learning to optimize nanocarrier properties and formulations.	Predicts particle size, surface charge, lipid composition; accelerates screening of nanomaterials; improves delivery efficiency and reduces toxicity.
Personalized RNAi Therapy	Tailoring RNAi therapeutics based on patient-specific genetic profiles.	Enables precise gene targeting, enhances efficacy, minimizes off-target effects, and aligns treatment with individual molecular characteristics.
Smart Nanomaterials	Self-assembling and stimuli-responsive materials for RNAi delivery, including micelles and hydrogels.	Facilitates efficient encapsulation and release, responds to pH or temperature changes, and biodegradable materials reduce toxicity and long-term accumulation.
Integration with Liquid Biopsy	Use of non-invasive biomarker analysis to guide RNAi therapy.	Enables real-time monitoring, dynamic therapy adjustment based on ctDNA/CTCs, and personalized treatment strategies to improve outcomes.

11. Challenges and Limitations of NanoRNAi Therapeutics

Despite substantial progress, several challenges hinder the clinical translation of nanotechnology-enhanced RNAi therapeutics:

11.1 Large-Scale Manufacturing and Reproducibility

Many advanced nanocarriers (lipid-polymer hybrids, exosome-nanoparticle systems, stimuli-responsive carriers) involve complex multistep processes. Ensuring Good Manufacturing Practice (GMP) compliance, reproducibility, and batch-to-batch consistency remains a bottleneck for large-scale production [52].

11.2 Long-Term Toxicity and Biodistribution

Short-term safety of lipid and polymeric nanoparticles has been favorable; however, inorganic nanomaterials (e.g., gold, silica, quantum dots) pose risks of long-term accumulation and toxicity. Biodegradation kinetics and immune clearance pathways remain poorly characterized in humans [53].

11.3 Regulatory Approval Hurdles

Unlike small molecules or biologics, RNAi–nanocarrier therapeutics face complex regulatory classification as drugs, biologics, or combination products. Lack of harmonized guidelines for characterization, safety evaluation, and quality control slows global approval [52].

11.4 Cost and Scalability

The high cost of RNAi synthesis, specialized lipid excipients, and complex nanocarrier systems limits accessibility. Large-scale GMP production is resource-intensive, raising concerns about affordability and equitable access in low- and middle-income countries [54].

11.5 Tumor Heterogeneity and Delivery Variability

The enhanced permeability and retention (EPR) effect, often relied upon for passive targeting, is highly variable across patients and tumor types. Dense extracellular matrix, hypoxia, and immunosuppressive tumor microenvironments further reduce delivery efficiency and predictability [55].

12. Conclusion

Nanotechnology-enhanced RNA interference (RNAi) has emerged as a powerful approach in precision oncology, addressing the inherent limitations of naked RNA such as instability, inefficient cellular uptake, and unintended off-target effects. Diverse nanocarrier platforms-including lipid-based, polymeric, inorganic, hybrid, and stimuli-responsive systems-have demonstrated the ability to improve RNA stability, enable tumor-specific delivery, and promote effective intracellular release of siRNAs and miRNAs. Integration of RNAi with chemotherapy, immunotherapy, or gene-editing modalities further amplifies therapeutic efficacy while minimizing systemic toxicity. Evidence from preclinical investigations and early-phase clinical trials underscores the translational promise of RNAi nanomedicine, although key hurdles related to safety, immunogenicity, and large-scale manufacturing remain. Looking ahead, innovations in smart and biodegradable delivery materials, combined with biomarker-driven and patient-tailored strategies, are anticipated to strengthen the precision and clinical impact of these platforms, positioning nanotechnology-assisted RNAi as a cornerstone of next-generation cancer therapeutics.

List of Abbreviations

AI (Artificial Intelligence); AuNPs (Gold Nanoparticles); CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats); CTCs (Circulating Tumor Cells); ctDNA (Circulating Tumor DNA); ECM (Extracellular Matrix); EPR (Enhanced Permeability and Retention); FDA (U.S. Food and Drug Administration); GMP (Good Manufacturing Practice); ICIs (Immune Checkpoint Inhibitors); IFP (Interstitial Fluid Pressure); LNPs (Lipid Nanoparticles); miRNA (MicroRNA); mRNA (Messenger RNA); ML (Machine Learning); MDR1 (Multidrug Resistance Protein 1); NPs (Nanoparticles); Onpattro® (Patisiran, the first FDA-approved siRNA drug); PD-1 (Programmed Death-1); PD-L1 (Programmed Death Ligand-1); PEG (Polyethylene Glycol); PI3K/AKT (Phosphatidylinositol-3-Kinase/Protein Kinase B pathway); PLGA (Poly(lactic-co-glycolic acid)); PRISMA (Preferred Reporting Items

for Systematic Reviews and Meta-Analyses); QDs (Quantum Dots); RISC (RNA-Induced Silencing Complex); RNAi (RNA Interference); shRNA (Short Hairpin RNA); siRNA (Small Interfering RNA); TME (Tumor Microenvironment); VEGF (Vascular Endothelial Growth Factor).

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Ethical Approval

Not applicable (review article based on publicly available data)

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Figure Originality

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