

Review

Dual Approaches in Oncology: The Promise of siRNA and Chemotherapy Combinations in Cancer Therapies

Carolina Sousa and Mafalda Videira *

Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisbon, Portugal; acmsousa@ff.ulisboa.pt

* Correspondence: mvideira@ff.ulisboa.pt; Tel.: +351-217946400

Simple Summary: The use of small interfering RNA (siRNA) combined with cancer drugs is a groundbreaking approach that aims to improve cancer treatment. By targeting specific genes that are active in cancer cells but not in healthy ones, siRNA can effectively block harmful processes that allow tumors to grow and spread. This review discusses important genes, like survivin, VEGF, and HER2, that siRNA can target to enhance the effectiveness of traditional cancer treatments. Clinical trials have shown that these siRNA therapies can be well tolerated, but challenges remain, such as delivering the siRNA effectively and minimizing side effects. Advances in delivery systems, like nanoparticles, are helping address these issues. Looking forward, researchers aim to improve how siRNA is delivered, tailor treatments to individual patients, and develop clearer guidelines for approval. Overall, siRNA-based therapies have the potential to revolutionize cancer treatment, providing new hope for patients facing tough-to-treat cancers.

Abstract: The integration of small interfering RNA (siRNA) with traditional cancer therapies represents a promising frontier in oncology aimed at enhancing treatment effectiveness, reducing side effects, and overcoming drug resistance. This review highlights the potential of siRNA to selectively silence genes that are overexpressed or uniquely expressed in cancer cells, thereby disrupting critical pathways that support tumor growth and survival. Key target genes discussed include survivin, VEGF, EGFR, c-MET, HER2, MUC1, and Bcl-2, all of which play vital roles in tumor proliferation, angiogenesis, and resistance to therapies. Clinical trials investigating various siRNA candidates, such as EZN-3042 and ALN-VSP, indicate that these therapies are generally well-tolerated; however, significant challenges persist, including the effective delivery and stability of siRNA. Recent advancements in nanoparticle-based delivery systems have shown promise in addressing these issues. Future research will focus on optimizing siRNA delivery methods, personalizing therapies based on individual genetic profiles, and establishing clearer regulatory guidelines for approval. As the field evolves, siRNA-based combination therapies are poised to become an integral part of precision oncology, offering new therapeutic options and hope for patients with difficult-to-treat cancers.

Keywords: siRNA; anticancer drugs; drug resistance; targeted therapy; nanoparticles



Academic Editor: Constantin N. Baxevanis

Received: 30 September 2024

Revised: 20 December 2024

Accepted: 25 December 2024

Published: 2 January 2025

Citation: Sousa, C.; Videira, M. Dual Approaches in Oncology: The Promise of siRNA and Chemotherapy Combinations in Cancer Therapies.

Onco **2025**, *5*, 2. <https://doi.org/10.3390/onco5010002>

Copyright: © 2025 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the Creative Commons

Attribution (CC BY) license

(<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Combination therapy using small interfering RNA (siRNA) and anticancer drugs represents a transformative advancement in oncology, addressing significant limitations of traditional treatments such as chemotherapy and targeted therapies [1–3]. While conventional

approaches have achieved considerable success, their effectiveness is often constrained by challenges such as non-specific cytotoxicity, the emergence of drug resistance, and debilitating side effects that comprise therapeutic outcomes and patient adherence. These obstacles underscore the urgent need for innovative and highly specific treatment modalities that can precisely target cancer cells while minimizing harm to healthy tissues [4–6].

The dual approach of combining siRNA with anticancer drugs offers a paradigm shift in precision medicine. siRNA, derived from the natural RNA interference (RNAi) mechanism, provides a unique capacity to selectively silence genes that drive cancer progression, metastasis, and treatment resistance. These double-stranded RNA molecules, typically 20–25 nucleotides long, operate with remarkable specificity by targeting messenger RNA (mRNA) sequences for degradation, thereby halting the synthesis of oncogenic or survival-promoting proteins [7–10]. This precise mode of action allows siRNA to directly modulate critical molecular pathways, enhancing the efficacy of anticancer drugs by sensitizing cancer cells to their effects. For example, siRNA targeting oncogenes such as Bcl-2 has been shown to promote apoptosis, amplifying the pro-apoptotic impact of chemotherapeutic agents [11,12], while silencing VEGF can suppress angiogenesis, thereby synergizing with anti-angiogenic drugs [13,14].

What distinguishes this dual therapeutic strategy is its ability to tackle cancer on multiple fronts simultaneously, offering a degree of specificity and adaptability that surpasses conventional monotherapies. By combining siRNA with existing chemotherapeutic or targeted agents, this approach enables the concurrent disruption of complementary molecular pathways, amplifying therapeutic efficacy while mitigating the toxicities associated with higher drug doses. Moreover, siRNA's capacity to selectively downregulate genes implicated in drug resistance mechanisms, such as ATP-binding cassette (ABC) transporters, addresses one of the most persistent challenges in cancer treatment: overcoming resistance to established therapies. Despite these promising attributes, several critical gaps remain in the field. Foremost among these is the challenge of the efficient and tumor-specific delivery of siRNA molecules. The instability of siRNA in biological environments, its susceptibility to rapid degradation, and potential off-target effects limit its clinical utility. Advances in nanotechnology and nanoparticle-based delivery systems have shown promise in addressing these issues by enhancing the stability, bioavailability, and tumor-targeting capacity of siRNA; yet, widespread clinical application remains constrained by logistical and regulatory hurdles [7–9].

Another pressing gap lies in the need to fully integrate siRNA-based therapies into personalized medicine frameworks. While siRNA offers unparalleled precision, its therapeutic success hinges on the accurate identification of patient-specific genetic vulnerabilities. This demands sophisticated diagnostic tools and biomarkers capable of guiding the selection of siRNA targets and optimizing combination regimens based on individual tumor profiles. Furthermore, the potential for siRNA to modulate the tumor microenvironment and augment immunotherapy responses remains an underexplored frontier that could unlock new therapeutic avenues [15–17].

This review seeks to advance the understanding of siRNA-based combination therapies by delving into the molecular mechanisms underlying siRNA activity and its role in complementing traditional anticancer drugs. We highlight critical gaps in the field, including delivery challenges and the need for a more robust integration of siRNA with personalized and immune-based therapies. By emphasizing these aspects, this review aims to set the stage for future research that will harness the full potential of this innovative therapeutic approach, ultimately transforming cancer treatment and offering new hope for patients with resistant or aggressive disease phenotypes. The following sections will

elaborate on the intracellular mechanisms of siRNA action and explore the advancements in delivery platforms and therapeutic strategies that are shaping the future of oncology.

2. Unraveling the Mechanism: How siRNA Silences Gene Expression in Cancer Cells

siRNA has emerged as a transformative tool in molecular biology and pharmacology, particularly in the field of oncology. It leverages the RNAi pathway, a natural cellular process for regulating gene expression and preserving genomic integrity [7,9,10]. This section explores the molecular mechanisms behind siRNA's action, highlighting its potential to improve cancer therapies outcomes by selectively silencing oncogenes involved in tumor growth, metastasis, and resistance to conventional drugs.

siRNA is a short, double-stranded RNA molecule, typically 20–25 nucleotides in length, engineered to precisely complement a target messenger RNA (mRNA) sequence. Discovered in *Caenorhabditis elegans* in 1998, the RNAi pathway is a highly conserved post-transcriptional gene-silencing mechanism. When siRNA is introduced into cells, it triggers a cascade of molecular events that lead to the degradation of complementary mRNA, preventing its translation into oncogenic proteins [18–20].

Effective intracellular delivery of this highly positive macromolecule requires complexation with advanced nanomedicine platforms, including lipid nanoparticles (LNPs), polymeric nanoparticles, viral vectors, liposomes, and dendrimers. These carriers can be functionalized with targeting ligands, such as aptamers or peptides, to facilitate specific cell-receptor-mediated endocytosis [8,20,21]. Once internalized, these delivery vehicles traffic through endosomal compartments. Strategies to enhance the endosomal escape include using pH-sensitive lipids or polymers that disrupt the endosomal membrane, thus releasing siRNA into the cytoplasm [8,20,22].

Once released into the cytoplasm, the synthetic siRNA is unwound by an ATP-dependent helicase, and the antisense (guide) strand is incorporated into the RNA-induced silencing complex (RISC) (Figure 1). The RISC's core protein, Argonaute (Ago), binds to the guide strand and facilitates its pairing with the complementary mRNA [1,9,10]. The guide strand's selection is thermodynamically determined, with the strand having the less-stable 5' end being loaded into the RISC, ensuring specificity and minimizing off-target effects. The other strand, known as the passenger strand, is usually degraded. This careful selection mechanism ensures that only the intended strand guides the RISC to the target mRNA, reducing off-target effects and enhancing the specificity of gene silencing [7,9,10] (Figure 1).

The activated RISC complex, loaded with the guide strand, scans the cytoplasm for complementary mRNA sequences. Silencing depends on near-perfect base pairing between the guide strand and target mRNA, with the seed region (positions 2–8 of the guide strand) playing a critical role in recognition. Even slight mismatches in this region can significantly reduce silencing efficiency, highlighting siRNA's high specificity (Figure 1). Upon binding to its target, the Ago2 protein catalyzes the cleavage of the mRNA, usually at the midpoint of the siRNA-mRNA duplex, leading to the rapid degradation of mRNA fragments by exonucleases, thereby preventing protein translation [7,9,10]. The RNAi process provides sustained gene silencing, as the RISC-siRNA complex can undergo multiple rounds of mRNA cleavage, amplifying the silencing effect. However, the duration of this effect depends on several factors, such as siRNA stability, mRNA turnover rate, and the half-life of the RISC complex. In cancer therapy, where high cell proliferation and rapid mRNA turnover are common, repeated siRNA administration may be required to maintain therapeutic efficacy [7,9,10,18].

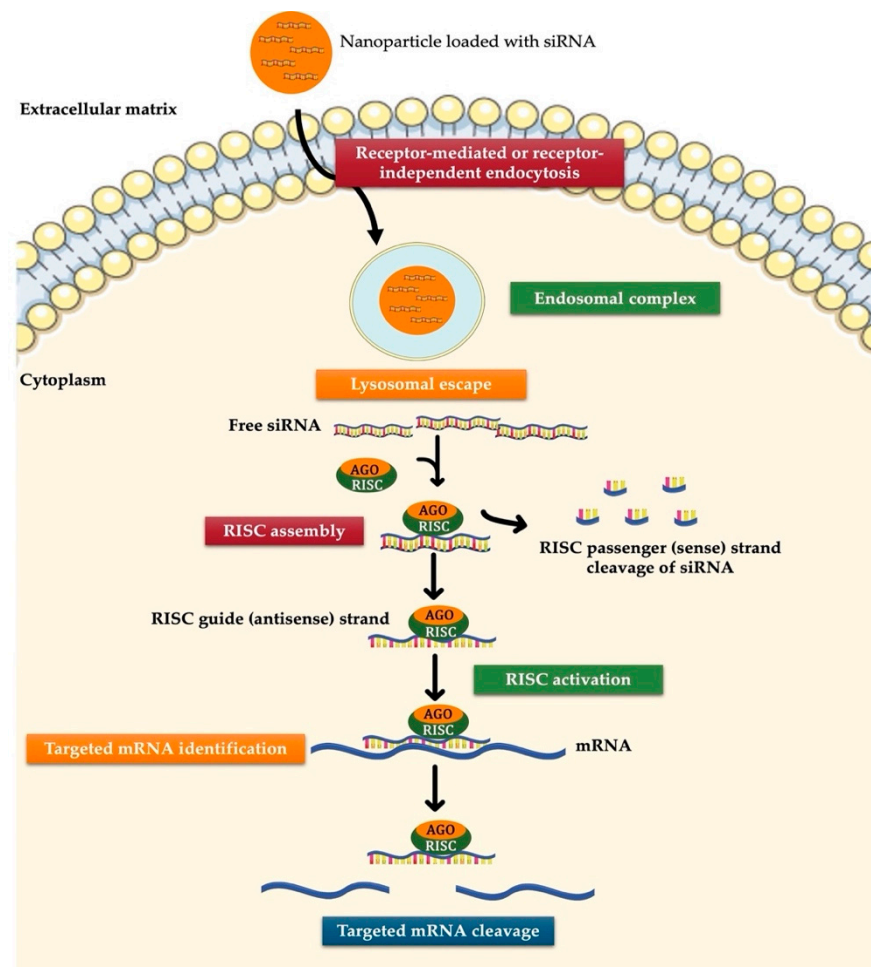


Figure 1. Mechanism of siRNA gene silencing via nanoparticle-based systems. Nanoparticles loaded with siRNA are delivered to target cells, utilizing either receptor-mediated or receptor-independent endocytosis for cellular internalization. This process ensures targeted delivery and minimizes off-target effects. Once internalized, nanoparticles are trapped within the endosomal compartment. Efficient endosomal escape, facilitated by nanoparticle design or pH-sensitive components, is critical for releasing free siRNA into the cytoplasm, avoiding degradation within lysosomes. In the cytoplasm, the free siRNA duplex undergoes strand separation, with the antisense (guide) strand incorporated into the RNA-induced silencing complex (RISC). The passenger (sense) strand is degraded during this period. The activated RISC, guided by the antisense strand, identifies complementary target mRNA within the cell. This high specificity ensures that only the intended mRNA sequence is targeted for degradation. Upon binding to the target mRNA, RISC facilitates precise cleavage and degradation, leading to effective gene silencing. This process disrupts the expression of genes implicated in tumorigenesis, drug resistance, or other pathological pathways.

The versatility of siRNA allows it to target multiple genes either simultaneously or sequentially, making it an ideal strategy for addressing the complex nature of cancer [7,9,10]. Unlike conventional small-molecule drugs, which often modulate protein function, siRNA directly reduces the expression of specific genes, potentially overcoming resistance mechanisms linked to gene amplification or mutation. Overall, understanding the molecular mechanisms of siRNA action lays a crucial foundation for its application in cancer therapy. By precisely silencing oncogenes, tumor suppressor genes, or genes involved in drug resistance, siRNA can significantly enhance the efficacy of conventional treatments, mitigate adverse effects, and potentially overcome treatment resistance.

3. Optimizing Synergistic Combinations: The Science Behind Rational siRNA and Drug Pairings

The development of combinatory therapies involving siRNA and anticancer drugs marks a transformative advance in cancer treatment, addressing the multifaceted challenges faced by traditional modalities [1,15,17]. While conventional chemotherapy and targeted therapies have made significant strides, their limitations—such as drug resistance, lack of specificity, and severe side effects—often hinder optimal patient outcomes [6,15]. Integrating siRNA, which can specifically silence genes driving cancer progression, drug resistance, and tumor survival, with conventional anticancer drugs presents an opportunity to enhance therapeutic efficacy and improve clinical outcome. The result is a dual mechanism where siRNA sensitizes tumor cells to the drug by inhibiting compensatory pathways, while the anticancer drug amplifies the effects of siRNA by creating an environment more conducive to gene silencing (e.g., via enhanced cellular uptake or stress-induced vulnerability) [9,10,18] (Figure 2).

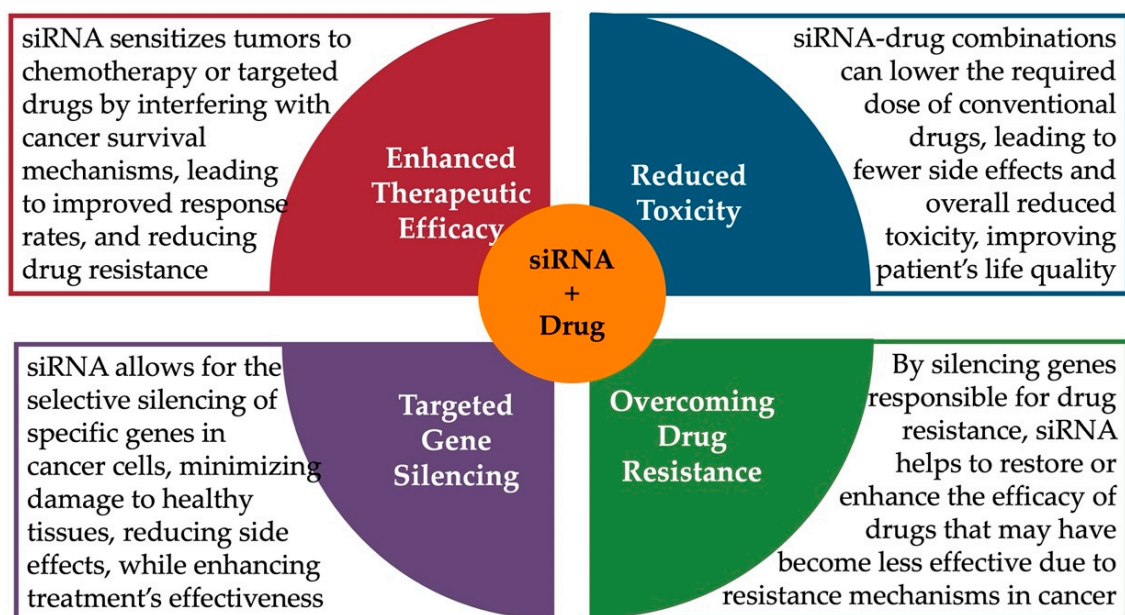


Figure 2. Primary advantages of siRNA-drug combination therapies in cancer treatment. There are four main advantages of siRNA-drug combination therapies: (1) targeted gene silencing; (2) enhanced therapeutic efficacy; (3) reduced toxicity; and (4) overcoming drug resistance. Each of these advantages contributes to a more effective, personalized, and safer approach to cancer treatment. First, targeted gene silencing is achieved through siRNA, which selectively silences genes that are overexpressed or uniquely expressed in cancer cells, minimizing off-target effects and preserving healthy tissues. This precision reduces side effects while effectively disrupting key oncogenic pathways. Second, the enhanced therapeutic efficacy of the combination is highlighted, as siRNA can sensitize tumors to chemotherapy or targeted therapies, improving overall response rates and overcoming resistance mechanisms. Third, the reduction in toxicity is demonstrated, as siRNA-drug combinations allow for the more precise targeting of cancer cells, reducing the required doses of conventional drugs and subsequently lowering treatment-related side effects. Finally, siRNA can block the expression of genes responsible for drug resistance, restoring the effectiveness of anticancer drugs that tumors may have become resistant to, and improving overall response to therapy.

A key motivation for combining siRNA with traditional anticancer drugs is to overcome drug resistance, a major obstacle in the successful treatment of cancer [23,24] (Figure 2). Drug resistance arises through multiple mechanisms, including overexpression of drug efflux pumps (e.g., P-glycoprotein (P-gp) encoded by the MDR1 gene), enhanced DNA

repair mechanisms, mutations in drug targets, and the activation of alternative survival pathways [25–27]. For example, the upregulation of the MDR1 gene leads to an increased efflux of chemotherapeutic agents, reducing their intracellular concentration and therapeutic efficacy [27]. By co-administering siRNA designed to target and downregulate MDR1, researchers have demonstrated the enhanced intracellular retention of chemotherapeutics such as doxorubicin and paclitaxel [28,29]. This dual approach not only boosts drug efficacy, but also allows for the targeting of complementary pathways, such as DNA repair pathways (e.g., PARP—(poly (ADP-ribose) polymerase) or anti-apoptotic genes (e.g., Bcl-2—B-cell lymphoma 2), further sensitizing tumor cells to treatment [30,31]. The inhibition of PARP, for example, can make cancer cells more susceptible to DNA-damaging agents, while targeting Bcl-2 enhances apoptosis induced by chemotherapeutic drugs [11,12,32].

Traditional chemotherapies often lack tumor specificity, resulting in significant toxicity to healthy tissues and dose-limiting side effects that compromise treatment outcome and patients' quality of life [3–5]. As previously explained, siRNA can be designed to specifically target oncogenes or genes involved in key cancer-promoting pathways, such as KRAS (Kirsten rat sarcoma virus), MYC, or VEGF (vascular endothelial growth factor), thereby selectively inhibiting tumor growth while sparing normal cells [18,33] (Figure 2). For example, siRNA targeting mutant KRAS has shown to be effective in preclinical studies by inhibiting tumor cell proliferation without affecting normal cells [34,35]. Therapeutic siRNA combined with conventional chemotherapy might aid these medicines to concentrate in tumor cells, whereas its ability to downregulate genes involved in cancer cell-specific processes, such as angiogenesis (e.g., VEGF), invasion (e.g., MMPs—matrix metalloproteinases), and metastasis (e.g., epithelial-to-mesenchymal-related genes), adds a layer of precision by targeting the unique vulnerabilities of cancer cells while minimizing collateral damage to healthy cells [2,15,16].

Combinatory therapy using siRNA also allows for a more focused attack on cancer cells, especially when paired with drugs that induce DNA damage, disrupt microtubule formation, or block enzymes essential for cell proliferation. Many of these drugs are cytotoxic to both rapidly dividing cancer cells and healthy cells, such as those in the bone marrow or gastrointestinal tract, leading to substantial side effects [6,15]. Combining siRNA that targets anti-apoptotic genes (e.g., Bcl-2, Bcl-xL—B-cell lymphoma-extra-large) with apoptosis-inducing agents (e.g., doxorubicin, cisplatin) enhances programmed cell death in cancer cells by both inducing DNA damage and blocking the cell's ability to repair that damage [12,32,36]. This synergistic effect can improve treatment efficacy and reduce the required drug dose, thereby lowering the risk of side effects (Figure 2).

Moreover, therapeutic doses of chemotherapy are frequently associated with systemic toxicities, including myelosuppression, neurotoxicity, cardiotoxicity, and gastrointestinal complications [4,6]. The combination of siRNA and anticancer drugs allows for dose reduction while maintaining therapeutic efficacy, as siRNA enhances the drugs' tumor-specific action [4,6,10] (Figure 2). As an example, siRNA targeting VEGF, when combined with paclitaxel, enhances tumor response by inhibiting angiogenesis, enabling lower doses of the cytotoxic agent [14,37]. This reduction in dose may alleviate the adverse effects commonly seen with standard chemotherapy regimens.

The rise of siRNA-based combination therapies aligns with the broader trend toward personalized and precision medicine in oncology. With the growing understanding of cancer's genetic and molecular heterogeneity, therapies can be tailored to the unique genetic profile of an individual's tumor. siRNA technology offers a customizable to target specific oncogenes, tumor suppressor genes, or other molecular drivers aberrantly expressed in each patient's cancer [7,9,18]. For instance, in cancers harboring mutations in genes like HER2 (human epidermal growth factor receptor 2) or EGFR (epidermal growth factor

receptor), siRNA can be specifically designed to silence these mutated alleles, providing a more personalized and precise treatment while sparing normal alleles [38–40]. Combining siRNA with chemotherapy or other targeted therapies facilitates addressing the unique molecular landscape of each tumor, potentially overcoming the limitations of one-size-fits-all approaches.

A critical challenge that remains in siRNA therapy is its delivery and stability [2,15,17]. Naked siRNA is rapidly degraded by nucleases in the bloodstream, experiences rapid renal clearance, and shows poor cellular uptake [9,18]. To overcome these challenges, advanced delivery systems, such as nanoparticles, liposomes, and viral vectors, have been developed to protect siRNA, improve its stability, and promote targeted delivery to tumors. These delivery platforms can be co-engineered to carry both siRNA and chemotherapeutic agents, allowing synchronized release at the tumor site and maximizing the therapeutic potential of the combination [20,21,41]. For example, lipid-based nanoparticles have been designed to co-deliver siRNA targeting HIF1 α (Hypoxia-Inducible Factor 1-alpha) and gemcitabine, improving drug retention and increasing tumor cell death in pancreatic cancer models [42,43].

Nevertheless, achieving these benefits critically depends on optimizing the dosage and timing of each component, which poses significant challenges. The intricate interplay between siRNA's gene-silencing effects and the pharmacological action of anticancer drugs complicates dosage optimization. siRNA must achieve sufficient intracellular concentrations to silence target gene expression effectively, while the anticancer drug must act on its specific molecular target or cellular process. The incorrect dosing of either agent can lead to suboptimal outcomes, including antagonistic effects where one agent interferes with the other's efficacy, or additive toxicities that exacerbate adverse side effects [15].

Additionally, siRNA delivery systems, such as nanoparticles, often exhibit distinct pharmacokinetics and biodistribution profiles compared to traditional drugs. These differences necessitate the careful synchronization of the delivery kinetics to ensure both agents reach the tumor microenvironment simultaneously and at therapeutically relevant concentrations. Timing is especially critical in therapies targeting dynamic processes, such as angiogenesis or cell cycle regulation, where precise coordination can maximize therapeutic synergy [44,45]. Advances in computational modeling, pharmacokinetic/pharmacodynamic (PK/PD) studies, and high-throughput screening are aiding in the design of optimal dosing regimens. However, these methods require extensive preclinical data and validation in relevant *in vivo* models, adding to the complexity and time required for preclinical development.

4. Next-Generation siRNA Therapeutics: Addressing Off-Target Challenges and Immune Responses

Despite its therapeutic potential, siRNA therapy faces several obstacles, primarily related to delivery. Challenges include ensuring siRNA stability in the bloodstream, enhancing delivery to target cells, achieving efficient endosomal escape, and avoiding off-target effects or immune activation [7,9,18]. Recent innovations in chemical modifications, nanoparticle design, and targeting strategies have significantly advanced the safety and efficiency of siRNA-based therapies. By addressing off-target effects, immune activation, and delivery challenges, these approaches pave the way for more precise and effective therapeutic applications, particularly in oncology [8–10].

4.1. Chemical Modifications to siRNA

Chemical modifications to siRNA are integral to advancing its application as a therapeutic platform, addressing key challenges such as poor stability, inefficient delivery,

immunogenicity, and off-target effects. Unmodified siRNA is prone to rapid degradation by endogenous nucleases, limiting its half-life and therapeutic efficacy in biological environments. To overcome this limitation, various modifications to the ribose sugar, phosphate backbone and nucleobases have been employed. Notable examples include 2'-O-methyl (2'-OMe) and 2'-fluoro (2'-F) modifications on the ribose sugar, which enhances nuclease resistance while retaining compatibility with the RISC. Additionally, phosphorothioate (PS) linkages, where oxygen atoms in the backbone are replaced with sulfur, further boost stability in serum and facilitate endosomal escape, enhancing intracellular delivery [21,46].

Another challenge with unmodified siRNA is its potential to activate innate immune responses via Toll-like receptors (TLRs), particularly TLR3, TLR7, and TLR8. This immunogenicity can result in cytokine release and systemic inflammation, posing significant safety risks in clinical settings. Modifications such as 2'-OMe and locked nucleic acids (LNAs) in specific regions of the siRNA duplex can mitigate these responses, maintaining target specificity while reducing immune activation. Beyond stability and immunogenicity, chemical modifications significantly improve siRNA specificity, minimizing the unintended silencing of off-target genes. Mismatches between the siRNA guide strand and off-target mRNA sequences can cause undesirable gene silencing, leading to toxicity. Modifications to the seed region (the first 2–8 nucleotides of the guide strand) have been shown to refine complementarity and reduce off-target effects, ensuring precise gene silencing [21,46].

Chemical modifications also play a pivotal role in optimizing siRNA delivery. Enhanced pharmacokinetic and pharmacodynamic properties allow siRNA to integrate more effectively with advanced delivery platforms, such as nanoparticle systems. Ligand conjugation, such as attaching N-acetylgalactosamine (GalNAc) to siRNA molecules, enables targeted delivery to specific cell types, like hepatocytes, through asialoglycoprotein-receptor-mediated endocytosis. This strategy minimizes off-target accumulation in non-relevant tissues, improving therapeutic outcomes. Hybrid RNA-DNA constructs and chemically modified siRNA aptamers are emerging as additional tools to expand delivery efficiency and specificity [21,46].

However, while chemical modifications enhance the therapeutic profile of siRNA, they must be carefully designed to avoid unintended cytotoxicity or impaired silencing function. Over-modification, particularly in the backbone, can disrupt the structural integrity of siRNA, affecting RISC loading or mRNA binding. Therefore, achieving the right balance between stability, immunocompatibility, and functionality is essential during the design phase.

4.2. Nanoparticle-Based Delivery Systems for siRNA Therapeutics

Nanoparticle-based delivery systems are at the cutting edge of siRNA therapeutic advancements, providing innovative solutions to overcome challenges associated with stability, bioavailability, targeted delivery, and biological barriers. These platforms safeguard siRNA from enzymatic degradation, enhance delivery efficiency, and enable tumor-specific targeting. However, each system has inherent trade-offs related to safety, biocompatibility, and efficiency. Here, key nanoparticle technologies and their advancements and limitations in, and their role in, optimizing siRNA delivery are highlighted [20,21].

Probably the most common utilized delivery nanosystem, lipid nanoparticles (LNPs) have emerged as the most clinically advanced vehicles for siRNA delivery, with their success exemplified by FDA-approved therapies such as patisiran [47,48]. LNPs typically consist of ionizable lipids, phospholipids, cholesterol, and PEG-lipids, forming a highly stable delivery system that enables effective siRNA encapsulation and endosomal escape. Recent advancements have introduced ionizable lipids with optimized pKa values to enhance endosomal release and minimize cytotoxicity. However, challenges remain

regarding potential immune activation and off-target effects. Strategies such as PEGylation or lipid chemical modifications have been employed to reduce immunogenicity and extend circulation time [47,48]. As an alternative to LNPs, polymeric nanoparticles, including biodegradable polymers such as poly (lactic-co-glycolic acid) (PLGA) and chitosan, provide a versatile platform for siRNA delivery. These systems offer controlled release profiles and high loading capacities. Innovations such as functionalized polymers with tumor-specific ligands (e.g., folic acid or RGD peptides) have enhanced selective tumor uptake. However, their immunogenic potential and potential for incomplete degradation *in vivo* are areas of active research to ensure safety and biocompatibility [47,48].

Gold nanoparticles (AuNPs) have gathered attention due to their tunable size, ease of functionalization, and inherent biocompatibility. siRNA can be attached to AuNPs through thiol or electrostatic interactions, enabling stable and targeted delivery. Recent innovations involve coating AuNPs with cell-penetrating peptides or tumor-targeting ligands to improve delivery efficiency [49,50]. Despite their promise, concerns regarding long-term accumulation and cytotoxicity must be addressed through rigorous preclinical evaluation. On the other hand, mesoporous silica nanoparticles (MSNs) offer unique advantages such as high surface area and pore volume, which enable efficient siRNA loading and controlled release. Functionalization with tumor-targeting molecules, pH-sensitive gates, or stimuli-responsive materials has been explored to improve their specificity and reduce off-target effects. While promising, their biodegradability and potential immunogenicity remain critical concerns for clinical translation [51,52].

Magnetic nanoparticles provide an innovative means to guide siRNA to target tissues using external magnetic fields. These systems can be functionalized with siRNA and surface ligands for active targeting. Additionally, their use in theranostics (therapy and diagnostics) offers unique opportunities for imaging-guided siRNA delivery. Safety concerns, particularly regarding iron overload and oxidative stress, must be addressed to advance these systems toward clinical applications [53]. In contrast, stimuli-responsive nanoparticles that release siRNA in response to specific triggers, such as pH, temperature, or enzymatic activity, have been developed to enhance precision. For example, pH-sensitive lipid or polymeric systems release siRNA in the acidic tumor microenvironment or endosomes, reducing off-target effects. This approach enhances therapeutic efficacy while minimizing systemic toxicity. Despite their potential, the scalability and robustness of such systems remain challenges for industrial production [20,24,46].

In recent years, researchers have been developing extracellular vesicles (EVs), including exosomes, that comprise a biologically inspired delivery system capable of leveraging the body's natural pathways for siRNA delivery. EVs are inherently biocompatible, exhibit low immunogenicity, and can cross biological barriers such as the blood–brain barrier. Recent advances include engineering EVs with tumor-homing peptides or siRNA cargo modifications to improve targeting efficiency. However, challenges in large-scale production and reproducibility remain barriers to widespread adoption [54,55]. Furthermore, hybrid nanoparticles, combining the benefits of multiple materials (e.g., lipid-polymer or lipid-inorganic hybrids), offer a promising approach to enhance siRNA delivery. These systems integrate the stability of polymers with the functional versatility of lipids or inorganic materials. For instance, lipid-coated PLGA nanoparticles have demonstrated improved endosomal escape and tumor-specific delivery. However, further studies are needed to optimize their physicochemical properties for clinical use [56].

While each nanoparticle system offers distinct advantages, none are without limitations. Immune activation, long-term safety concerns, and scalability challenges persist across platforms. Ongoing research is essential to refine these technologies, focusing on enhancing specificity, biocompatibility, and delivery efficiency. Collaborative efforts between

academia, industry, and regulatory agencies are crucial to translating these advancements into clinically viable siRNA-based therapies.

4.3. Targeting Strategies to Minimize Off-Target Effects

One of the primary challenges in siRNA therapy is the mitigation of off-target effects, which can lead to unintended gene silencing or toxicity in non-target cells. Recent advances in targeting strategies offer innovative solutions to enhance the precision and efficacy of siRNA delivery, significantly reducing its uptake by non-target cells. Below, we detail three key approaches that exemplify the cutting-edge strategies employed to achieve this goal: (1) ligand-based targeting; (2) pH-responsive and stimuli-responsive systems; and (3) cleavable linkers [20,21,41].

Functionalizing nanoparticles or siRNA with ligands that bind specifically to receptors overexpressed on tumor cells has emerged as a highly effective targeting mechanism. Receptors such as transferrin, folate, or specific tumor-associated antigens serve as molecular beacons for ligand-conjugated delivery systems. For example, transferrin-functionalized nanoparticles exploit the high expression of transferrin receptors in rapidly proliferating tumor cells, facilitating selective uptake. Alternatively, folate-conjugated systems leverage the ubiquitous overexpression of folate receptors in certain cancers, ensuring enhanced precision. Last but not least, aptamer-based targeting, which uses short nucleic acid sequences, offers a high degree of specificity by recognizing unique molecular signatures on the tumor surface. This approach minimizes siRNA exposure to normal tissues, reducing systemic toxicity while improving therapeutic outcomes [16,20,57].

Furthermore, tumor microenvironments are characterized by unique conditions, such as acidic pH, hypoxia, and elevated glutathione levels, which can be exploited to enhance delivery specificity. Nanoparticles designed with stimuli-responsive materials release their siRNA payloads in response to these triggers: (1) pH-sensitive materials destabilize under acidic conditions, releasing siRNA specifically in the tumor milieu or within the acidic endosomal compartments of cancer cells, (2) hypoxia-responsive systems utilize oxygen-sensitive linkers or materials that degrade in low-oxygen environments typical of tumors, and (3) glutathione-sensitive nanoparticles release siRNA in the presence of elevated intracellular glutathione levels, common in many cancer cells [58,59]. Such systems reduce off-target delivery by ensuring controlled release at the tumor site while sparing healthy tissues.

One final strategy is the incorporation of cleavable linkers sensitive to intracellular enzymes that provide another layer of delivery specificity. These linkers ensure that siRNA is released only within target cells, minimizing unintended effects in non-target tissues. They can either be enzyme-sensitive linkers, such as those cleaved by cathepsins or esterases, which are activated in the lysosomal or cytosolic environment of target cells, or dual-cleavable linkers, which respond to both pH and enzymatic triggers, further enhancing the precision of release [60,61].

4.4. Advances in Endosomal Escape Mechanisms for siRNA Delivery

Endosomal escape remains one of the most significant hurdles in achieving effective siRNA-based therapeutics. After internalization via endocytosis, siRNA must escape the endosomal compartment to avoid lysosomal degradation and exert its gene-silencing effects in the cytoplasm. Innovations in this field have focused on designing systems and methodologies that enhance endosomal disruption while maintaining safety and efficiency [62,63]. As previously explained, pH-sensitive nanoparticles exploit the acidic environment within endosomes (pH ~5–6) to trigger conformational changes or membrane disruption. These nanoparticles are typically designed with materials that remain

stable in neutral pH but destabilize under acidic conditions, facilitating endosomal escape. For example, ionizable lipids in LNPs acquire a positive charge in acidic environments, promoting interaction with negatively charged endosomal membranes, which results in destabilization and the release of the siRNA payload. Additionally, polymers like poly(β -amino esters) exhibit pH-dependent swelling or degradation, aiding in the rupture of endosomal membranes [62,63].

Incorporating fusogenic peptides or membrane-disrupting polymers into delivery systems has proven highly effective in improving endosomal escape. Fusogenic peptides, such as those derived from viral proteins, mimic the mechanisms viruses use to enter the cytoplasm, destabilizing endosomal membranes through lipid bilayer fusion. Similarly, cationic polymers like polyethyleneimine (PEI) exert a “proton sponge effect”, causing osmotic swelling and endosomal rupture. Advances in peptide engineering have also led to the development of pH-responsive and tumor-targeted fusogenic peptides that selectively enhance escape while minimizing systemic toxicity [60,62,63].

Emerging technologies such as photothermal or ultrasound-assisted endosomal escape have gained attention for their ability to trigger endosomal disruption through external stimuli. Photothermal approaches employ nanoparticles that convert light energy into heat, which destabilizes endosomal membranes. For instance, gold nanoparticles or carbon nanotubes functionalized with siRNA are irradiated with near-infrared light, generating localized heat to release their cargo. Similarly, ultrasound-assisted methods utilize microbubbles or nanoparticles that respond to ultrasonic waves, producing mechanical forces or heat that disrupt endosomes. These strategies are particularly attractive for localized and precise delivery applications [60,62,63].

While these innovations represent significant progress, challenges remain in optimizing their safety profiles and scalability. pH-sensitive systems must balance efficacy with the risk of premature release or nonspecific interactions, while membrane-disrupting polymers and peptides need further refinement to reduce cytotoxicity and immunogenicity. Photothermal and ultrasound techniques, though promising, require the precise control of external stimuli to avoid tissue damage.

5. Nanoparticle-Assisted Targeting of Oncogenes in Combinatorial Therapy

The efficacy of siRNA-drug combination therapies is heavily influenced by the identification of appropriate gene targets and, critically, the development of sophisticated delivery systems [1,2,64]. Nanoparticle-based platforms have become the leading method for siRNA delivery, providing a versatile tool to co-deliver both siRNA and chemotherapeutic agents by tuning their physical and chemical properties [8,20,21]. This section delves into the selection of key gene targets for siRNA-drug combinatorial therapies and the pivotal role that nanoparticle systems play in enhancing intracellular delivery, stability, and therapeutic outcomes.

Gene targets for siRNA in cancer therapy can be broadly classified into three categories: (1) genes involved in drug resistance; (2) genes essential for tumor survival, and (3) genes selectively expressed in cancerous tissues [24,64] (Figure 3). However, one of the major obstacles to effective siRNA-based therapy is the inherent instability of siRNA molecules in biological systems. Their rapid degradation in the bloodstream, short half-life due to renal clearance, and potential for immune activation compromise therapeutic efficacy [7,18,64]. As explained in the previous section, nanoparticles address these limitations by providing a protective carrier for siRNA, ensuring enhanced delivery, cellular uptake, and stability. The ability to functionalize nanoparticles with ligands, such as hyaluronic acid (HA), peptides, or antibodies, enables the precise targeting of cancer cells via ligand–receptor interactions.

This targeted approach significantly enhances the accumulation of nanoparticles at tumor sites, reducing off-target effects and limiting the toxicity commonly seen with conventional therapies [16,20,21]. Nanoparticles can also be engineered for stimulus-responsive release, enabling controlled drug and siRNA delivery based on specific conditions in the tumor microenvironment (Figure 3). As an example, pH-sensitive nanoparticles release their cargo in response to the acidic environment commonly found in tumors, enhancing localized therapeutic effects while minimizing systemic toxicity [8,20]. This stimulus-responsive behavior allows for precise control over drug and siRNA bioavailability at the tumor site, further enhancing the therapeutic index.

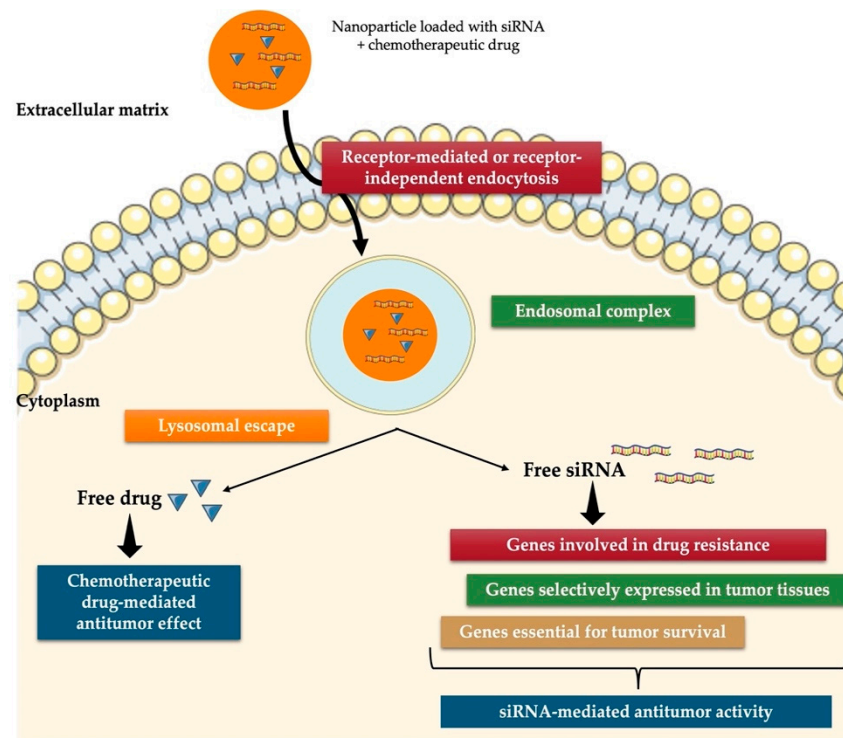


Figure 3. Mechanism of action for siRNA-drug combination therapy in cancer treatment. The nanoparticles are engineered to carry both siRNA and chemotherapeutic agents, facilitating targeted delivery to cancer cells. These nanoparticles are taken up by tumor cells through receptor-mediated or receptor-independent endocytosis. Once inside the cell, these systems must escape from the endosomal complex to prevent degradation in the lysosomes. Successful escape releases both siRNA and the chemotherapeutic drug into the cytoplasm. On the one hand, the (free) siRNA silences specific genes involved in tumor survival proliferation or drug resistance by binding to mRNA and preventing protein synthesis. This gene silencing inhibits critical pathways for cancer cell growth and survival, such as genes uniquely expressed in tumor tissues or those involved in resistance mechanisms. On the other hand, the released chemotherapeutic drug exerts its antitumor effect, attacking cancer cells through traditional cytotoxic mechanisms. The combination of siRNA-mediated gene silencing and drug-induced cytotoxicity enhances the overall therapeutic efficacy, helping to overcome drug resistance and improve patient outcomes. This dual approach also targets tumors more precisely while minimizing off-target effects and damage to healthy tissues.

Various nanoparticle types, including LNPs, polymeric nanoparticles, dendrimers, and MSNs, have been extensively explored for this purpose. Among these, LNPs are the most clinically advanced and have shown efficacy in delivering siRNA in multiple cancer models. For instance, LNPs loaded with siRNA targeting KRAS mutations have demonstrated marked success in reducing tumor growth in models of lung cancer [34]. The protective lipid bilayer of LNPs not only shields siRNA from degradation but can also be modified

with surface ligands to enhance the targeting of specific tumor cells [21,65]. Polymeric nanoparticles, such as those made from PLGA, provide another robust delivery strategy. These systems can co-encapsulate both siRNA and chemotherapeutics, enabling synchronized controlled release at the tumor site, and enhancing therapeutic synergy [8,20,21]. A notable example is PLGA nanoparticles co-loaded with siRNA targeting FAK (focal adhesion kinase), and the chemotherapeutic drug paclitaxel, which demonstrated enhanced apoptosis in epithelial ovarian cancer cells through their combined action [66].

The co-delivery potential of nanoparticle systems represents a significant advance in cancer therapy, as they follow the simultaneous targeting of multiple pathways involved in cancer progression and drug resistance [16,20] (Figure 3). MSNs, with their high surface area and pore volume, are particularly well-suited for loading both small molecule drugs and siRNA. Functionalizing with ligands, such as antibodies or peptides, improves tumor-specific delivery [28], as demonstrated by studies in which MSNs loaded with siRNA targeting ABCB1 and the chemotherapeutic agent doxorubicin successfully reversed multidrug resistance in cervix cancer cells [67], greatly enhancing the cytotoxic effect of doxorubicin [36]. By synchronizing the release of both agents, nanoparticle-based systems can achieve a more potent therapeutic effect while potentially lowering the required doses of each drug, reducing toxicity.

The success of nanoparticle-based delivery systems is contingent on efficient cellular uptake and the ability to release their therapeutic payloads within the target cells [57]. Many nanoparticles exploit receptor-mediated endocytosis to enter cancer cells by targeting overexpressed receptors such as transferrin or folate receptors. Once internalized, these systems must escape the endosomal compartment to release their siRNA payloads into the cytoplasm (Figure 3), where RISC can process and execute gene silencing [7,9,10]. pH-sensitive nanoparticles can trigger endosomal escape by destabilizing the endosomal membrane in response to acidic environment, facilitating siRNA release into the cytoplasm [16,22,33]. Cationic lipids, frequently used in LNPs, also promote endosomal escape through mechanisms like membrane fusion or pore formation, enhancing the bioavailability of siRNA inside the target cells [22,43].

Despite the promise of nanoparticle-based systems, several challenges remain, such as avoiding rapid clearance by the reticuloendothelial system (RES), achieving adequate tumor penetration, and minimizing off-target effects [8,9,19]. Surface modification strategies, such as PEGylation (coating nanoparticles with PEG), can extend circulation time by reducing immune system recognition and clearance. Additionally, stimuli-responsive nanoparticles that release their payload in response to specific tumor microenvironment cues, such as low pH or high glutathione levels, provide enhanced control over delivery and reduce systemic side effects [41].

In summary, nanoparticle-based delivery systems have revolutionized siRNA-based combinatorial therapies by addressing critical challenges associated with the stability, specificity, and co-delivery of therapeutic agents. These platforms offer innovative solutions for the precise targeting and silencing of key genes involved in drug resistance, tumor survival, and cancer progression, thereby enhancing the efficacy of siRNA in combination with conventional chemotherapeutics. Continued advancements in nanoparticle design and functionalization hold significant promise for translating these combination therapies into clinical practice, opening new avenues in precision oncology.

5.1. Suppressing Cancer Resistance Genes with siRNA: Enhancing Chemotherapy Efficacy

Drug resistance remains a critical obstacle in cancer therapy, often leading to therapeutic failure and poor patient prognosis. Resistance arises from a range of molecular mechanisms, including drug efflux, enhanced DNA repair, mutations in drug targets, and

the evasion of apoptosis. Key genes involved in these processes play significant roles in diminishing the effectiveness of anticancer drugs [27,33,68].

Among these ABC transporters, particularly ABCB1 (also known as MDR1 or P-gp) and ABCG2 (Breast Cancer Resistance Protein, BCRP), are well-established mediators of multidrug resistance in cancer. These membrane-bound proteins actively pump chemotherapeutic drugs out of cancer cells, lowering intracellular drug concentrations and thereby reducing their cytotoxic effects [27,65,69]. ABCB1 is a notable efflux transporter associated with resistance to several key chemotherapeutic agents, including paclitaxel, doxorubicin, and vinblastine [27,65,69]. The overexpression of ABCB1 is frequently documented in cancers, such as ovarian [29], breast [70], and colorectal cancer [71]. For example, high ABCB1 expression in ovarian cancer cells has been linked to reduced sensitivity to paclitaxel, a standard chemotherapeutic agent [29]. More recently, Yang et al. (2021) demonstrated that combining MDR1-targeted siRNA with doxorubicin in human serum albumin-based nanoparticles in MCF-7/ADR mouse model suppressed MDR1's protein expression, thereby promoting the retention of doxorubicin within the cancer cells, leading to increased cytotoxicity. The combined action of doxorubicin-induced cytotoxicity and the MDR1 siRNA-mediated reversal of drug resistance results in enhanced tumor cell death and the inhibition of tumor growth [72]. This dual approach prevents efflux-mediated resistance while maintaining the chemotherapeutic efficacy of doxorubicin [28]. ABCG2 plays a similar role in drug resistance, particularly against agents like mitoxantrone, topotecan, and methotrexate [73]. It is highly expressed in various cancers, including colon carcinoma and lung cancer [74], where it confers resistance to topoisomerase inhibitors [75]. A study by Bai and coworkers (2015) using siRNA to target ABCG2 in combination with Adriamycin in MCF-7 breast cancer cells demonstrated that silencing ABCG2 enhanced Adriamycin drug retention and potentiated its cytotoxic effect [76]. The siRNA-mediated silencing of ABC transporters effectively inhibits their synthesis, thereby restoring intracellular drug accumulation. By preventing active drug efflux, this approach ensures that chemotherapeutic agents, such as doxorubicin or paclitaxel, remain sequestered within cancer cells for prolonged periods, allowing sufficient intracellular concentrations to induce robust cytotoxic effects. This combinatorial strategy not only overcomes transporter-mediated drug resistance, but also maximizes the therapeutic efficacy of conventional anticancer agents, highlighting the potential of siRNA-based interventions in multidrug-resistant cancers.

Another key mechanism of resistance involves the upregulation of DNA repair enzymes, such as PARP1 and ERCC1 (Excision Repair Cross-Complementation Group 1). These proteins are essential for maintaining genomic stability, but also contribute to resistance against DNA-damaging agents, including platinum-based chemotherapies and PARP inhibitors [25,26]. PARP1 plays a vital role in the base excision repair pathway [25,26], and its overexpression is associated with resistance to both PARP inhibitors, such as Olaparib [77], and platinum drugs, such as cisplatin [78]. In cancers harboring BRCA1/2 mutations, PARP inhibitors have shown clinical efficacy, though resistance frequently emerges due to upregulated PARP1 activity [30,79]. siRNA-based therapies provide a promising approach to address this resistance by selectively targeting DNA repair genes. By silencing key repair factors like PARP1 and ERCC1, siRNA can sensitize cancer cells to DNA-damaging agents, including cisplatin and doxorubicin. The knockdown of these genes impairs cancer cells' ability to repair chemotherapy-induced DNA damage, thereby enhancing the efficacy of the treatment and overcoming chemoresistance. This strategy exploits the reliance of resistant cancer cells on hyperactivated repair pathways, leading to synthetic lethality and selective tumor cell death, particularly in repair-deficient tumors. A 2018 study demonstrated that siRNA-mediated PARP1 silencing significantly enhanced the efficacy of docetaxel in prostate cancer cells by promoting apoptosis through the suppres-

sion of EGFR and reduced the phosphorylation of GSK3 β [80]. This effect was primarily achieved via the inhibition of the EGF/Akt/FOXO1 signaling pathway [31], highlighting the role of PARP1 in sustaining survival pathways in resistant cancer cells. Similarly, ERCC1, a crucial component of the nucleotide excision repair (NER) pathway, is essential for repairing DNA crosslinks induced by platinum-based chemotherapeutics [81]. Elevated ERCC1 expression has been strongly correlated with resistance to platinum drugs, particularly in non-small cell lung cancer (NSCLC) [82,83] and other solid tumors [84,85]. In a recent study, Xie and colleagues (2021) demonstrated that siRNA-mediated ERCC1, in combination with olaparib, synergistically enhanced cisplatin sensitivity in NSCLC cells [86]. Mechanistically, ERCC1 silencing induced cell cycle arrest at the G1 phase and triggered apoptosis through the PI3K/AKT-caspase-3 signaling pathway [87]. These findings underscore the therapeutic potential of targeting DNA repair mechanisms with siRNA to overcome chemoresistance and sensitize cancer cells to DNA-damaging agents.

Resistance to apoptosis, a defining hallmark of cancer, is frequently driven by the overexpression of anti-apoptotic proteins such as Bcl-2 and Mcl-1 (myeloid cell leukemia 1). These proteins suppress the intrinsic apoptotic pathway, enabling cancer cells to evade programmed cell death in response to therapeutic agents [88–90]. Bcl-2, for instance, prevents mitochondrial outer membrane permeabilization (MOMP), a critical step in apoptosis [88,90]. Its overexpression is closely associated with resistance to chemotherapeutics such as cisplatin [91], venetoclax [32], and doxorubicin [36], particularly in cancers such as chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphoma [92]. Recent advancements have explored nanoparticle-based co-delivery systems to target Bcl-2. For example, Zhou et al. (2018), demonstrated that co-delivering doxorubicin with siRNA targeting Bcl-2 significantly enhanced apoptosis induction, underscoring the synergistic potential of this combination therapy [93]. Additionally, Vu and coworkers (2020) reported that silencing Bcl-2 with specific siRNA, in combination with doxorubicin, promoted both apoptosis and autophagy through Bcl-2 downregulation and the upregulation of key autophagy markers ATG5 (Autophagy Protein 5) and Beclin 1 [94]. Similarly, Mcl-1, another member of the Bcl-2 family, is a pivotal regulator of chemoresistance, particularly in multiple myeloma, acute myeloid leukemia (AML) [88–90], and breast cancer [95]. High Mcl-1 levels have been linked to resistance against various therapies, including anthracyclines, proteasome inhibitors, and BH3-mimetics [32,88,89]. Recent studies indicated that silencing Mcl-1 using siRNA enhances the efficacy of anticancer drugs, such as andrographolide nanosuspensions [96] and doxorubicin [97], by promoting apoptosis in MCF-7 breast cancer cells. Similarly, the co-delivery of the lipid-based delivery system, composed of an siRNA targeting Mcl-1 and gemcitabine, exhibited increased cellular uptake, enhanced Mcl-1 downregulation efficacy, and inhibited the tumor in both in vitro and in vivo carcinoma models, as demonstrated by Wang and colleagues (2019) [98]. Collectively, silencing key antiapoptotic proteins like Bcl-2 and Mcl-1 using siRNA in combination with chemotherapeutic agents effectively disrupts critical survival pathways, enhances the apoptotic response, and helps to overcome drug resistance mechanisms. These findings highlight the therapeutic potential of targeting apoptotic regulators to improve treatment outcomes in resistant cancers.

Oncogenic mutations and the overactivation of key signaling pathways, such as KRAS and PI3K/AKT/mTOR, are pivotal drivers of cancer cell proliferation, survival, and drug resistance [99–101]. Mutations in the KRAS gene are particularly prevalent in various cancers, including pancreatic, colorectal, and lung cancers [34]. These mutations result in the constitutive activation of the RAS-MAPK signaling cascade, which drives uncontrolled cell growth, enhances survival mechanisms, and contributes to resistance against targeted therapies and chemotherapy agents [101,102]. Mechanistically, KRAS mutations impair

GTPase activity, keeping KRAS in its active GTP-bound state, which continuously stimulates downstream effectors such as RAF, MEK and ERK, fostering oncogenic signaling. In addition, KRAS mutations interact with the PI3K/Akt/mTOR pathway, a critical signaling axis regulating cell metabolism, survival, and proliferation. Crosstalk between these pathways amplifies drug resistance and adaptive responses, making them prime therapeutic targets [101]. To address this, combinatorial strategies involving the siRNA-based silencing of KRAS have shown considerable promise. Zarredar et al. (2019) demonstrated that the combination of KRAS-targeted siRNA with the novel EGFR inhibitor AZD8931 in A549 lung adenocarcinoma cells synergistically induced apoptosis and reduced cell proliferation rates [35]. This synergy likely arises from the simultaneous disruption of KRAS-driven MAPK signaling and EGFR-mediated survival pathways, effectively limiting compensatory signaling loops. Similar findings were reported in a recent study combining KRAS-specific siRNA with the pan-PI3K inhibitor GDC-0941, which targets both the PI3K/Akt/mTOR and RAS signaling pathways in <https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/ovary-cancer> (accessed on 24 December 2024) ovarian cancer cells [103]. This dual inhibition exerted synergistic antitumor effects by simultaneously blocking KRAS-dependent proliferation and PI3K-drive survival signaling, preventing pathway redundancy and adaptive resistance. These findings underscore the potential of combinatorial siRNA-based therapies to target key oncogenic nodes, dismantling critical signaling networks that drive tumor progression and therapeutic resistance.

The PI3K/AKT/mTOR signaling pathway is a central regulator of cellular processes, including growth, proliferation, metabolism, and survival. The hyperactivation of this pathway, often resulting from genetic mutations, amplifications, or the overexpression of its components, is a hallmark of many cancers. Such hyperactivation not only promotes tumorigenesis, but also confers resistance to a range of treatments, including chemotherapy, radiotherapy, and targeted agents [99,101,104]. The molecular mechanism underlying this resistance involves enhanced survival signaling, reduced apoptotic responses, and increased DNA repair efficiency, which collectively sustain tumor viability under therapeutic stress. Recent advancements underscore the critical role of targeting the PI3K/Akt/mTOR axis to overcome therapeutic resistance. Notably, a genome-wide siRNA screen conducted by Gupte and coworkers (2015) in genetically engineered murine models of human osteosarcoma provided compelling evidence for the therapeutic potential of this approach. The study integrated genetic and chemical screens with and without the DNA-damaging doxorubicin. Both approaches revealed a significant overlap, with a profound enrichment of the PI3K and mTOR pathways as critical nodes in osteosarcoma survival. The inhibition of these pathways, either through siRNA-mediated knockdown or pharmacological inhibitors, led to heightened apoptosis and notable tumor regression. Further mechanistic insight suggests that targeting PI3K/Akt/mTOR reduces survival signaling and sensitizes cancer cells to apoptosis by downregulation and antiapoptotic proteins (e.g., Bcl-2) and impairing DNA repair pathways [105].

Targeting genes that contribute to drug resistance, such as ABC transporters, DNA repair enzymes, apoptosis regulators, and components of oncogenic signaling pathways, represents a promising strategy for enhancing the efficacy of cancer therapies. Clinical trials leveraging siRNA to downregulate these genes are showing encouraging results, providing a foundation for the development of new combination therapies that could overcome resistance and improve outcomes for cancer patients. Continued research and clinical validation will be critical to fully realize the potential of siRNA-based strategies in overcoming drug resistance.

5.2. Enhancing Cancer Cell Death by Silencing Survival Genes with siRNA and Chemotherapy

Tumor survival is sustained by a complex network of genes that prevent apoptosis, enable unchecked cell cycle progression, and facilitate adaptation to the hostile tumor microenvironment. In many cancers, these survival pathways are constitutively active, enabling malignant cells to resist conventional treatments, evade immune surveillance, and adapt to metabolic stress [90,101,106]. Critical mediators of these processes include the Bcl-2 family of proteins, inhibitors of apoptosis proteins (IAPs), and regulators of hypoxia responses such as HIF-1 α [90,101,107], which are promising targets for siRNA-based therapies.

The Bcl-2 family is pivotal in regulating mitochondrial-mediated apoptosis, controlling the balance between pro-apoptotic proteins (such as BAX and BAK) and anti-apoptotic proteins (such as Bcl-2, Bcl-xL, and Mcl-1). The overexpression of antiapoptotic members, such as Bcl-2, is a hallmark of many cancers, contributing to resistance against therapies that induce apoptosis [90,92]. For example, Bcl-2 is overexpressed in numerous hematologic malignancies, including CLL and follicular lymphoma, where it inhibits the release of cytochrome c from mitochondria, blocking the caspase cascade required for apoptosis [90,92]. Recent studies highlight the potential of siRNA targeting Bcl-2 to enhance the efficacy of standard chemotherapeutic agents [32,90]. For example, preclinical investigations demonstrated that combining Bcl-2-specific siRNA with doxorubicin or AT-101 (a Bcl-2 inhibitor) significantly increased apoptosis in MCF-7 breast cancer [12] and head and neck cancer cells [11]. Additionally, a 2021 phase III trial (NCT04269902) demonstrated that venetoclax, in combination with Obinutuzumab, significantly improved progression-free survival in relapsed or refractory CLL [108]. While this trial did not directly involve siRNA, the findings highlight the potential of targeting Bcl-2, which could be further optimized by siRNA-based approaches to downregulate Bcl-2 expression more specifically and effectively [109]. Mcl-1, another member of the Bcl-2 family, is frequently overexpressed in breast cancer, multiple myeloma, and AML, where it acts as a key survival factor by sequestering pro-apoptotic proteins and preventing apoptosis [88,95]. Unlike Bcl-2, Mcl-1's rapid turnover and dynamic regulation make it particularly challenging to target. siRNA targeting Mcl-1 has been shown to reduce tumor growth and enhance sensitivity to BH3 mimetics, a class of drugs that inhibit anti-apoptotic proteins [110]. For example, preclinical studies have demonstrated that Mcl-1 siRNA can synergize with chemotherapeutic agents like bortezomib and doxorubicin to induce apoptosis in resistant multiple myeloma cells [111] and breast cancer cells [97]. Recently, ABBV-467, a highly selective Mcl-1 inhibitor, has entered a phase I clinical trial (NCT04178902). ABBV-467 was shown to trigger rapid mechanism-based apoptosis in Mcl-1-dependent tumor cells and inhibit tumor growth in xenograft models of multiple myeloma and AML. The phase I trial revealed that, while ABBV-467 demonstrated disease control in one patient, it also caused increased plasma cardiac troponin levels in 4 of 8 patients, likely reflecting a class effect of Mcl-1 inhibition [112]. These findings highlight both the promise and challenges of targeting Mcl-1 in cancer therapy. Incorporating the targeting of Mcl-1 by siRNA could enhance specificity, reduce off-target effects, and overcome limitations of small-molecule inhibitors.

IAPs are a family of proteins that suppress apoptosis by directly inhibiting caspases, the central executioners of the apoptotic pathway. Among the most studied IAPs are XIAP (X-linked inhibitor of apoptosis protein), survivin (BIRC5), and c-IAP1/2 (cellular inhibitor of apoptosis proteins 1 and 2). These proteins are often overexpressed in cancers, contributing to therapy resistance by preventing the execution of apoptosis [113,114]. Survivin, in particular, is highly expressed in most cancers, including lung, colorectal, and breast cancers, while being minimally expressed in normal adult tissues [115]. Survivin not only inhibits apoptosis by suppressing caspase-3 and -7, but also regulates cell division

and the mitotic spindle checkpoint [116–118]. siRNA targeting survivin has been shown to reduce cancer cell proliferation, enhance apoptosis, and sensitize tumors to chemotherapy and radiotherapy in various preclinical models [119–121]. For instance, survivin siRNA enhanced the cytotoxic effects of paclitaxel in NSCLC cell lines [122,123]. A phase I clinical trial explored the use of LY2181308, an antisense oligonucleotide targeting survivin mRNA, in patients with advanced solid tumors [124]. Although the trial did not meet its primary endpoint, it provided valuable insights into the challenges of targeting survivin and the need for more effective delivery systems, potentially using siRNA with nanoparticle carriers for improved stability and tumor targeting. Similarly, siRNA targeting XIAP has shown promise in sensitizing cancer cells to chemotherapeutic agents like methotrexate and cytarabine [125–127]. Xu et al. (2022) designed a preclinical study using XIAP siRNA combined with a naturally occurring anticancer agent (gambogic acid) in a chitosan-based cationic nanoemulsion and demonstrated enhanced apoptosis lung cancer cell models [128], suggesting a potential strategy for overcoming resistance to conventional therapies.

HIF-1 α is a transcription factor that is critical for cellular responses to hypoxia, a hallmark of the tumor microenvironment. Through stabilization under hypoxic conditions, HIF-1 α transactivates genes involved in angiogenesis, glycolysis, and cell survival. The overexpression of HIF-1 α is associated with poor prognosis in many cancers, including breast, prostate, and pancreatic cancers [129–131]. siRNA targeting HIF-1 α has emerged as a promising strategy to inhibit tumor adaption to hypoxia [129,131]. Preclinical studies have shown that siRNA against HIF-1 α reduces angiogenesis, tumor growth, and metastasis [42,132,133]. For instance, HIF-1 α siRNA combined with gemcitabine, a first-line chemotherapeutic drug, enhanced anti-tumor activity and reduced metastasis in pancreatic cancer mouse models [42]. In 2013, a pilot trial investigated the use of EZN-2968, an antisense oligonucleotide targeting HIF-1 α , in patients with refractory solid tumors [134]. Although siRNA specifically targeting HIF-1 α has not yet reached clinical trials [134], this study provided the proof of concept of the potential of targeting hypoxia pathways to improve treatment outcomes, especially when combined with conventional therapies.

The development of siRNA-based combinatorial therapies targeting tumor survival genes represents a transformative strategy to overcome resistance and improve treatment efficacy. Targeting the Bcl-2 family, IAPs, and HIF-1 α with siRNA holds significant promise, particularly when integrated into nanoparticle delivery systems for enhanced specificity and stability. While challenges remain, including delivery efficiency and regulatory approval, innovative approaches combining siRNA with chemotherapeutic agents are paving the way for more effective and durable cancer treatments.

5.3. Exploiting Tumor-Specific Gene Expression for Targeted Therapy

Targeting genes that are selectively expressed or overexpressed in cancerous tissues, while minimally or not at all expressed in normal tissues, represents a highly promising strategy for developing effective, precise, and less toxic anticancer therapies. This specificity reduces the risk of off-target effects, minimizes collateral damage to healthy cells, and enhances the therapeutic index of the treatment [135,136]. Engineered siRNA can silence these cancer-specific genes, halting tumor progression, inducing apoptosis, or sensitizing tumors to other therapeutic agents [7,18,33]. Below, we provide an overview of key oncogenic targets, their roles in cancer, and advancements in siRNA-based therapies, including combination approaches and recent preclinical or clinical studies exploring their potential.

VEGF is a master regulator of angiogenesis, enabling the formation of new blood vessels to sustain tumor growth and metastasis by supplying oxygen and nutrients to rapidly dividing cancer cells. VEGF is overexpressed in several cancers, including glioblastoma, renal cell carcinoma, and colorectal cancer [137,138]. siRNA targeting VEGF downregulates

angiogenic pathways, effectively reducing tumor vascularization and growth. For example, preclinical studies demonstrate that VEGF-siRNA, when combined with chemotherapy agents like itraconazole or paclitaxel, significantly inhibited tumor burden in breast [139] and lung cancer models [14,140]. Molecularly, VEGF silencing interrupts the VEGF receptor (VEGFR) signaling cascade, leading to endothelial cell apoptosis and impaired tumor vascularization. A phase I clinical trial involving ALN-VSP, a lipid nanoparticle formulation of siRNA targeting VEGF and kinesin spindle protein (KSP), provided early evidence of clinical applicability [37]. However, challenges related to delivery efficiency highlighted the need for improved delivery strategies, such as exosome-based systems, which offer increased biocompatibility and precision.

EGFR, a receptor tyrosine kinase, activates signaling pathways like MAPK, PI3K/AKT, and JAK/STAT, promoting the proliferation, survival, and migration of cancer cells. Overexpressed or mutated EGFR is prevalent in cancers, such as NSCLC, head and neck squamous cell carcinoma (HNSCC), and colorectal cancer [101,141–143]. siRNA targeting EGFR disrupts these oncogenic pathways, inhibiting tumor cell proliferation and survival. For instance, EGFR silencing enhances NSCLC sensitivity to tyrosine kinase inhibitors (TKIs) like cetuximab [40] and gefitinib [144]. Mechanistically, EGFR-siRNA downregulates receptor phosphorylation, effectively blunting downstream signaling. A recent study by Majumder and Minko (2021) demonstrated a 10-fold improvement in anticancer activity when EGFR-targeting siRNA was co-delivered with gefitinib and paclitaxel using lipid nanoparticles [145], highlighting the potential of combining siRNA with traditional therapies to overcome resistance.

MUC1, a transmembrane glycoprotein, is aberrantly glycosylated and overexpressed in adenocarcinomas, including breast, ovarian, pancreatic, and lung cancers. MUC1 promotes tumor progression, metastasis, and therapeutic resistance by activating signaling pathways like PI3K/Akt and NF- κ B [146–148]. siRNA targeting MUC1 has been shown to sensitize tumors to chemotherapeutic agents [149–151] and enhance immune responses [152]. For example, MUC1-siRNA improves the efficacy of trastuzumab in breast cancer cells [150], and lapatinib in endometrial cancer cells [151], by inducing apoptosis and suppressing proliferation. In 2018, in a preclinical study, Liu et al. (2018) investigated the effects of an MUC1-targeted mRNA nano-vaccine in combination with immune checkpoint inhibitors (CTLA-4) in triple-negative breast cancer 4T1 cells. Preliminary results suggested enhanced in vivo T-cell immune responses and tumor regression, supporting the potential of MUC1 as a therapeutic target. Molecularly, MUC1 silencing disrupts its interaction with β -catenin, impairing tumor cell invasiveness and stemness [153].

c-MET, or hepatocyte growth factor receptor (HGFR), is another critical oncogenic driver. The overexpression or mutation of c-MET is associated with enhanced tumor growth, angiogenesis, and metastasis in several cancers, including gastric, lung, and liver cancers. [154–156]. As an example, c-MET-targeted siRNA has been shown to inhibit cellular growth and the invasion of hepatocellular carcinoma (HCC) cells by downregulating the MEK/ERK pathway [157], which is often upregulated in these cells. Nonetheless, in gastric cancer MKN-5 cells, although the inhibition of c-Met's significantly diminished the phosphorylation levels of PI3K and Akt, it did not enhance the cells' sensitivity to gefitinib [158]. In opposition to this, Zhang and colleagues (2020) demonstrated that an siRNA-targeting c-MET delivered via exosome into gastric cancer cells was able to decrease cellular drug resistance, thus sensitizing these cells to cisplatin [159]. In recent years, two phase I clinical trials have tested the efficacy of two inhibitors of c-MET, HS-10241 [160] and ABN401 [161], in patients with advanced NSCLC. Even though neither of these trials involved the use of an RNA-based therapy, preliminary results indicated that

both inhibitors had an acceptable safety profile and good tolerability for the patients, while simultaneously offering promising antitumor activity against solid tumors [160,161].

HER2 is overexpressed in approximately 20–25% of breast cancers, as well as in some gastric, ovarian, and bladder cancers, and is associated with aggressive disease, poor prognosis, and resistance to standard therapies [38,162,163]. siRNA targeting HER2 effectively suppresses cell proliferation [164,165] and sensitizes tumors to HER2-targeted therapies, such as trastuzumab [166]. Mechanistically, HER2-siRNA reduces receptor expression, limiting downstream PI3K/Akt signaling and induces apoptosis. In a preclinical study performed by Archana and coworkers (2024), HER2-siRNA loaded into nanoparticles co-delivered with doxorubicin demonstrated significant antitumor activity in HER2-positive SKBR3 breast cancer cells as it resulted in enhanced cytotoxicity via the induction of apoptosis and the outstanding downregulation of HER2 [167]. Additionally, Ngamcherdtrakul et al. (2022) developed a single therapy based on trastuzumab-conjugated nanoparticles for the co-delivery of docetaxel and siRNA against HER2 in a drug-resistant orthopedic HER2⁺ HCC1954 tumor mouse model, which significantly inhibited tumor growth [39], highlighting the promise of integrating siRNA into multimodal treatment regimens.

Targeting selectively expressed or overexpressed genes in cancer provides a powerful approach to enhance therapeutic efficacy while minimizing off-target effects. Advances in siRNA engineering and delivery systems, particularly nanoparticle- and exosome-based platforms, are driving the clinical translation of these strategies. By addressing barriers such as delivery, stability, and tumor specificity, siRNA-based therapies hold immense potential for reshaping cancer treatment paradigms.

6. Regulatory Framework for siRNA-Based Combinatory Therapies in Cancer

The development of combination therapies using siRNA and anticancer drugs is at the cutting edge of oncology, offering new avenues of precision medicine. However, the path from bench to bedside is complex, with significant regulatory hurdles. Ensuring safety, efficacy, and quality is critical for obtaining regulatory approval from agencies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [168–170].

The FDA and EMA have established comprehensive guidelines to regulate the development and approval of RNA-based therapeutics, including siRNA. The guidelines focus on demonstrating a favorable risk–benefit profile, establishing robust manufacturing processes, and conducting rigorous preclinical and clinical testing [171–177]. However, combining siRNA with traditional anticancer drugs adds another layer of complexity, necessitating adherence to multiple regulatory frameworks. siRNA therapies are classified under “Advanced Therapy Medicinal Products” (ATMPs) in the European Union and “Drug/Biologic Products” in the United States depending on their mechanism of action and the presence of chemical modifications or delivery vehicles like nanoparticles. This classification affects the regulatory pathway, as ATMPs must meet stringent quality standards, undergo detailed characterization, and provide comprehensive safety and efficacy data [173,178,179]. When siRNA is combined with conventional drugs, the regulatory process typically follows the more stringent pathway, which requires evidence for both the individual and combined effects of the therapeutic agents.

Preclinical studies are critical for establishing the foundation of any new therapeutic strategy. For siRNA-drug combinations, preclinical data must demonstrate the mechanism of action, pharmacokinetics (PK), pharmacodynamics (PD), toxicity, and proof of concept in relevant models. Both the siRNA and the anticancer drug must be evaluated separately and in combination to assess potential synergistic or antagonistic effects. One of the primary

challenges is demonstrating the specificity and selectivity of siRNA for its target gene. Off-target effects must be thoroughly investigated, as they can lead to unintended gene silencing and toxicity [173,178,179]. Furthermore, the delivery system, often involving nanoparticles, needs to be evaluated for its own safety profile, distribution characteristics, and potential immunogenicity. The combination's pharmacokinetic and pharmacodynamic profiles must also be carefully studied. The interplay between the siRNA's gene-silencing effects and the pharmacological action of the drug must be clearly understood to optimize dosing regimens. Animal models should mimic human physiology as closely as possible, especially concerning tumor microenvironment, to provide reliable safety and efficacy data [172–178].

Clinical trials for siRNA-drug combination therapies must follow a well-defined phased approach typically involving phase I, II, and III trials. The design of these trials must consider both the individual agents and their combined effects. Early-phase trials focus on safety, tolerability, and dose optimization. Phase I trials should aim to determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of the combination. The trial design should account for potential adverse events arising from both the siRNA and the drug, including immunogenic responses to siRNA or its delivery vehicle. Phase II and III trials focus on efficacy, often requiring endpoints to assess the therapeutic benefits of combination therapy [172,175,178]. Traditional endpoints, such as progression-free survival (PFS) and overall survival (OS), may not fully capture the benefits of siRNA-drug combinations, particularly if they target specific molecular pathways. Biomarker-driven studies may be necessary to identify patient populations most likely to benefit from the therapy, supporting a more personalized approach to treatment. Additionally, the FDA and EMA emphasize the need for adaptive trial designs that allow modifications based on interim results [180,181]. Given the novel mechanisms of action of siRNA, trial designs may incorporate a broader range of safety endpoints, including immune responses, off-target effects, and long-term follow-up for delayed adverse events.

Ensuring the quality and consistency of siRNA and its delivery systems presents unique challenges. siRNA therapeutics require precise chemical synthesis, sequence verification, and stringent controls over chemical modifications to maintain stability and avoid degradation [171–174]. The manufacturing process must be validated to ensure reproducibility and batch-to-batch consistency. The delivery vehicle, often a lipid nanoparticle (LNP)- or polymer-based system, must also be rigorously characterized for size, charge, encapsulation efficiency, and biocompatibility [182]. The production of combination therapies necessitates integrated manufacturing processes to ensure both components (siRNA and drug) meet quality standards.

The safety assessment of siRNA-drug combinations requires a comprehensive evaluation of both the drug and the RNA molecule, individually and in combination. Key concerns include off-target gene silencing, unintended immune activation, and potential toxicities arising from the delivery vehicle. The regulatory guidelines emphasize the need for robust preclinical models that adequately predict human responses to minimize risks during clinical development [168,173,176]. Immunogenicity remains a critical concern, particularly for siRNA therapy nanoparticles or other delivery systems that may provoke an immune response. Regulatory agencies require developers to conduct thorough immunogenicity assessments, including cytokine release assays, complement activation studies, and in vivo models of immune response [173,176,179]. Strategies to mitigate immunogenicity, such as chemical modifications of siRNA (e.g., 2'-O-methyl modifications) or the use of biocompatible nanoparticles, must be documented and justified in regulatory submissions.

Ethical considerations play a pivotal role in the regulatory evaluation of siRNA-drug combination therapies. Ensuring informed consent, particularly in early-phase trials where

the risk profile of the combination is still being established, is crucial. The potential risks associated with new technologies, such as off-target gene silencing or long-term immunogenicity, should be clearly communicated to patients. Regulatory agencies also scrutinize the ethical aspects of patient selection, trial design, and data transparency [168,173,176]. Trials should be designed to maximize benefits while minimizing risks, and data should be shared transparently with both regulators and the scientific community to facilitate the development of future siRNA-based therapies. Given the experimental nature of many siRNA therapies, compassionate use protocols and expanded access programs may also come under regulatory scrutiny [175,178].

To successfully navigate the regulatory landscape, developers should engage in early and continuous dialogue with regulatory agencies, such as pre-IND (Investigational New Drug) meetings with the FDA or Scientific Advice meetings with the EMA. Such interactions help clarify expectations, provide guidance on trial design and endpoints, and address specific regulatory concerns. Developers should also consider leveraging existing regulatory pathways designed to expedite the development of innovative therapies, such as the FDA's Fast Track, Breakthrough Therapy, and Accelerated Approval designations [183], or the EMA's PRIME (Priority Medicines) scheme [184]. These pathways can provide early and frequent communication with regulators, potentially shortening the time to market.

As siRNA-based combination therapies continue to evolve, there is a need for the greater harmonization of regulatory standards across different regions. Collaboration efforts between regulatory agencies, such as the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), are essential to develop consistent guidelines that facilitate global development and approval. Harmonized standard would simplify the development process, reduce costs, and speed up access to these potentially life-saving therapies. Efforts are also underway to create standardized frameworks for evaluating novel endpoints and biomarkers in clinical trials, which are particularly relevant for siRNA-based therapies targeting specific molecular pathways [180,181]. Incorporating patient-reported outcomes and real-world evidence may further refine the assessment of siRNA-drug combinations' therapeutic value.

The regulatory landscape for siRNA and anticancer drug combination therapies is complex, reflecting the innovative nature of these therapies and the need to ensure patient safety and efficacy. By understanding and navigating the regulatory requirements, pharmaceutical developers can optimize their strategies for bringing these promising therapies to market, ultimately advancing precision medicine in oncology.

7. Conclusions

The use of siRNA in combination with anticancer drugs represents a groundbreaking strategy in cancer therapy, offering enhanced efficacy, reduced toxicity, and the potential to overcome drug resistance. By selectively silencing genes that are overexpressed or uniquely expressed in cancerous tissues, siRNA provides a targeted means to disrupt critical oncogenic pathways, inhibit tumor growth, and sensitize tumors to existing therapies.

This review highlights several key genes as ideal targets for siRNA in combinatorial therapies, including survivin, VEGF, EGFR, c-MET, HER2, MUC1, and Bcl-2. These genes play pivotal roles in tumor survival, proliferation, angiogenesis, and resistance mechanisms. For example, silencing survivin enhances apoptosis and the effects of chemotherapeutic agents, while targeting VEGF inhibits angiogenesis. Similarly, downregulating EGFR and c-MET disrupts critical signaling pathways involved in cell growth and metastasis, and targeting HER2, MUC1, and Bcl-2 sensitizes tumors to targeted therapies and chemotherapy.

To fully realize the potential of siRNA-based therapies, several key strategies should be prioritized in future research and clinical development. These include advancements in

delivery systems, integration with personalized medicine, combination with immunotherapies, the optimization of dosing regimens, the identification of biomarkers, and regulatory improvements. The effective delivery of siRNA remains a critical challenge. Multifunctional nanocarriers, such as ligand-targeted lipid nanoparticles and exosomes, can selectively bind to tumor-specific receptors, ensuring that siRNA reaches its target while minimizing off-target effects. Additionally, stimuli-responsive systems designed to release siRNA in response to tumor-specific conditions, such as acidic pH or high enzymatic activity, are promising innovations to improve therapeutic precision.

Personalized medicine can greatly enhance the efficacy of siRNA therapies. The genomic profiling of tumors using next-generation sequencing and transcriptomic analyses can identify patient-specific genetic vulnerabilities, enabling tailored treatment approaches. For instance, patients with BRCA mutations could benefit from a combination of PARP inhibitors and siRNA targeting DNA repair genes like PARP1 or RAD51. Adaptive therapy approaches that allow for the real-time monitoring of tumor gene expression can further enable the dynamic adjustment of siRNA targets and drug combinations, ensuring sustained therapeutic responses and minimizing resistance. Combining siRNA therapies with immune checkpoint inhibitors, such as anti-PD-1 or anti-CTLA-4 antibodies, has the potential to modulate the tumor microenvironment and achieve durable responses. Silencing genes like MUC1, which enhances tumor immunogenicity, can improve the efficacy of immune-based therapies. Furthermore, siRNA can target genes involved in immune evasion, such as PD-L1 or IDO1, to create synergies with existing immunotherapy regimens.

Clinical trials should focus on determining the optimal dosing regimens for siRNA and co-administered drugs. Evaluating whether sequential or simultaneous delivery yields better outcomes is essential for maximizing therapeutic efficacy. The benefits of combining siRNA with other treatment modalities, such as radiotherapy, chemotherapy, and novel small-molecule inhibitors, also need to be rigorously assessed. For example, combining VEGF-siRNA with tyrosine kinase inhibitors could provide anti-angiogenic synergy. Identifying predictive biomarkers is vital for selecting patients most likely to respond to siRNA therapies. For instance, tumors with high VEGF expression may benefit from VEGF-targeting siRNA, particularly in angiogenesis-dependent cancers. Liquid biopsy techniques, such as the analysis of circulating tumor DNA (ctDNA) or RNA, can facilitate the real-time monitoring of siRNA efficacy and tumor adaptation during treatment.

The regulatory landscape for siRNA-based therapies needs to evolve to address unique challenges, such as dual-drug delivery systems and nanoparticle formulations. Clear guidelines for clinical trials, safety evaluations, and quality control are essential for accelerating clinical translation. Moreover, scalable and cost-effective manufacturing processes for siRNA and nanocarriers are needed to ensure accessibility for patients worldwide.

By addressing these key areas, siRNA-based therapies can overcome current challenges and become a cornerstone of precision oncology. These strategies aim to maximize therapeutic efficacy, minimize side effects, and, ultimately, provide new hope for patients with challenging cancer types.

Author Contributions: Conceptualization, C.S.; investigation, C.S.; writing—original draft preparation, C.S.; writing—review and editing, C.S. and M.V.; supervision, M.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Kumar, K.; Rani, V.; Mishra, M.; Chawla, R. New Paradigm in Combination Therapy of SiRNA with Chemotherapeutic Drugs for Effective Cancer Therapy. *Curr. Res. Pharmacol. Drug Discov.* **2022**, *3*, 100103. [\[CrossRef\]](#) [\[PubMed\]](#)
- Mokhtari, R.B.; Homayouni, T.S.; Baluch, N.; Morgatskaya, E.; Kumar, S.; Das, B.; Yeger, H. Combination Therapy in Combating Cancer. *Oncotarget* **2017**, *8*, 38022. [\[CrossRef\]](#)
- Palmer, A.C.; Sorger, P.K. Combination Cancer Therapy Can Confer Benefit via Patient-to-Patient Variability without Drug Additivity or Synergy. *Cell* **2017**, *171*, 1678. [\[CrossRef\]](#)
- Kilgallen, A.B.; Štibler, U.; Printezi, M.I.; Putker, M.; Punt, C.J.A.; Sluijter, J.P.G.; May, A.M.; van Laake, L.W. Comparing Conventional Chemotherapy to Chronomodulated Chemotherapy for Cancer Treatment: Protocol for a Systematic Review. *JMIR Res. Protoc.* **2020**, *9*, e18023. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kiani Shahvandi, M.; Souri, M.; Tavasoli, S.; Moradi Kashkooli, F.; Kar, S.; Soltani, M. A Comparative Study between Conventional Chemotherapy and Photothermal Activated Nano-Sized Targeted Drug Delivery to Solid Tumor. *Comput. Biol. Med.* **2023**, *166*, 107574. [\[CrossRef\]](#) [\[PubMed\]](#)
- Anand, U.; Dey, A.; Chandel, A.K.S.; Sanyal, R.; Mishra, A.; Pandey, D.K.; De Falco, V.; Upadhyay, A.; Kandimalla, R.; Chaudhary, A.; et al. Cancer Chemotherapy and beyond: Current Status, Drug Candidates, Associated Risks and Progress in Targeted Therapeutics. *Genes Dis.* **2023**, *10*, 1367–1401. [\[CrossRef\]](#) [\[PubMed\]](#)
- Hu, B.; Zhong, L.; Weng, Y.; Peng, L.; Huang, Y.; Zhao, Y.; Liang, X.J. Therapeutic SiRNA: State of the Art. *Signal Transduct. Target. Ther.* **2020**, *5*, 101. [\[CrossRef\]](#) [\[PubMed\]](#)
- Subhan, M.A.; Torchilin, V.P. Efficient Nanocarriers of SiRNA Therapeutics for Cancer Treatment. *Transl. Res.* **2019**, *214*, 62–91. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sajid, M.I.; Moazzam, M.; Kato, S.; Cho, K.Y.; Tiwari, R.K. Overcoming Barriers for SiRNA Therapeutics: From Bench to Bedside. *Pharmaceuticals* **2020**, *13*, 294. [\[CrossRef\]](#) [\[PubMed\]](#)
- Isazadeh, H.; Oruji, F.; Shabani, S.; Behrooz, J.; Nasiri, H.; Isazadeh, A.; Akbari, M. Advances in SiRNA Delivery Approaches in Cancer Therapy: Challenges and Opportunities. *Mol. Biol. Rep.* **2023**, *50*, 9529–9543. [\[CrossRef\]](#)
- Lin, Y.L.; Yuksel Durmaz, Y.; Nör, J.E.; Elsayed, M.E.H. Synergistic Combination of Small Molecule Inhibitor and RNA Interference Against Anti-Apoptotic Bcl-2 Protein in Head and Neck Cancer Cells. *Mol. Pharm.* **2013**, *10*, 2730. [\[CrossRef\]](#)
- Tunç, C.Ü.; Aydin, O. Co-Delivery of Bcl-2 SiRNA and Doxorubicin through Gold Nanoparticle-Based Delivery System for a Combined Cancer Therapy Approach. *J. Drug Deliv. Sci. Technol.* **2022**, *74*, 103603. [\[CrossRef\]](#)
- Sun, Q.; Wang, X.; Cui, C.; Li, J.; Wang, Y. Doxorubicin and Anti-VEGF SiRNA Co-Delivery via Nano-Graphene Oxide for Enhanced Cancer Therapy in Vitro and in Vivo. *Int. J. Nanomed.* **2018**, *13*, 3713–3728. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zhang, C.; Zhao, Y.; Zhang, E.; Jiang, M.; Zhi, D.; Chen, H.; Cui, S.; Zhen, Y.; Cui, J.; Zhang, S. Co-Delivery of Paclitaxel and Anti-VEGF SiRNA by Tripeptide Lipid Nanoparticle to Enhance the Anti-Tumor Activity for Lung Cancer Therapy. *Drug Deliv.* **2020**, *27*, 1397–1411. [\[CrossRef\]](#)
- Jin, H.; Wang, L.; Bernards, R. Rational Combinations of Targeted Cancer Therapies: Background, Advances and Challenges. *Nat. Rev. Drug Discov.* **2022**, *22*, 213–234. [\[CrossRef\]](#)
- Babu, A.; Munshi, A.; Ramesh, R. Combinatorial Therapeutic Approaches with RNAi and Anticancer Drugs Using Nanodrug Delivery Systems. *Drug Dev. Ind. Pharm.* **2017**, *43*, 1391–1401. [\[CrossRef\]](#)
- Zhou, Z.; Edil, B.H.; Li, M. Combination Therapies for Cancer: Challenges and Opportunities. *BMC Med.* **2023**, *21*, 171. [\[CrossRef\]](#)
- Singh, A.; Trivedi, P.; Jain, N.K. Advances in SiRNA Delivery in Cancer Therapy. *Artif. Cells Nanomed. Biotechnol.* **2018**, *46*, 274–283. [\[CrossRef\]](#)
- Huang, L.; Guo, S. Nanoparticles Escaping RES and Endosome: Challenges for SiRNA Delivery for Cancer Therapy. *J. Nanomater.* **2011**, *2011*, 742895. [\[CrossRef\]](#)
- Gupta, N.; Rai, D.B.; Jangid, A.K.; Pooja, D.; Kulhari, H. Nanomaterials-Based SiRNA Delivery: Routes of Administration, Hurdles and Role of Nanocarriers. In *Nanotechnology in Modern Animal Biotechnology: Recent Trends and Future Perspectives*; Springer: Singapore, 2019; pp. 67–114. [\[CrossRef\]](#)
- Mahmoodi Chalbatani, G.; Dana, H.; Gharagouzloo, E.; Grijalvo, S.; Eritja, R.; Logsdon, C.D.; Memari, F.; Miri, S.R.; Rad, M.R.; Marmari, V. Small Interfering RNAs (SiRNAs) in Cancer Therapy: A Nano-Based Approach. *Int. J. Nanomed.* **2019**, *14*, 3111–3128. [\[CrossRef\]](#) [\[PubMed\]](#)
- Foroozandeh, P.; Aziz, A.A. Insight into Cellular Uptake and Intracellular Trafficking of Nanoparticles. *Nanoscale Res. Lett.* **2018**, *13*, 339. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ganesh, S.; Iyer, A.K.; Weiler, J.; Morrissey, D.V.; Amiji, M.M. Combination of SiRNA-Directed Gene Silencing with Cisplatin Reverses Drug Resistance in Human Non-Small Cell Lung Cancer. *Mol. Ther. Nucleic Acids* **2013**, *2*, e110. [\[CrossRef\]](#)
- Creixell, M.; Peppas, N.A. Co-Delivery of SiRNA and Therapeutic Agents Using Nanocarriers to Overcome Cancer Resistance. *Nano Today* **2012**, *7*, 367–379. [\[CrossRef\]](#)

25. Li, L.Y.; Guan, Y.D.; Chen, X.S.; Yang, J.M.; Cheng, Y. DNA Repair Pathways in Cancer Therapy and Resistance. *Front. Pharmacol.* **2020**, *11*, 629266. [\[CrossRef\]](#)
26. Jurkovicova, D.; Neophytou, C.M.; Gašparović, A.Č.; Gonçalves, A.C. DNA Damage Response in Cancer Therapy and Resistance: Challenges and Opportunities. *Int. J. Mol. Sci.* **2022**, *23*, 14672. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Pote, M.S.; Gacche, R.N. ATP-Binding Cassette Efflux Transporters and MDR in Cancer. *Drug Discov. Today* **2023**, *28*, 103537. [\[CrossRef\]](#)
28. Wang, D.; Xu, X.; Zhang, K.; Sun, B.; Wang, L.; Meng, L.; Liu, Q.; Zheng, C.; Yang, B.; Sun, H. Codelivery of Doxorubicin and MDR1-SiRNA by Mesoporous Silica Nanoparticles-Polymerpolyethylenimine to Improve Oral Squamous Carcinoma Treatment. *Int. J. Nanomed.* **2018**, *13*, 187. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Gao, B.; Russell, A.; Beesley, J.; Chen, X.Q.; Healey, S.; Henderson, M.; Wong, M.; Emmanuel, C.; Galletta, L.; Johnatty, S.E.; et al. Paclitaxel Sensitivity in Relation to ABCB1 Expression, Efflux and Single Nucleotide Polymorphisms in Ovarian Cancer. *Sci. Rep.* **2014**, *4*, 4669. [\[CrossRef\]](#)
30. McMullen, M.; Karakasis, K.; Madariaga, A.; Oza, A.M. Overcoming Platinum and PARP-Inhibitor Resistance in Ovarian Cancer. *Cancers* **2020**, *12*, 1607. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Wu, W.; Kong, Z.; Duan, X.; Zhu, H.; Li, S.; Zeng, S.; Liang, Y.; Iliakis, G.; Gui, Z.; Yang, D. Inhibition of PARP1 by Small Interfering RNA Enhances Docetaxel Activity against Human Prostate Cancer PC3 Cells. *Biochem. Biophys. Res. Commun.* **2013**, *442*, 127–132. [\[CrossRef\]](#)
32. Vernooij, L.; Kamili, A.; Ober, K.; van Arkel, J.; Lankhorst, L.; Vermeulen, E.; Al-Khakany, H.; Tax, G.; van den Boogaard, M.L.; Fletcher, J.I.; et al. Preclinical Assessment of Combined BCL-2 and MCL-1 Inhibition in High-Risk Neuroblastoma. *EJC Paediatr. Oncol.* **2024**, *3*, 100168. [\[CrossRef\]](#)
33. Gandhi, N.S.; Tekade, R.K.; Chougule, M.B. Nanocarrier Mediated Delivery of SiRNA/MiRNA in Combination with Chemotherapeutic Agents for Cancer Therapy: Current Progress and Advances. *J. Control. Release* **2014**, *194*, 238–256. [\[CrossRef\]](#)
34. Anthiya, S.; Öztürk, S.C.; Yanik, H.; Tavukcuoglu, E.; Şahin, A.; Datta, D.; Charisse, K.; Álvarez, D.M.; Loza, M.I.; Calvo, A.; et al. Targeted SiRNA Lipid Nanoparticles for the Treatment of KRAS-Mutant Tumors. *J. Control. Release* **2023**, *357*, 67–83. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Zarredar, H.; Pashapour, S.; Ansarin, K.; Khalili, M.; Baghban, R.; Farajnia, S. Combination Therapy with KRAS SiRNA and EGFR Inhibitor AZD8931 Suppresses Lung Cancer Cell Growth in Vitro. *J. Cell. Physiol.* **2019**, *234*, 1560–1566. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Chen, A.M.; Zhang, M.; Wei, D.; Stueber, D.; Taratula, O.; Minko, T.; He, H. Co-Delivery of Doxorubicin and Bcl-2 SiRNA by Mesoporous Silica Nanoparticles Enhances the Efficacy of Chemotherapy in Multidrug-Resistant Cancer Cells. *Small* **2009**, *5*, 2673–2677. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Tabernero, J.; Shapiro, G.I.; LoRusso, P.M.; Cervantes, A.; Schwartz, G.K.; Weiss, G.J.; Paz-Ares, L.; Cho, D.C.; Infante, J.R.; Alsina, M.; et al. First-in-Humans Trial of an RNA Interference Therapeutic Targeting VEGF and KSP in Cancer Patients with Liver Involvement. *Cancer Discov.* **2013**, *3*, 406–417. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Horimoto, Y.; Ishizuka, Y.; Ueki, Y.; Higuchi, T.; Arakawa, A.; Saito, M. Comparison of Tumors with HER2 Overexpression versus HER2 Amplification in HER2-Positive Breast Cancer Patients. *BMC Cancer* **2022**, *22*, 242. [\[CrossRef\]](#)
39. Ngamcherdrakul, W.; Bejan, D.S.; Cruz-Muñoz, W.; Reda, M.; Zaidan, H.Y.; Siriwon, N.; Marshall, S.; Wang, R.; Nelson, M.A.; Rehwaltd, J.P.C.; et al. Targeted Nanoparticle for Co-Delivery of HER2 SiRNA and a Taxane to Mirror the Standard Treatment of HER2+ Breast Cancer: Efficacy in Breast Tumor and Brain Metastasis. *Small* **2022**, *18*, 2107550. [\[CrossRef\]](#)
40. Chen, G.; Kronenberger, P.; Teugels, E.; Umelo, I.A.; De Grève, J. Effect of SiRNAs Targeting the EGFR T790M Mutation in a Non-Small Cell Lung Cancer Cell Line Resistant to EGFR Tyrosine Kinase Inhibitors and Combination with Various Agents. *Biochem. Biophys. Res. Commun.* **2013**, *431*, 623–629. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Suk, J.S.; Xu, Q.; Kim, N.; Hanes, J.; Ensign, L.M. PEGylation as a Strategy for Improving Nanoparticle-Based Drug and Gene Delivery. *Adv. Drug Deliv. Rev.* **2016**, *99*, 28–51. [\[CrossRef\]](#)
42. Zhao, X.; Li, F.; Li, Y.; Wang, H.; Ren, H.; Chen, J.; Nie, G.; Hao, J. Co-Delivery of HIF1 α SiRNA and Gemcitabine via Biocompatible Lipid-Polymer Hybrid Nanoparticles for Effective Treatment of Pancreatic Cancer. *Biomaterials* **2015**, *46*, 13–25. [\[CrossRef\]](#)
43. Loney, C.; Vandenbranden, M.; Ruysschaert, J.M. Cationic Lipids Activate Intracellular Signaling Pathways. *Adv. Drug Deliv. Rev.* **2012**, *64*, 1749–1758. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Miles, D.; Von Minckwitz, G.; Seidman, A.D. Combination Versus Sequential Single-Agent Therapy in Metastatic Breast Cancer. *Oncologist* **2002**, *7*, 13–19. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Michel, M.C.; Staskin, D. Study Designs for Evaluation of Combination Treatment: Focus on Individual Patient Benefit. *Biomedicines* **2022**, *10*, 270. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Magalhães, M.; Alvarez-Lorenzo, C.; Concheiro, A.; Figueiras, A.; Santos, A.C.; Veiga, F. RNAi-Based Therapeutics for Lung Cancer: Biomarkers, MicroRNAs, and Nanocarriers. *Expert Opin. Drug Deliv.* **2018**, *15*, 965–982. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Yonezawa, S.; Koide, H.; Asai, T. Recent Advances in SiRNA Delivery Mediated by Lipid-Based Nanoparticles. *Adv. Drug Deliv. Rev.* **2020**, *154–155*, 64–78. [\[CrossRef\]](#)

48. El Moukhtari, S.H.; Garbayo, E.; Amundarain, A.; Pascual-Gil, S.; Carrasco-León, A.; Prosper, F.; Agirre, X.; Blanco-Prieto, M.J. Lipid Nanoparticles for SiRNA Delivery in Cancer Treatment. *J. Control. Release* **2023**, *361*, 130–146. [[CrossRef](#)]
49. Morgan, E.; Wupperfeld, D.; Morales, D.; Reich, N. Shape Matters: Gold Nanoparticle Shape Impacts the Biological Activity of SiRNA Delivery. *Bioconjugate Chem.* **2019**, *30*, 853–860. [[CrossRef](#)] [[PubMed](#)]
50. Artiga, Á.; Serrano-Sevilla, I.; De Matteis, L.; Mitchell, S.G.; De La Fuente, J.M. Current Status and Future Perspectives of Gold Nanoparticle Vectors for SiRNA Delivery. *J. Mater. Chem. B* **2019**, *7*, 876–896. [[CrossRef](#)]
51. Kanungo, A.; Tripathy, N.S.; Sahoo, L.; Acharya, S.; Dilnawaz, F. Theranostic SiRNA Loaded Mesoporous Silica Nanoplatfoms: A Game Changer in Gene Therapy for Cancer Treatment. *OpenNano* **2024**, *15*, 100195. [[CrossRef](#)]
52. Xie, X.; Yue, T.; Gu, W.; Cheng, W.Y.; He, L.; Ren, W.Y.; Li, F.; Piao, J.G. Recent Advances in Mesoporous Silica Nanoparticles Delivering SiRNA for Cancer Treatment. *Pharmaceutics* **2023**, *15*, 2483. [[CrossRef](#)] [[PubMed](#)]
53. Mykhaylyk, O.; Sanchez-Antequera, Y.; Vlaskou, D.; Cerda, M.B.; Bokharaei, M.; Hammerschmid, E.; Anton, M.; Plank, C. Magnetic Nanoparticle and Magnetic Field Assisted SiRNA Delivery In Vitro. In *RNA Interference: Challenges and Therapeutic Opportunities*; Humana Press: New York, NY, USA, 2015; pp. 53–106. [[CrossRef](#)]
54. Amiri, A.; Bagherifar, R.; Ansari Dezfouli, E.; Kiaie, S.H.; Jafari, R.; Ramezani, R. Exosomes as Bio-Inspired Nanocarriers for RNA Delivery: Preparation and Applications. *J. Transl. Med.* **2022**, *20*, 125. [[CrossRef](#)]
55. Ubanako, P.; Mirza, S.; Ruff, P.; Penny, C. Exosome-Mediated Delivery of SiRNA Molecules in Cancer Therapy: Triumphs and Challenges. *Front. Mol. Biosci.* **2024**, *11*, 1447953. [[CrossRef](#)]
56. Shi, J.; Xu, Y.; Xu, X.; Zhu, X.; Pridgen, E.; Wu, J.; Votrubá, A.R.; Swami, A.; Zetter, B.R.; Farokhzad, O.C. Hybrid Lipid-Polymer Nanoparticles for Sustained SiRNA Delivery and Gene Silencing. *Nanomedicine* **2014**, *10*, e897–e900. [[CrossRef](#)] [[PubMed](#)]
57. Behzadi, S.; Serpooshan, V.; Tao, W.; Hamaly, M.A.; Alkawareek, M.Y.; Dreaden, E.C.; Brown, D.; Alkilany, A.M.; Farokhzad, O.C.; Mahmoudi, M. Cellular Uptake of Nanoparticles: Journey inside the Cell. *Chem. Soc. Rev.* **2017**, *46*, 4218–4244. [[CrossRef](#)] [[PubMed](#)]
58. Zhou, H.; Chen, S.; Hu, C.J.; Hong, X.; Shi, J.; Xiao, Y. Stimuli-Responsive Nanotechnology for RNA Delivery. *Adv. Sci.* **2023**, *10*, 2303597. [[CrossRef](#)] [[PubMed](#)]
59. Eloy, J.O.; Petrilli, R.; Lopez, R.F.V.; Lee, R.J. Stimuli-Responsive Nanoparticles for SiRNA Delivery. *Curr. Pharm. Des.* **2015**, *21*, 4131–4144. [[CrossRef](#)]
60. Moazzam, M.; Zhang, M.; Hussain, A.; Yu, X.; Huang, J.; Huang, Y. The Landscape of Nanoparticle-Based SiRNA Delivery and Therapeutic Development. *Mol. Ther.* **2024**, *32*, 284–312. [[CrossRef](#)]
61. Ebrahimi, N.; Manavi, M.S.; Nazari, A.; Momayezi, A.; Faghihkhorsani, F.; Rasool Riyadh Abdulwahid, A.H.; Rezaei-Tazangi, F.; Kavei, M.; Rezaei, R.; Mobarak, H.; et al. Nano-Scale Delivery Systems for SiRNA Delivery in Cancer Therapy: New Era of Gene Therapy Empowered by Nanotechnology. *Environ. Res.* **2023**, *239*, 117263. [[CrossRef](#)] [[PubMed](#)]
62. Smith, S.A.; Selby, L.I.; Johnston, A.P.R.; Such, G.K. The Endosomal Escape of Nanoparticles: Toward More Efficient Cellular Delivery. *Bioconjugate Chem.* **2019**, *30*, 263–272. [[CrossRef](#)]
63. Grau, M.; Wagner, E. Strategies and Mechanisms for Endosomal Escape of Therapeutic Nucleic Acids. *Curr. Opin. Chem. Biol.* **2024**, *81*, 102506. [[CrossRef](#)] [[PubMed](#)]
64. Xue, R.; Pan, Y.; Xia, L.; Li, J. Non-Viral Vectors Combined Delivery of SiRNA and Anti-Cancer Drugs to Reverse Tumor Multidrug Resistance. *Biomed. Pharmacother.* **2024**, *178*, 117119. [[CrossRef](#)]
65. Xue, X.; Liang, X.J. Overcoming Drug Efflux-Based Multidrug Resistance in Cancer with Nanotechnology. *Chin. J. Cancer* **2012**, *31*, 100. [[CrossRef](#)]
66. Byeon, Y.; Lee, J.W.; Choi, W.S.; Won, J.E.; Kim, G.H.; Kim, M.G.; Wi, T.I.; Lee, J.M.; Kang, T.H.; Jung, I.D.; et al. CD44-Targeting PLGA Nanoparticles Incorporating Paclitaxel and FAK SiRNA Overcome Chemoresistance in Epithelial Ovarian Cancer. *Cancer Res.* **2018**, *78*, 6247–6256. [[CrossRef](#)] [[PubMed](#)]
67. Meng, H.; Liong, M.; Xia, T.; Li, Z.; Ji, Z.; Zink, J.I.; Nel, A.E. Engineered Design of Mesoporous Silica Nanoparticles to Deliver Doxorubicin and P-Glycoprotein SiRNA to Overcome Drug Resistance in a Cancer Cell Line. *ACS Nano* **2010**, *4*, 4539–4550. [[CrossRef](#)] [[PubMed](#)]
68. Mansoori, B.; Mohammadi, A.; Davudian, S.; Shirjang, S.; Baradaran, B. The Different Mechanisms of Cancer Drug Resistance: A Brief Review. *Adv. Pharm. Bull.* **2017**, *7*, 339–348. [[CrossRef](#)] [[PubMed](#)]
69. Robey, R.W.; Pluchino, K.M.; Hall, M.D.; Fojo, A.T.; Bates, S.E.; Gottesman, M.M. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat. Rev. Cancer* **2018**, *18*, 452. [[CrossRef](#)] [[PubMed](#)]
70. Chen, H.K.; Chen, Y.L.; Wang, C.Y.; Chung, W.P.; Fang, J.H.; Lai, M.D.; Hsu, H.P. ABCB1 Regulates Immune Genes in Breast Cancer. *Breast Cancer Targets Ther.* **2023**, *15*, 801–811. [[CrossRef](#)]
71. Lei, Z.N.; Teng, Q.X.; Wu, Z.X.; Ping, F.F.; Song, P.; Wurpel, J.N.D.; Chen, Z.S. Overcoming Multidrug Resistance by Knockout of ABCB1 Gene Using CRISPR/Cas9 System in SW620/Ad300 Colorectal Cancer Cells. *MedComm* **2021**, *2*, 765. [[CrossRef](#)] [[PubMed](#)]
72. Yang, X.; Wang, Y.; Chen, S.; Zhang, S.; Cui, C. Cetuximab-Modified Human Serum Albumin Nanoparticles Co-Loaded with Doxorubicin and Mdr1 SiRNA for the Treatment of Drug-Resistant Breast Tumors. *Int. J. Nanomed.* **2021**, *16*, 7051–7069. [[CrossRef](#)]

73. Kukal, S.; Guin, D.; Rawat, C.; Bora, S.; Mishra, M.K.; Sharma, P.; Paul, P.R.; Kanojia, N.; Grewal, G.K.; Kukreti, S.; et al. Multidrug Efflux Transporter ABCG2: Expression and Regulation. *Cell. Mol. Life Sci.* **2021**, *78*, 6887. [\[CrossRef\]](#)
74. Wu, Z.X.; Mai, Q.; Yang, Y.; Wang, J.Q.; Ma, H.; Zeng, L.; Chen, Z.S.; Pan, Y. Overexpression of Human ATP-Binding Cassette Transporter ABCG2 Contributes to Reducing the Cytotoxicity of GSK1070916 in Cancer Cells. *Biomed. Pharmacother.* **2021**, *136*, 111223. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Omori, M.; Noro, R.; Seike, M.; Matsuda, K.; Hirao, M.; Fukuizumi, A.; Takano, N.; Miyanaga, A.; Gemma, A. Inhibitors of ABCB1 and ABCG2 Overcame Resistance to Topoisomerase Inhibitors in Small Cell Lung Cancer. *Thorac. Cancer* **2022**, *13*, 2142. [\[CrossRef\]](#)
76. Bai, M.; Shen, M.; Teng, Y.; Sun, Y.; Li, F.; Zhang, X.; Xu, Y.; Duan, Y.; Du, L. Enhanced Therapeutic Effect of Adriamycin on Multidrug Resistant Breast Cancer by the ABCG2-SiRNA Loaded Polymeric Nanoparticles Assisted with Ultrasound. *Oncotarget* **2015**, *6*, 43779–43790. [\[CrossRef\]](#)
77. Gilibert, M.; Launay, S.; Ginestier, C.; Bertucci, F.; Audebert, S.; Pophillat, M.; Toiron, Y.; Baudalet, E.; Finetti, P.; Noguchi, T.; et al. Poly(ADP-Ribose) Polymerase 1 (PARP1) Overexpression in Human Breast Cancer Stem Cells and Resistance to Olaparib. *PLoS ONE* **2014**, *9*, e104302. [\[CrossRef\]](#)
78. Michels, J.; Vitale, I.; Galluzzi, L.; Adam, J.; Olaussen, K.A.; Kepp, O.; Senovilla, L.; Talhaoui, I.; Guegan, J.; Enot, D.P.; et al. Cisplatin Resistance Associated with PARP Hyperactivation. *Cancer Res.* **2013**, *73*, 2271–2280. [\[CrossRef\]](#)
79. Jackson, L.M.; Moldovan, G.L. Mechanisms of PARP1 Inhibitor Resistance and Their Implications for Cancer Treatment. *NAR Cancer* **2022**, *4*, zcac042. [\[CrossRef\]](#) [\[PubMed\]](#)
80. Lai, Y.; Kong, Z.; Zeng, T.; Xu, S.; Duan, X.; Li, S.; Cai, C.; Zhao, Z.; Wu, W. PARP1-SiRNA Suppresses Human Prostate Cancer Cell Growth and Progression. *Oncol. Rep.* **2018**, *39*, 1901–1909. [\[CrossRef\]](#) [\[PubMed\]](#)
81. D'Souza, A.; Blee, A.M.; Chazin, W.J. Mechanism of Action of Nucleotide Excision Repair Machinery. *Biochem. Soc. Trans.* **2022**, *50*, 375. [\[CrossRef\]](#)
82. Simon, G.R.; Sharma, S.; Cantor, A.; Smith, P.; Bepler, G. ERCC1 Expression Is a Predictor of Survival in Resected Patients with Non-Small Cell Lung Cancer. *Chest* **2005**, *127*, 978–983. [\[CrossRef\]](#)
83. Postel-Vinay, S.; Soria, J.C. ERCC1 as Predictor of Platinum Benefit in Non-Small-Cell Lung Cancer. *J. Clin. Oncol.* **2016**, *35*, 384–386. [\[CrossRef\]](#)
84. Du, P.; Wang, Y.; Chen, L.; Gan, Y.; Wu, Q. High ERCC1 Expression Is Associated with Platinum-Resistance, but Not Survival in Patients with Epithelial Ovarian Cancer. *Oncol. Lett.* **2016**, *12*, 857. [\[CrossRef\]](#)
85. Wan, J.; Chao, L.; Lee, A.C.; Chen, Q. Higher Expression of ERCC1 May Be Associated with Resistance to Adjuvant Platinum-Based Chemotherapy in Gastric Cancer. *Cancer Investig.* **2017**, *35*, 85–91. [\[CrossRef\]](#)
86. Xie, K.; Ni, X.; Lv, S.; Zhou, G.; He, H. Synergistic Effects of Olaparib Combined with ERCC1 on the Sensitivity of Cisplatin in Non-small Cell Lung Cancer. *Oncol. Lett.* **2021**, *21*, 365. [\[CrossRef\]](#)
87. Zhao, T.; Ye, W.; Zhang, R.; Zhu, X.; Shi, Q.; Xu, X.; Chen, W.; Xu, L.; Meng, Y. Dual-regulated Oncolytic Adenovirus Carrying ERCC1-siRNA Gene Possesses Potent Antitumor Effect on Ovarian Cancer Cells. *Mol. Med. Rep.* **2024**, *30*, 120. [\[CrossRef\]](#)
88. Roberts, A.W.; Wei, A.H.; Huang, D.C.S. BCL2 and MCL1 Inhibitors for Hematologic Malignancies. *Blood* **2021**, *138*, 1120–1136. [\[CrossRef\]](#)
89. Widden, H.; Placzek, W.J. The Multiple Mechanisms of MCL1 in the Regulation of Cell Fate. *Commun. Biol.* **2021**, *4*, 1029. [\[CrossRef\]](#)
90. Kaloni, D.; Diepstraten, S.T.; Strasser, A.; Kelly, G.L. BCL-2 Protein Family: Attractive Targets for Cancer Therapy. *Apoptosis* **2022**, *28*, 20–38. [\[CrossRef\]](#)
91. Beale, P.J.; Rogers, P.; Boxall, F.; Sharp, S.Y.; Kelland, L.R. BCL-2 Family Protein Expression and Platinum Drug Resistance in Ovarian Carcinoma. *Br. J. Cancer* **1999**, *82*, 436–440. [\[CrossRef\]](#) [\[PubMed\]](#)
92. Perini, G.F.; Ribeiro, G.N.; Pinto Neto, J.V.; Campos, L.T.; Hamerschlag, N. BCL-2 as Therapeutic Target for Hematological Malignancies. *J. Hematol. Oncol.* **2018**, *11*, 65. [\[CrossRef\]](#)
93. Zhou, M.; Zhang, X.; Xu, X.; Chen, X.; Zhang, X. Doxorubicin@Bcl-2 SiRNA Core@Shell Nanoparticles for Synergistic Anticancer Chemotherapy. *ACS Appl. Bio Mater.* **2018**, *1*, 289–297. [\[CrossRef\]](#)
94. Vu, M.; Kassouf, N.; Ofili, R.; Lund, T.; Bell, C.; Appiah, S. Doxorubicin Selectively Induces Apoptosis through the Inhibition of a Novel Isoform of Bcl-2 in Acute Myeloid Leukaemia MOLM-13 Cells with Reduced Beclin 1 Expression. *Int. J. Oncol.* **2020**, *57*, 113–121. [\[CrossRef\]](#) [\[PubMed\]](#)
95. Williams, M.M.; Cook, R.S. Bcl-2 Family Proteins in Breast Development and Cancer: Could Mcl-1 Targeting Overcome Therapeutic Resistance? *Oncotarget* **2015**, *6*, 3519. [\[CrossRef\]](#)
96. Pengnam, S.; Charoensuksai, P.; Yingyongnarongkul, B.E.; Saeeng, R.; Uludağ, H.; Patrojanasophon, P.; Opanasopit, P.; Plianwong, S. SiRNA Targeting Mcl-1 Potentiates the Anticancer Activity of Andrographolide Nanosuspensions via Apoptosis in Breast Cancer Cells. *Pharmaceutics* **2022**, *14*, 1196. [\[CrossRef\]](#) [\[PubMed\]](#)

97. Pengnam, S.; Plianwong, S.; Patrojanasophon, P.; Radchatawedchakoon, W.; Yingyongnarongkul, B.E.; Opanasopit, P.; Charoen-suksai, P. Synergistic Effect of Doxorubicin and SiRNA-Mediated Silencing of Mcl-1 Using Cationic Niosomes against 3D MCF-7 Spheroids. *Pharmaceutics* **2021**, *13*, 550. [CrossRef]
98. Wang, Y.; Gao, F.; Jiang, X.; Zhao, X.; Wang, Y.; Kuai, Q.; Nie, G.; He, M.; Pan, Y.; Shi, W.; et al. Co-Delivery of Gemcitabine and Mcl-1 SiRNA via Cationic Liposome-Based System Enhances the Efficacy of Chemotherapy in Pancreatic Cancer. *J. Biomed. Nanotechnol.* **2019**, *15*, 966–978. [CrossRef]
99. Dey, N.; De, P.; Leyland-Jones, B. PI3K-AKT-MTOR Inhibitors in Breast Cancers: From Tumor Cell Signaling to Clinical Trials. *Pharmacol. Ther.* **2017**, *175*, 91–106. [CrossRef]
100. Porta, C.; Paglino, C.; Mosca, A. Targeting PI3K/Akt/MTOR Signaling in Cancer. *Front. Oncol.* **2014**, *4*, 64. [CrossRef] [PubMed]
101. Sousa, C.; Silva-Lima, B.; Videira, M. Akt/MTOR Activation in Lung Cancer Tumorigenic Regulators and Their Potential Value as Biomarkers. *Onco* **2022**, *2*, 36–55. [CrossRef]
102. Huang, C.; Zhang, X.; Jiang, L.; Zhang, L.; Xiang, M.; Ren, H. FoxM1 Induced Paclitaxel Resistance via Activation of the FoxM1/PHB1/RAF-MEK-ERK Pathway and Enhancement of the ABCA2 Transporter. *Mol. Ther. Oncolytics* **2019**, *14*, 196–212. [CrossRef]
103. Kim, M.J.; Lee, S.J.; Ryu, J.H.; Kim, S.H.; Kwon, I.C.; Roberts, T.M. Combination of KRAS Gene Silencing and PI3K Inhibition for Ovarian Cancer Treatment. *J. Control. Release* **2020**, *318*, 98–108. [CrossRef]
104. Manning, B.D.; Toker, A. AKT/PKB Signaling: Navigating the Network. *Cell* **2017**, *169*, 381–405. [CrossRef]
105. Gupte, A.; Baker, E.K.; Wan, S.S.; Stewart, E.; Loh, A.; Shelat, A.A.; Gould, C.M.; Chalk, A.M.; Taylor, S.; Lackovic, K.; et al. Systematic Screening Identifies Dual PI3K and MTOR Inhibition as a Conserved Therapeutic Vulnerability in Osteosarcoma. *Clin. Cancer Res.* **2015**, *21*, 3216–3229. [CrossRef] [PubMed]
106. Datta, S.R.; Brunet, A.; Greenberg, M.E. Cellular Survival: A Play in Three Akts. *Genes Dev.* **1999**, *13*, 2905–2927. [CrossRef] [PubMed]
107. Itoh, N.; Semba, S.; Ito, M.; Takeda, H.; Kawata, S.; Yamakawa, M. Phosphorylation of Akt/PKB Is Required for Suppression of Cancer Cell Apoptosis and Tumor Progression in Human Colorectal Carcinoma. *Cancer* **2002**, *94*, 3127–3134. [CrossRef]
108. Stephens, D.M. Testing Early Treatment for Patients with High-Risk Chronic Lymphocytic Leukemia (CLL) or Small Lymphocytic Leukemia (SLL), EVOLVE CLL/SLL Study. NCI. Available online: <https://www.cancer.gov/research/participate/clinical-trials-search/v?id=NCT04269902> (accessed on 19 December 2024).
109. Kater, A.P.; Wu, J.Q.; Kipps, T.; Eichhorst, B.; Hillmen, P.; D’Rozario, J.; Assouline, S.; Owen, C.; Robak, T.; de la Serna, J.; et al. Venetoclax plus Rituximab in Relapsed Chronic Lymphocytic Leukemia: 4-Year Results and Evaluation of Impact of Genomic Complexity and Gene Mutations from the MURANO Phase III Study. *J. Clin. Oncol.* **2020**, *38*, 4042–4054. [CrossRef] [PubMed]
110. Ewald, L.; Dittmann, J.; Vogler, M.; Fulda, S. Side-by-Side Comparison of BH3-Mimetics Identifies MCL-1 as a Key Therapeutic Target in AML. *Cell Death Dis.* **2019**, *10*, 917. [CrossRef]
111. Gomez-Bougie, P.; Wuillème-Toumi, S.; Ménoret, E.; Trichet, V.; Robillard, N.; Philippe, M.; Bataille, R.; Amiot, M. Noxa Up-Regulation and Mcl-1 Cleavage Are Associated to Apoptosis Induction by Bortezomib in Multiple Myeloma. *Cancer Res.* **2007**, *67*, 5418–5424. [CrossRef] [PubMed]
112. Yuda, J.; Will, C.; Phillips, D.C.; Abraham, L.; Alvey, C.; Avigdor, A.; Buck, W.; Besenhofer, L.; Boghaert, E.; Cheng, D.; et al. Selective MCL-1 Inhibitor ABBV-467 Is Efficacious in Tumor Models but Is Associated with Cardiac Troponin Increases in Patients. *Commun. Med.* **2023**, *3*, 154. [CrossRef]
113. Nachmias, B.; Ashhab, Y.; Ben-Yehuda, D. The Inhibitor of Apoptosis Protein Family (IAPs): An Emerging Therapeutic Target in Cancer. *Semin. Cancer Biol.* **2004**, *14*, 231–243. [CrossRef] [PubMed]
114. Liang, J.; Zhao, W.; Tong, P.; Li, P.; Zhao, Y.; Li, H.; Liang, J. Comprehensive Molecular Characterization of Inhibitors of Apoptosis Proteins (IAPs) for Therapeutic Targeting in Cancer. *BMC Med. Genom.* **2020**, *13*, 7. [CrossRef] [PubMed]
115. Sakthivel, R.; Ramamoorthy, A.; Jeddy, N.; Singaram, M. Evaluation and Expression of Survivin in Potentially Malignant Lesions and Squamous Cell Carcinoma: A Comparative Study. *Cureus* **2020**, *12*, e7551. [CrossRef] [PubMed]
116. Jaiswal, P.K.; Goel, A.; Mittal, R.D. Survivin: A Molecular Biomarker in Cancer. *Indian J. Med. Res.* **2015**, *141*, 389. [CrossRef]
117. Garg, H.; Suri, P.; Gupta, J.C.; Talwar, G.P.; Dubey, S. Survivin: A Unique Target for Tumor Therapy. *Cancer Cell Int.* **2016**, *16*, 49. [CrossRef]
118. Li, Y.; Lu, W.; Yang, J.; Edwards, M.; Jiang, S. Survivin as a Biological Biomarker for Diagnosis and Therapy. *Expert Opin. Biol. Ther.* **2021**, *21*, 1429–1441. [CrossRef] [PubMed]
119. Zhang, Z.; Wang, T.; Liu, Z.; Tang, S.; Yue, M.; Feng, S.; Hu, M.; Xuan, L.; Chen, Y. Small Interfering RNA Targeting of the Survivin Gene Inhibits Human Tumor Cell Growth in Vitro. *Exp. Ther. Med.* **2017**, *14*, 35. [CrossRef] [PubMed]
120. Li, Y.; Liu, D.; Zhou, Y.; Li, Y.; Xie, J.; Lee, R.J.; Cai, Y.; Teng, L. Silencing of Survivin Expression Leads to Reduced Proliferation and Cell Cycle Arrest in Cancer Cells. *J. Cancer* **2015**, *6*, 1187. [CrossRef] [PubMed]

121. Kesharwani, P.; Sheikh, A.; Abourehab, M.A.S.; Salve, R.; Gajbhiye, V. A Combinatorial Delivery of Survivin Targeted SiRNA Using Cancer Selective Nanoparticles for Triple Negative Breast Cancer Therapy. *J. Drug Deliv. Sci. Technol.* **2023**, *80*, 104164. [\[CrossRef\]](#)
122. Akbaba, H.; Erel-Akbaba, G.; Kotmakçı, M.; Başpınar, Y. Enhanced Cellular Uptake and Gene Silencing Activity of Survivin-SiRNA via Ultrasound-Mediated Nanobubbles in Lung Cancer Cells. *Pharm. Res.* **2020**, *37*, 165. [\[CrossRef\]](#)
123. Zhang, C.; Zhang, S.; Zhi, D.; Zhao, Y.; Cui, S.; Cui, J. Co-Delivery of Paclitaxel and Survivin SiRNA with Cationic Liposome for Lung Cancer Therapy. *Colloids Surf. A Physicochem. Eng. Asp.* **2020**, *585*, 124054. [\[CrossRef\]](#)
124. Tanioka, M.; Nokihara, H.; Yamamoto, N.; Yamada, Y.; Yamada, K.; Goto, Y.; Fujimoto, T.; Sekiguchi, R.; Uenaka, K.; Callies, S.; et al. Phase I Study of LY2181308, an Antisense Oligonucleotide against Survivin, in Patients with Advanced Solid Tumors. *Cancer Chemother. Pharmacol.* **2011**, *68*, 505–511. [\[CrossRef\]](#)
125. Xie, S.; Yin, S.; Li, D.; Nie, D.; Li, Y.; Ma, L.; Wang, X.; Xiao, J. The Effect of SiRNA Down-Regulating XIAP On the Apoptosis and Chemo-Sensitivity of K562 Cells in Vitro. *Blood* **2009**, *114*, 5053. [\[CrossRef\]](#)
126. Chen, J.; Xiao, X.Q.; Deng, C.M.; Su, X.S.; Li, G.Y. Downregulation of XIAP Expression by Small Interfering RNA Inhibits Cellular Viability and Increases Chemosensitivity to Methotrexate in Human Hepatoma Cell Line HepG2. *J. Chemother.* **2006**, *18*, 525–531. [\[CrossRef\]](#) [\[PubMed\]](#)
127. Li, Y.; Jian, Z.; Xia, K.; Li, X.; Lv, X.; Pei, H.; Chen, Z.; Li, J. XIAP Is Related to the Chemoresistance and Inhibited Its Expression by RNA Interference Sensitize Pancreatic Carcinoma Cells to Chemotherapeutics. *Pancreas* **2006**, *32*, 288–296. [\[CrossRef\]](#) [\[PubMed\]](#)
128. Xu, M.; Zhang, L.; Guo, Y.; Bai, L.; Luo, Y.; Wang, B.; Kuang, M.; Liu, X.; Sun, M.; Wang, C.; et al. Nanoemulsion Co-Loaded with XIAP SiRNA and Gambogic Acid for Inhalation Therapy of Lung Cancer. *Int. J. Mol. Sci.* **2022**, *23*, 14294. [\[CrossRef\]](#)
129. Chen, Z.; Han, F.; Du, Y.; Shi, H.; Zhou, W. Hypoxic Microenvironment in Cancer: Molecular Mechanisms and Therapeutic Interventions. *Signal Transduct. Target. Ther.* **2023**, *8*, 70. [\[CrossRef\]](#)
130. Infantino, V.; Santarsiero, A.; Convertini, P.; Todisco, S.; Iacobazzi, V. Cancer Cell Metabolism in Hypoxia: Role of HIF-1 as Key Regulator and Therapeutic Target. *Int. J. Mol. Sci.* **2021**, *22*, 5703. [\[CrossRef\]](#)
131. Zhao, Y.; Xing, C.; Deng, Y.; Ye, C.; Peng, H. HIF-1 α Signaling: Essential Roles in Tumorigenesis and Implications in Targeted Therapies. *Genes Dis.* **2024**, *11*, 234–251. [\[CrossRef\]](#) [\[PubMed\]](#)
132. Yan, Y.; Li, H.; Yao, H.; Cheng, X. Nanodelivery Systems Delivering Hypoxia-Inducible Factor-1 Alpha Short Interfering RNA and Antisense Oligonucleotide for Cancer Treatment. *Front. Nanotechnol.* **2022**, *4*, 932976. [\[CrossRef\]](#)
133. Liao, H.; Wang, G.; Huang, S.; Li, Y.; Cai, S.; Zhang, J.; Chen, H.; Wu, W. HIF-1 α Silencing Suppresses Growth of Lung Adenocarcinoma A549 Cells through Induction of Apoptosis. *Mol. Med. Rep.* **2014**, *9*, 911–915. [\[CrossRef\]](#)
134. Jeong, W.; Rapisarda, A.; Park, S.R.; Kinders, R.J.; Chen, A.; Melillo, G.; Turkbey, B.; Steinberg, S.M.; Choyke, P.; Doroshow, J.H.; et al. Pilot Trial of EZN-2968, an Antisense Oligonucleotide Inhibitor of Hypoxia-Inducible Factor-1 Alpha (HIF-1 α), in Patients with Refractory Solid Tumors. *Cancer Chemother. Pharmacol.* **2014**, *73*, 343. [\[CrossRef\]](#) [\[PubMed\]](#)
135. Montaña-Samaniego, M.; Bravo-Estupiñan, D.M.; Méndez-Guerrero, O.; Alarcón-Hernández, E.; Ibáñez-Hernández, M. Strategies for Targeting Gene Therapy in Cancer Cells with Tumor-Specific Promoters. *Front. Oncol.* **2020**, *10*, 605380. [\[CrossRef\]](#)
136. Kumar, A.; Das, S.K.; Emdad, L.; Fisher, P.B. Applications of Tissue-Specific and Cancer-Selective Gene Promoters for Cancer Diagnosis and Therapy. *Adv. Cancer Res.* **2023**, *160*, 253–315. [\[CrossRef\]](#)
137. Yang, Y.; Cao, Y. The Impact of VEGF on Cancer Metastasis and Systemic Disease. *Semin. Cancer Biol.* **2022**, *86*, 251–261. [\[CrossRef\]](#) [\[PubMed\]](#)
138. Duffy, A.M.; Bouchier-Hayes, D.J.; Harmey, J.H. Vascular Endothelial Growth Factor (VEGF) and Its Role in Non-Endothelial Cells: Autocrine Signalling by VEGF. In *Madame Curie Bioscience Database [Internet]*; Landes Bioscience: Austin, TX, USA, 2013.
139. Jin, M.; Zeng, B.; Liu, Y.; Jin, L.; Hou, Y.; Liu, C.; Liu, W.; Wu, H.; Chen, L.; Gao, Z.; et al. Co-Delivery of Repurposing Itraconazole and VEGF SiRNA by Composite Nanoparticulate System for Collaborative Anti-Angiogenesis and Anti-Tumor Efficacy against Breast Cancer. *Pharmaceutics* **2022**, *14*, 1369. [\[CrossRef\]](#)
140. Li, F.; Wang, Y.; Chen, W.L.; Wang, D.D.; Zhou, Y.J.; You, B.G.; Liu, Y.; Qu, C.X.; Yang, S.D.; Chen, M.T.; et al. Co-Delivery of VEGF SiRNA and Etoposide for Enhanced Anti-Angiogenesis and Anti-Proliferation Effect via Multi-Functional Nanoparticles for Orthotopic Non-Small Cell Lung Cancer Treatment. *Theranostics* **2019**, *9*, 5886–5898. [\[CrossRef\]](#) [\[PubMed\]](#)
141. Uribe, M.L.; Marrocco, I.; Yarden, Y. EGFR in Cancer: Signaling Mechanisms, Drugs, and Acquired Resistance. *Cancers* **2021**, *13*, 2748. [\[CrossRef\]](#) [\[PubMed\]](#)
142. Thomas, R.; Weihua, Z. Rethink of EGFR in Cancer with Its Kinase Independent Function on Board. *Front. Oncol.* **2019**, *9*, 468754. [\[CrossRef\]](#)
143. Videira, M.A.; Llop, J.; Sousa, C.; Kreutzer, B.; Cossío, U.; Forbes, B.; Vieira, I.; Gil, N.; Silva-Lima, B. Pulmonary Administration: Strengthening the Value of Therapeutic Proximity. *Front. Med.* **2020**, *7*, 50. [\[CrossRef\]](#) [\[PubMed\]](#)
144. Lu, Y.; Liu, L.; Wang, Y.; Li, F.; Zhang, J.; Ye, M.; Zhao, H.; Zhang, X.; Zhang, M.; Zhao, J.; et al. SiRNA Delivered by EGFR-Specific ScFv Sensitizes EGFR-TKI-Resistant Human Lung Cancer Cells. *Biomaterials* **2016**, *76*, 196–207. [\[CrossRef\]](#) [\[PubMed\]](#)

145. Majumder, J.; Minko, T. Multifunctional Lipid-Based Nanoparticles for Codelivery of Anticancer Drugs and Sirna for Treatment of Non-Small Cell Lung Cancer with Different Level of Resistance and Egfr Mutations. *Pharmaceutics* **2021**, *13*, 1063. [[CrossRef](#)] [[PubMed](#)]
146. Qing, L.; Li, Q.; Dong, Z. MUC1: An Emerging Target in Cancer Treatment and Diagnosis. *Bull. Cancer*. **2022**, *109*, 1202–1216. [[CrossRef](#)] [[PubMed](#)]
147. Lan, Y.; Ni, W.; Tai, G. Expression of MUC1 in Different Tumours and Its Clinical Significance. *Mol. Clin. Oncol.* **2022**, *17*, 161. [[CrossRef](#)] [[PubMed](#)]
148. Chen, X.; Sandrine, I.K.; Yang, M.; Tu, J.; Yuan, X. MUC1 and MUC16: Critical for Immune Modulation in Cancer Therapeutics. *Front. Immunol.* **2024**, *15*, 1356913. [[CrossRef](#)]
149. Ham, S.Y.; Kwon, T.; Bak, Y.; Yu, J.H.; Hong, J.; Lee, S.K.; Yu, D.Y.; Yoon, D.Y. Mucin 1-Mediated Chemo-Resistance in Lung Cancer Cells. *Oncogenesis* **2016**, *5*, e185. [[CrossRef](#)] [[PubMed](#)]
150. Hosseinzadeh, A.; Merikhian, P.; Naseri, N.; Eisavand, M.R.; Farahmand, L. MUC1 Is a Potential Target to Overcome Trastuzumab Resistance in Breast Cancer Therapy. *Cancer Cell Int.* **2022**, *22*, 110. [[CrossRef](#)]
151. Engel, B.J.; Bowser, J.L.; Broaddus, R.R.; Carson, D.D. MUC1 Stimulates EGFR Expression and Function in Endometrial Cancer. *Oncotarget* **2016**, *7*, 32796–32809. [[CrossRef](#)]
152. Hattrup, C.L.; Gendler, S.J. MUC1 Alters Oncogenic Events and Transcription in Human Breast Cancer Cells. *Breast Cancer Res.* **2006**, *8*, R37. [[CrossRef](#)] [[PubMed](#)]
153. Liu, L.; Wang, Y.; Miao, L.; Liu, Q.; Musetti, S.; Li, J.; Huang, L. Combination Immunotherapy of MUC1 mRNA Nano-Vaccine and CTLA-4 Blockade Effectively Inhibits Growth of Triple Negative Breast Cancer. *Mol. Ther.* **2018**, *26*, 45–55. [[CrossRef](#)]
154. Zhang, Y.; Xia, M.; Jin, K.; Wang, S.; Wei, H.; Fan, C.; Wu, Y.; Li, X.; Li, X.; Li, G.; et al. Function of the C-Met Receptor Tyrosine Kinase in Carcinogenesis and Associated Therapeutic Opportunities. *Mol. Cancer* **2018**, *17*, 45. [[CrossRef](#)]
155. Raj, S.; Kesari, K.K.; Kumar, A.; Rath, B.; Sharma, A.; Gupta, P.K.; Jha, S.K.; Jha, N.K.; Slama, P.; Roychoudhury, S.; et al. Molecular Mechanism(s) of Regulation(s) of c-MET/HGF Signaling in Head and Neck Cancer. *Mol. Cancer* **2022**, *21*, 45. [[CrossRef](#)]
156. Faiella, A.; Riccardi, F.; Carteni, G.; Chiurazzi, M.; Onofrio, L. The Emerging Role of C-Met in Carcinogenesis and Clinical Implications as a Possible Therapeutic Target. *J. Oncol.* **2022**, *2022*, 5179182. [[CrossRef](#)]
157. Xie, B.; Xing, R.; Chen, P.; Gou, Y.; Li, S.; Xiao, J.; Dong, J. Down-Regulation of c-Met Expression Inhibits Human HCC Cells Growth and Invasion by RNA Interference. *J. Surg. Res.* **2010**, *162*, 231–238. [[CrossRef](#)] [[PubMed](#)]
158. Ma, J.; Hu, C.; Li, W.; Ren, J.; Zou, F.; Zhou, D.; Zou, W.; Wei, Y.; Zhou, Y. Downregulation of C-Met Expression Does Not Enhance the Sensitivity of Gastric Cancer Cell Line MKN-45 to Gefitinib. *Mol. Med. Rep.* **2014**, *11*, 2269–2275. [[CrossRef](#)]
159. Zhang, Q.; Zhang, H.; Ning, T.; Liu, D.; Deng, T.; Liu, R.; Bai, M.; Zhu, K.; Li, J.; Fan, Q.; et al. Exosome-Delivered c-Met siRNA Could Reverse Chemoresistance to Cisplatin in Gastric Cancer. *Int. J. Nanomed.* **2020**, *15*, 2323. [[CrossRef](#)] [[PubMed](#)]
160. Dong, X.; Li, X.; Chen, J.; Ma, S.; Mu, D.; Hu, J.; Lu, S. Phase 1 Study of the Selective C-MET Inhibitor, HS-10241, in Patients With Advanced Solid Tumors. *JTO Clin. Res. Rep.* **2023**, *4*, 100449. [[CrossRef](#)]
161. Lee, D.H.; Roohullah, A.; Cho, B.C.; Lemech, C.R.; de Souza, P.L.; Millward, M.; Choi, J.Y.; Park, K.E.; Kim, N.Y.; Kim, E.; et al. A Phase 1 Dose-Escalation Study of the ABN401 (c-MET Inhibitor) in Patients with Solid Tumors. *J. Clin. Oncol.* **2022**, *40*, 3105. [[CrossRef](#)]
162. Galogre, M.; Rodin, D.; Pyatnitskiy, M.; Mackelprang, M.; Koman, I. A Review of HER2 Overexpression and Somatic Mutations in Cancers. *Crit. Rev. Oncol. Hematol.* **2023**, *186*, 103997. [[CrossRef](#)]
163. Cheng, X. A Comprehensive Review of HER2 in Cancer Biology and Therapeutics. *Genes* **2024**, *15*, 903. [[CrossRef](#)] [[PubMed](#)]
164. Faltus, T.; Yuan, J.; Zimmer, B.; Krämer, A.; Loibl, S.; Kaufmann, M.; Strebhardt, K. Silencing of the HER2/Neu Gene by siRNA Inhibits Proliferation and Induces Apoptosis in HER2/Neu-Overexpressing Breast Cancer Cells. *Neoplasia* **2004**, *6*, 786. [[CrossRef](#)]
165. Balgobind, A.; Daniels, A.; Ariatti, M.; Singh, M. HER2/Neu Oncogene Silencing in a Breast Cancer Cell Model Using Cationic Lipid-Based Delivery Systems. *Pharmaceutics* **2023**, *15*, 1190. [[CrossRef](#)] [[PubMed](#)]
166. Gu, S.; Hu, Z.; Ngamcherdtrakul, W.; Castro, D.J.; Morry, J.; Reda, M.M.; Gray, J.W.; Yantasee, W. Therapeutic siRNA for Drug-Resistant HER2-Positive Breast Cancer. *Oncotarget* **2016**, *7*, 14727. [[CrossRef](#)]
167. Archana, M.G.; Anusree, K.S.; Unnikrishnan, B.S.; Reshma, P.L.; Syama, H.P.; Sreekutty, J.; Joseph, M.M.; Sreelekha, T.T. HER2 siRNA Facilitated Gene Silencing Coupled with Doxorubicin Delivery: A Dual Responsive Nanoplatfrom Abrogates Breast Cancer. *ACS Appl. Mater. Interfaces* **2024**, *16*, 25710–25726. [[CrossRef](#)]
168. Vervaeke, P.; Borgos, S.E.; Sanders, N.N.; Combes, F. Regulatory Guidelines and Preclinical Tools to Study the Biodistribution of RNA Therapeutics. *Adv. Drug Deliv. Rev.* **2022**, *184*, 114236. [[CrossRef](#)]
169. Sharma, P.; Jhawar, V.; Mathur, P.; Dutt, R. Innovation in Cancer Therapeutics and Regulatory Perspectives. *Med. Oncol.* **2022**, *39*, 76. [[CrossRef](#)] [[PubMed](#)]
170. Jones, P.S.; Jones, D. New Regulatory Framework for Cancer Drug Development. *Drug Discov. Today* **2012**, *17*, 227–231. [[CrossRef](#)] [[PubMed](#)]

171. Committee for Advanced Therapies (CAT). *ICH Q5E Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process: Comparability of Biotechnological/Biological Products—Scientific Guideline*; European Medicines Agency: Amsterdam, The Netherlands, 2005.
172. Committee for Advanced Therapies (CAT). *ICH Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products—Scientific Guideline*; European Medicines Agency: Amsterdam, The Netherlands, 1999.
173. Center for Drug Evaluation and Research. *Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics*; European Medicines Agency: Amsterdam, The Netherlands, 2024.
174. Committee for Advanced Therapies (CAT). *Gene Therapy Product Quality Aspects in the Production of Vectors and Genetically Modified Somatic Cells—Scientific Guideline*; European Medicines Agency: Amsterdam, The Netherlands, 1995.
175. Center for Biologics Evaluation and Research. *Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products*; European Medicines Agency: Amsterdam, The Netherlands, 2015.
176. Center for Biologics Evaluation and Research. *Considerations for the Use of Human and Animal-Derived Materials in the Manufacture of Cell and Gene Therapy and Tissue-Engineered Medical Products*; European Medicines Agency: Amsterdam, The Netherlands, 2024.
177. Committee for Advanced Therapies (CAT). *ICH Q11 Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities)—Scientific Guideline*; European Medicines Agency: Amsterdam, The Netherlands, 2012.
178. Committee for Advanced Therapies (CAT). *Quality, Preclinical and Clinical Aspects of Gene Therapy Medicinal Products—Scientific Guideline*; European Medicines Agency: Amsterdam, The Netherlands, 2014.
179. Center for Biologics Evaluation and Research. *Human Gene Therapy Products Incorporating Human Genome Editing*; European Medicines Agency: Amsterdam, The Netherlands, 2024.
180. Center for Biologics Evaluation and Research; Center for Drug Evaluation and Research. *Adaptive Design Clinical Trials for Drugs and Biologics Guidance for Industry*; European Medicines Agency: Amsterdam, The Netherlands, 2019.
181. Committee for Medicinal Products for Human Use (CHMP). *Methodological Issues in Confirmatory Clinical Trials Planned with an Adaptive Design—Scientific Guideline*; European Medicines Agency: Amsterdam, The Netherlands, 2007.
182. Committee for Veterinary Medicinal Products (CVMP). *Development of a Guideline on the Safety of Nanoparticles—In the Context of the Establishment of Maximum Residue Limits and Veterinary Marketing Authorisations*; European Medicines Agency: Amsterdam, The Netherlands, 2024.
183. Food and Drug Administration. Fast Track, Breakthrough Therapy, Accelerated Approval, Priority Review. Available online: <https://www.fda.gov/patients/learn-about-drug-and-device-approvals/fast-track-breakthrough-therapy-accelerated-approval-priority-review> (accessed on 28 September 2024).
184. European Medicines Agency. PRIME: Priority Medicines. Available online: <https://www.ema.europa.eu/en/human-regulatory-overview/research-development/prime-priority-medicines> (accessed on 28 September 2024).

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.