RNA Interference-Based Cancer Drugs: The Roadblocks, and the “Delivery” of the Promise

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Nucleic acid-based therapeutics like synthetic small interfering RNAs have been exploited to modulate gene function, taking advantage of RNA interference (RNAi), an evolutionally conserved biological process. Recently, the world's first RNAi drug was approved for a rare genetic disorder in the liver. However, there are significant challenges that need to be resolved before RNAi can be translated in other genetic diseases like cancer. Current drug delivery platforms for therapeutic silencing RNAs are tailored to hepatic targets. RNAi therapies for nonhepatic conditions are still at early clinical phases. In this study, we discuss the critical design considerations in anticancer RNAi drug development, insights gained from initial clinical trials, and new strategies that are entering clinical development, shaping the future of RNAi in cancer.

Keywords: siRNA, gene silencing, RNA interference, cancer therapy

The Journey of RNA Interference: An Overview

FROM MODEL ORGANISMS to the Nobel Prize in Medicine in 2006 leading to world's first approved RNA interference (RNAi) drug in 2018 with Alnylam's Patisiran™, using RNA to manipulate gene function has come a long way [1,2]. RNAi is a biological process using single antisense or double-stranded RNA to suppress genetic expression, facilitated by hybridization between the administered RNA and endogenous messenger RNA (mRNA) [1]. This strategy of genetic interference can be exploited clinically using exogenous nucleic acids like short hairpin RNA, micro RNA (miRNA), and synthetic small interfering RNA (siRNA) to modulate specific gene function.

The first clinical translation came in 2017 when Alnylam Pharmaceuticals reported a successful Phase III trial (NCT01960348) using Patisiran, an RNAi drug against transthyretin, in the treatment of hereditary transthyretin amyloidosis [3], leading to the regulatory approval by US Food and Drug Administration (FDA) in August 10, 2018.

Andrew Z. Fire wondered in his Nobel Prize lecture in 2006 if it will be feasible to target a gene associated with cancer using a double-stranded RNA, to inhibit tumor growth [2]. Currently, we lack advanced clinical data from human cancer patients harnessing RNAi mediated gene silencing; however, promising results from preclinical animal models of cancer and a limited number of early phase clinical trials encourage further development of RNAi based anticancer drugs [4–9]. Nevertheless, there are numerous clinical chal-

lenges associated with delivery of siRNA and other nucleic acid mediated therapeutics, and the complexity of cancers as disease targets, that need to be addressed to advance the development of RNAi drugs in anticancer therapy.

Drug Delivery Systems for RNAi: Design Considerations

RNAi drugs require appropriate drug delivery systems. The efficiency of naked molecules of siRNA to enter the cytoplasm of the cells, where RNAi-based manipulation is facilitated, is rather low [10]. RNAi mediators like siRNAs undergo rapid renal clearance, degraded by endogenous RNases, and recognized by innate immune receptors leading to immune related adverse events. Thus, there is a need for optimizing drug delivery systems to facilitate internalization into cells, release of the drug from the delivery system and endosomes, and allowing delivery into the cytoplasmic compartment of cells, further minimizing off-target delivery and immune activation [11–13]. However, for solid tumors that are often associated with immunosuppression, immune activation may be beneficial [14].

Lipid nanoparticle formulations

For systemic administration of siRNAs, lipid nanoparticle (LNP) is the most clinically optimized drug delivery platform. The recently approved Patisiran also exploits LNPs for siRNA delivery. LNPs are not a new entity in cancer drug development, and regulatory agencies globally had approved

multiple LNP based anticancer drugs [15]. LNPs exploit the capability of lipids to self-assemble into bilayer structures and encapsulate nucleic acids when subjected to dispersion in an aqueous medium. For drug delivery in solid tumors, this strategy can be particularly useful as nanoparticles can selectively extravasate into tumor sites after systemic administration, and preferentially accumulate, taking advantage of the enhanced permeability and retention effect [16].

The identity of the lipids in LNP design can be critical to mitigate delivery challenges in RNAi. Cationic lipids had been traditionally used for transfection purposes, *in vitro*, and *in vivo*, to facilitate interaction with negatively charged RNAi molecules and cell membrane, driving internalization, and release from the endosomal compartment by forming ion pairs with the anionic lipids of the membrane [17,18].

However, the permanent positive surface charge may contribute to dose-limiting toxicity and rapid clearance by the reticuloendothelial system [19]. This inspired the development of a new generation of cationic lipids that are ionizable, to minimize toxicity and immunogenicity [20]. These lipids typically have a $pK_a < 7$ allowing complexation with nucleic acids at low pH due to protonation of the amine head group, while maintaining a neutral to slightly positive charge at physiological pH. Patisiran exploits a formulation based on an ionizable lipid called DLin-MC3-DMA, with proven potency for hepatic gene silencing *in vivo* [21].

Treating cancer requires strategies to target cells beyond the liver. Recent preclinical studies have exploited siRNA delivery in cancer using strategies beyond LNPs, such as the incorporation of targeting ligand on lipopolyplexes by post-modification [6], aptamer-based targeting using DNA nanostructures [22], and spherical nucleic acid nanoparticles coated with polyethyleneimine [23], among others. Several siRNA-based anticancer drugs have completed Phase I of clinical trials at this point. Majority of them are nontargeted LNPs, polymeric nanoparticles being the other platform exploited as drug delivery vehicles [9].

Challenges of RNAi Drug Development in Cancer

Dosing of therapeutics

Designing anticancer RNAi therapeutics requires considering certain factors that are unique to the inherent complexity of cancer as a disease. It has been shown in past that dilution of siRNA due to cell division may govern the duration of gene silencing [24]. This is a critical consideration in determining clinical dosing schedules with rapidly dividing cancer cells, which are distinct from hepatocytes, undergoing cell division at a slower rate. So far, there has been much variability in dosage frequency when it comes to Phase I clinical trials in cancer RNAi, ranging from once to twice per week to twice a month [25–29].

In contrast to these studies, the liver-targeted Patisiran was dosed once every 3 weeks during the Phase III trial [3]. However, the dose may play a critical role in determining therapeutic efficacy. Silence Therapeutics investigated Atu027, a siRNA-lipoplex targeting protein kinase N3, in a recent Phase Ib/IIa trial with 23 metastatic pancreatic adenocarcinoma patients. The study demonstrated a mean progression-free survival of 5.3 versus 1.8 months in the treatment arm dosed twice per week over the arm with once-weekly dosing [26]. Repeated dosing may further facilitate

delivery of the RNAi therapeutic to a maximum number of cancer cells. A systemic route of delivery is also preferable as it allows better access to metastatic tumors.

Delivery to extrahepatic targets

Another critical issue with RNAi therapeutic development in cancer is an off-target accumulation of the delivery vehicle and its cargo. LNP formulations are optimized to accumulate in the hepatic tissues due to the binding of ionizable lipids with apolipoprotein E, driving uptake in hepatocytes [30], which make them attractive for targeting genetic conditions in the liver. However, this can become an issue with drug delivery to solid tumors.

In Alnylam's LNP based siRNA formulation of siRNAs against vascular endothelial growth factor A (VEGF-A) and kinesin spindle protein (KSP), the most prominent mRNA cleavage results were obtained primarily from normal hepatic tissues [28]. This preferential accumulation may lead to liver toxicities if the chosen target for gene silencing has a high basal level expression in normal tissues like liver, warranting a careful consideration of the gene expression of the therapeutic target in different tissues. It is also important to optimize formulations and consider approaches like tumor tissue targeting to shift biodistribution of the formulation. So far, clinical exploration of targeted formulations against non-hepatic targets in the domain of RNAi drugs is limited.

Calando Pharmaceutical's CALAA-01, a siRNA therapeutic against ribonucleotide reductase regulatory subunit M2 (RRM2), remains a limited clinical example of an experimental therapeutic exploiting a targeting ligand [29]. The formulation above uses transferrin as a targeting agent to enhance delivery to cancer cells that overexpress transferrin receptors [31]. While transferrin targeting can be beneficial in drug delivery, the authors noted drug instability in the targeted formulations in the previous CALAA-01 study, which may have contributed to adverse effects in the Phase I trials. This is an important clinical lesson and requires careful optimization of formulation stability and quality control in future clinical trials.

Furthermore, incorporating a targeting agent adds additional regulatory hurdles and may significantly increase costs associated with developing therapeutics, requiring justification of the benefits associated with multifunctional nanoparticles [32].

It is also worthwhile to look beyond LNPs as drug delivery vehicles for gene delivery to nonhepatic tumor targets in a clinical setting. Alnylam had developed "enhanced stabilization chemistry" to design chemically modified siRNAs that are metabolically stable [33]. These *N*-acetylgalactosamine (GalNAc) conjugated siRNAs have 2'-deoxy-2'-fluoro (2'-F) and 2'-O-methyl (2'-OMe) modifications on the ribose sugar on both strands with phosphorothioate linkages at the termini, which provide protection against degradation mediated by 5' and 3' exonucleases [34].

Currently, new RNAi formulations are entering early phase clinical trials to investigate alternate strategies in anticancer therapy. One of these studies (NCT03608631) for patients with metastatic pancreatic cancer is planning to explore exosomes derived from mesenchymal stromal cells as a delivery vehicle for siRNA against KRas^{G12D}, the most common mutant form of the *KRAS* gene, a driver oncogene in pancreatic ductal adenocarcinoma (PDAC) [35]. There is another ongoing Phase II

trial targeting KRas^{G12D} in patients with locally advanced pancreatic cancer, using a bio polymer matrix implant [36] for siRNA delivery to the unresectable tumor.

KRas driven cancers such as PDAC remain challenging to treat, “undruggable” [37], and subsequently, these trials using an RNAi approach against oncogenes arose optimism. Furthermore, designing specific RNAi therapeutics such as KRas^{G12D} siRNA that selectively targets mutated genes expressed on cancer cells may further aid to mitigate toxicities associated with off-target delivery.

Safety

One of the most promising aspects of these early phase clinical trials with RNAi drugs against cancer was the associated safety profile. The therapeutics were relatively well tolerated, and cytokine release and infusion-related adverse events were manageable with supportive treatments [9]. Complement activation was observed with some formulations, although transient. Furthermore, none of these delivery systems showed any major signs of antibody-mediated rapid clearance affecting pharmacokinetics after multiple dosing.

Combining RNAi with small molecule agents

Multidrug resistance and toxicity to healthy cells can limit the efficacy of chemotherapeutic drugs. Combination of chemotherapy with suitable siRNA drugs had been exploited to reverse drug resistance and further allow synergistic therapeutic effect. Multiple studies had investigated combinations of drugs like Doxorubicin or Paclitaxel with siRNAs targeting drug efflux pump related proteins like P-glycoprotein, which are overexpressed in several cancers, to enhance the therapeutic efficacy of chemotherapeutic agents [38]. Chemoresistance can also be driven by cancer stem cells (CSCs), and miRNAs targeting the CSCs in combination with chemotherapeutic drugs such as gemcitabine were explored [39].

The development of co-delivery systems combining chemotherapeutics with siRNAs silencing antiapoptotic genes is another effective strategy to improve therapeutic outcome in cancer [40,41]. In recent years, RNAi-based synthetic lethality screens had been conducted to enhance the efficacy of targeted kinase inhibitor monotherapy [42]. Using these screens, genetic targets can be identified that compensate for signaling perturbed by the original drug, and the information about the feedback mechanism can be used to develop rational combination therapies using a targeted kinase inhibitor and siRNAs targeting the compensatory signaling pathways [43]. However, designing delivery platforms involving a combination of siRNAs and chemotherapeutic drugs can be challenging.

In recent years, a multitude of nanoparticle-based drug delivery platforms had been optimized to co-encapsulate drugs with different physicochemical properties, exploiting electrostatic and hydrophobic interactions. For nucleic acid reacting drugs, damage of the siRNA can occur during the co-encapsulation process, which may limit drug-siRNA pairing for co-delivery.

Harnessing RNAi to cancer immunotherapy

While immune-related adverse events are not desired for a safe therapeutic in the clinic, immune stimulation can be a useful clinical strategy in multiple malignancies. The year

TABLE 1. ONGOING CLINICAL TRIALS FOR RNA INTERFERENCE-BASED CANCER DRUGS

Condition	Intervention	Phase	Sponsor	Clinical trial identifier
Metastatic pancreatic ductal adenocarcinoma	KRas ^{G12D} siRNA loaded in exosomes from mesenchymal stem cells	Phase I	M.D. Anderson Cancer Center	NCT03608631
Advanced solid tumors	EPHA2 siRNA in DOPC liposomes	Phase I	M.D. Anderson Cancer Center	NCT01591356
Recurrent/metastatic pancreatic cancer, colorectal cancer, and other surgically unresectable solid tumors	PBMC of individual patients silenced <i>ex-vivo</i> with cbl-b siRNA	Phase I	Wake Forest University Health Sciences	NCT03087591
Hematological malignancies	Minor histocompatibility antigen-loaded PD-L1/L2-silenced donor dendritic cells	Phase I/II	Radboud University	NCT02528682
Unresectable locally advanced pancreatic cancer	KRas ^{G12D} siRNA biopolymeric matrix implant	Phase II	Silenseed Ltd.	NCT01676259

DOPC, dipalmitoylphosphatidylcholine; PBMC, peripheral blood mononuclear cell; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; siRNA, small interfering RNA.

2018 witnessed the Nobel Prize in Medicine going to cancer immunotherapy for the work involving immune checkpoint inhibitors that revolutionized cancer treatment by offering strategies to interfere with immune regulation exploited by cancer cells to evade eradication by the immune system [44,45].

While checkpoint inhibitor therapies may allow long-term clinical responses, the therapies only work for a small subset of patients. Current efforts are focused on increasing the efficacy of checkpoint inhibitors and identifying other nodes to interfere with immune regulation mediated by checkpoint signaling. One early phase clinical trial (NCT02528682) is currently exploring a cancer vaccine for hematological malignancies using checkpoint inhibitor silenced dendritic cells, with silencing mediated by *ex vivo* transfection with siRNAs against programmed death-ligand 1 (PD-L1) and the related molecule programmed death-ligand 2 (PD-L2).

Recently, targeted LNPs had been used for co-delivery of mRNA encoding for tyrosinase-related protein 2 antigen and PD-L1 siRNA, in a preclinical melanoma model [46]. This is not the only example of harnessing RNAi to cancer immunotherapy. Casitas-B-lineage lymphoma protein-b, a critical immune regulator [47], belonging to the class of proteins called E3 ubiquitin ligase was silenced *ex vivo* in peripheral blood mononuclear cells and investigated in patients with metastatic tumors in another Phase I (NCT03087591) study [48]. It is also possible to design siRNA-based therapeutics harnessing RNAi and sequence-dependent immune activation by actuating pattern recognition receptors that can stimulate the rapid onset innate immune system [49,50].

Conclusions

Many of the early phase RNAi trials in cancer were not specific to a single cancer type, the patients were often heavily pretreated, and the genetic profile of the patient's tumors was unknown. In the future study designs, a more focused approach, preselecting a patient population with a genetic signature predictive of therapeutic response will be necessary to improve clinical outcomes [51]. As of October 2018, at least five studies are investigating anticancer RNAi therapeutics (Table 1) and are at different stages of recruitment (NCT03608631, NCT01591356, NCT03087591, NCT02528682, and NCT01676259). While the lack of market interest can be disappointing, this pattern is not new to RNAi drug development in general.

Multiple RNAi therapeutic development programs were shut down after early clinical trial failures in the previous decade [52]. The tide started to turn around in the early 2010s when results from human trials focused on liver delivery were available from formulations based on DLin-MC3-DMA and GalNAc conjugated siRNAs [53]. Currently, many firms are strategizing and focusing their resources on liver-targeted therapies, driven by the therapeutic opportunities available with the platforms tailored to hepatic delivery [54]. However, there is enough to be hopeful about, and liver is probably just the beginning of the clinical journey of RNAi.

The results from the Phase I clinical trials in anticancer RNAi demonstrated that it is possible to deliver therapeutic siRNA to human tumors with limited and mostly manageable immune-related toxicities. The field is moving at an extraordinary pace, and despite its challenges, the fortunes for

RNAi therapy in liver cancer and extrahepatic solid tumors may begin to turn around soon. Newer formulation platforms are being investigated to resolve delivery challenges [55], bringing hope for oncogene driven malignancies with limited therapeutic options.

Author Disclosure Statement

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