Molecular Therapy
Nucleic Acids
Review

Therapeutic miRNA and siRNA: Moving from Bench to Clinic as Next Generation Medicine

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In the past few years, therapeutic microRNA (miRNA) and small interfering RNA (siRNA) are some of the most important biopharmaceuticals that are in commercial space as future medicines. This review summarizes the patents of miRNA- and siRNA-based new drugs, and also provides a snapshot about significant biopharmaceutical companies that are investing for the therapeutic development of miRNA and siRNA molecules. An insightful view about individual siRNA and miRNA drugs has been depicted with their present status, which is gaining attention in the therapeutic landscape. The efforts of the biopharmaceuticals are discussed with the status of their preclinical and/or clinical trials. Here, some of the setbacks have been highlighted during the biopharmaceutical development of miRNA and siRNA as individual therapeutics. Finally, a snapshot is illustrated about pharmacokinetics, pharmacodynamics with absorption, distribution, metabolism, and excretion (ADME), which is the fundamental development process of these therapeutics, as well as the delivery system for miRNA- and siRNA-based drugs.

INTRODUCTION

Victor Ambros[1] and his groups from Harvard University led to a new milestone in research by discovering microRNAs (miRNAs) in 1993. The first miRNA gene discovered was lin-4, which contains sequences complementary to a repeated sequence element in the 3′ UTR of the lin-14 mRNA from *Caenorhabditis elegans*.[1] Next, lin-4 was considered a worm genetics discovery. However, it was not until the discovery of a second miRNA, let-7, that miRNAs were found to be highly conserved among all groups of animals, including humans.[2,3] This discovery augmented the research and development of numerous miRNAs in *C. elegans*, *Drosophila*, and human cell lines, especially in HeLa cells. The increased research on miRNAs leads to studies linking miRNA dysregulation and human disease; such a study was published in 2002. In this work, Calin et al.[4] describe two human miRNA genes, mir-15a and mir-16-1, that are downregulated or frequently deleted chronic lymphocytic leukemia (CLL) diseases. Presently, thousands of miRNAs have been found in plants and animals including humans. In 2011, Kozomara and Griffiths-Jones[5] recorded that 18,226 miRNAs have been noted in animals, plants, and viruses, and that 1,921 miRNAs were encrypted in the human genome. Significantly, the world's first miRNA therapeutic, miravirsen, a short locked nucleic acid (LNA) for miR-122, is currently moving toward the market. This drug is currently in phase II clinical trials for the treatment of hepatitis C virus (HCV) infection.[6]

Small interfering RNA (siRNA) is a potent tool for target-specific gene silencing through RNAi. Gene silencing by RNAi was first observed in 1998 by Craig Mello and his co-researchers in *C. elegans*.[7] For this work, Mello and Fire received the Nobel Prize in 2006.[8] Now, Dr. Craig Mello is one of the founders of RXi Pharmaceuticals, a company that develops RNAi compounds. The most important idea behind RXi Pharmaceuticals Corporation is the unique ability of their compounds to be "self-delivering," meaning that no additional delivery vehicles are needed for specific targeting.[9] Currently, "Big Pharma" companies are watching the clinical trial trends for RNAi therapeutics.

To date, approximately 20 clinical trials have been initiated using miRNA- and siRNA-based therapeutics. These drugs are running platforms driven by four leading RNA-therapeutic companies. Only one miRNA therapeutic, the compound SPC3649 (miravirsen), which is an inhibitor of miR-122 developed by Santaris Pharma from Denmark, is entered in a clinical trial. Several other miRNA therapeutics are in the preclinical stage and aiming to enter clinical trials. In contrast, several siRNA-based therapeutics have been introduced into clinical trials. Some researchers believe that miRNAs fall into the category of RNAi-based therapeutics.[10] The biogenesis and mechanism of action of miRNAs are quite similar to siRNAs with respect to post-transcriptional gene silencing.[11,12] It has been noted that miRNAs are endogenous short RNAs that combine with Argonaute proteins to regulate gene expression. At the translational level, these two categories of small RNAs are important for the gene regulatory landscape in the present scientific world. miRNAs are known as regulators of endogenous genes.[13] In contrast, siRNAs help to maintain genome stability. Both are single-stranded forms and were found to associate with effector associations[14]

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that are recognized as RNA-induced silencing complexes (RISCs).[15] However, there are differences between miRNA and siRNA modes of action, because both of these ribonucleic acids act differently. miRNAs mostly use eight nucleotides from their 5′ end to identify target mRNA sequences and utilize their inhibitory activities on the translation process. In contrast, siRNAs use approximately their full lengths to recognize target sequences and mediate cleavage of the target mRNA.[16]

In this paper, we examine the patent landscape of therapeutic miRNAs and siRNAs. We present insights regarding significant siRNAs and miRNAs in the therapeutic landscape and their useful therapeutic modalities. We also focus on their parent biopharmaceutical companies and the status of their preclinical and/or clinical trials. Some of the bottlenecks involved have been highlighted in the discussion. Finally, we also tried to elucidate the fundamental views on pharmacokinetics, pharmacodynamics, and efficient delivery systems for therapeutic RNAs.

Patent Landscape for Therapeutic miRNAs and siRNAs

The potential importance of patent rights is increasing day by day in the area of innovation. Patent rights are not limited to pharmaceuticals, as it has become a common practice for other innovations as well. It has been noted that newly developed modern biology techniques, such as monoclonal antibodies and rDNA techniques, are subject to patent rights.[17] Additionally, biological drug candidates and small-molecule drugs have also been subject to patent rights. This instance resulted in the transfer of academic technologies to industries. The government has provided licenses to industry for several biomolecules. Presently, innovation and patents play a crucial role in biopharmaceuticals, as well as for the biopharmaceutical industry.[18] Over the past decade, the number of patents issued to biopharmaceutical companies has increased substantially. Therefore, obtaining patent rights for small RNA-based therapeutics is a significant area. However, the market value that each company can derive from the supporting technologies also depends on their ability to protect the patents with well-licensed purposes. The companies have to keep them as trade secrets to get a competitive advantage in areas that are curtailed for miRNA or RNAi technological success. Laitala-Leinonen[19] has reviewed recent patent applications regarding miRNA biology[20] and described the patent search through the Delphion patent database, where 1,661 American miRNA-related patent papers were found, as documented by International Patent Classification (IPC) codes. miRNA-related patent documents cover approximately 60 IPC code categories. Nearly 50% of USA patent papers were categorized as relating to some type of medicinal preparation consisting of pharmaceutical compositions that encompass miRNA-modulating compounds or the methods related to miRNA-modulating activity for the treatment of diseases. The researchers also describe a large number of patent filings related to methods for treating cancer, significantly more than any other illness or disorder. In the case of other diseases, less than 10% of the patents were issued for each other disorder. The authors also show that the first miRNA-based patent was published in Europe in 2008.[21] However, we found from the Google Scholar database that a patent was granted for miRNA analysis (US 20070092882 A1); this is an American patent. It has been noted that the initial patents for specific miRNAs were filed by several advanced research institutes, universities, or pharmaceutical companies throughout the globe, including in Europe (Max Planck Society for the Advancement of Science, Munich, Germany); the United States (University of Massachusetts, Massachusetts Institute of Technology, and Rockefeller University, New York, NY); Asia (College of Medicine, Pochon Cha University Industry-Academic Cooperation Foundation, Gyeonggi-do, South Korea and Council of Scientific & Industrial Research, India); Western Asia (Rosetta Genomics, Rehovot, Israel), etc. Methods for the functional analysis of miRNA, as well as anti-miRNAs with improved activities and efficacies, have also been patented by companies and universities such as Regulus Therapeutics, Stanford University, etc.[21] The US Patent and Trademark Office's (USPTO's) Utility Guidelines were published in 2001 for the controversy related to the patenting of gene sequences developed in the late 1990s. The goal of the USPTO's Utility Guidelines was to propagate patent requirements and satisfy the requirements.[22,23] We performed a search for the total number of patents with the terms "microRNA" and "siRNA" from the USA patent search database (http://patft.uspto.gov/) and European Patent Office database (http://www.epo.org/searching-for-patents.html), and found that more patents have been granted for siRNA compared with miRNAs in both databases (Figure 1). However, we found a difference in the trends between the number of US and European patent filings. We have also performed a search regarding the number of patents with the terms "microRNA" and "siRNA" with different diseases, such as cancer, viral infections, inflammatory disorders, cardiovascular disorders, neurological disorders, ocular disorders, and metabolic disorders. In the US patent database, we found that the highest number of patents was granted for "siRNA and cancer" (6,560 patents), whereas the lowest number of patents was granted for "miRNA and ocular disorder" (17 patent). These filings reveal that there is a problem with miRNA therapeutics with respect to ocular disease; this may be because of problems with nucleic acid delivery systems in eye-related diseases.

We performed an analysis on the US patents for different cancers and found that the highest number of patents was granted for "siRNA and breast cancer" (3,284 patents). These findings demonstrate that this is an important research area. In contrast, the lowest number of patents was granted for "miRNA and renal cancer" (109 patents). These searches may assist us in identifying intellectual properties (IPs) that may help us better understand the real potential of miRNA- and siRNA-based products for market uniqueness. Table 1 shows a summary of several important US and European patents related to miRNA and siRNA. This table denotes several important properties of the patents, including the patent application number, content of the patent statement, inventors, and applicant institute.

Biopharmaceutical Industry and Therapeutic miRNAs and siRNAs

The biotechnology industry is currently flourishing. It has been noted that US biotechnology industry revenues have increased from $20 billion in 1996 to $70.1 billion in 2008. In contrast, biotechnology research and development (R&D) expenditures in industry increased

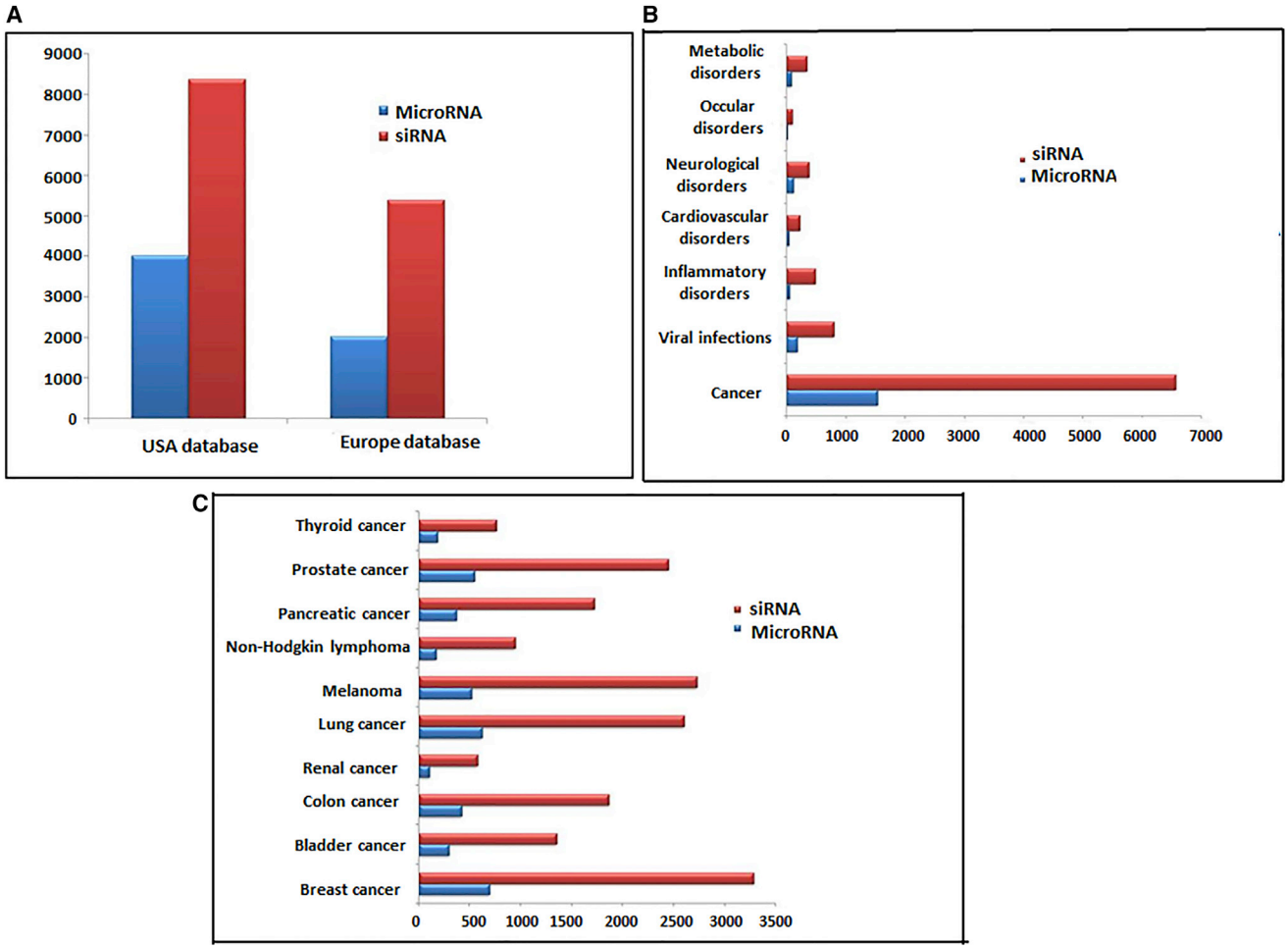

Figure 1. miRNA and siRNA Patents

(A) Total number of keyword searches ("microRNA" and "siRNA") performed in both the US patent search database and the European patent office database. (B) Number of patents in "microRNA" and "siRNA" in case of the different diseases. (C) Number of patents in "microRNA" and "siRNA" in event of the different type of cancers.

from $10.8 billion to $30.4 billion.[24] The biopharmaceutical industry plays a major role in the US biotechnology industry. Biopharmaceutical industries are investing in the development of therapeutic miRNAs and siRNAs. Several biopharmaceutical industries, such as Alnylam Pharmaceuticals, Rosetta Genomics, and Regulus Therapeutics, were established during the last 25 years (Figure 2). These biopharma companies are investing in the development of miRNA- and siRNA-based therapeutic molecules. However, there is a challenge for small biotechnology companies because there is some financial volatility in this area.[25,26] Big Pharma is using small companies to develop molecules for R&D to clinical trials. Big Pharma is investing in this new area to enter into the market with new therapeutic miRNA and siRNA molecules as early as possible.

Significant siRNA Therapeutics in Clinical Trials and Their Biopharmaceutical Companies

It is well established that siRNAs are therapeutic for gene silencing.[27] Therefore, nucleic-acid-based biopharmaceuticals are entering the market with the help of new biopharmaceutical companies. It has been noted that more than 14 RNAi therapeutic programs have entered clinical trials in the past decade (Figure 3, top left). The NIH in the United States has developed a database regarding the molecules that have completed clinical trials or are in ongoing clinical trials (https://www.clinicaltrials.gov). Seven out of 14 RNAi therapeutics are for localized and topical use (4 for the eye, 2 for the respiratory tract, and 1 for the skin). Approximately 1,500 patients and healthy volunteers have been enrolled in RNAi clinical programs that use siRNA therapeutics.[28] We will discuss some of the important therapeutic siRNAs with ongoing clinical trials below. Snapshots of these therapeutics are presented in Table 2.

ALN-RSV01 and ALN-TTR from Alnylam Pharmaceuticals

Alnylam Pharmaceuticals has strategic alliances with two pharmaceutical companies (Cubist Pharmaceuticals and Kyowa Hakko Kirin) and has initiated siRNA therapeutic (molecular name: ALN-RSV01) human clinical trials for the treatment of respiratory syncytial

Table 1. Some Relevant Issued Patents Related to MicroRNA and siRNA

Patent No.	Patent Type	Application No.	Publication Date	Content of the Patent Statement	Inventors	Applicant
CA2404890 C	USA	PCT/US2001/010188	November 19, 2013	RNA sequence-specific mediators of RNAi	Thomas Tuschl et al.	Whitehead Institute for Biomedical Research and seven more
EP 1309726 B1	European	EP20010922870	December 2, 2009	RNA sequence-specific mediators of RNAi	Thomas Tuschl et al.	Whitehead Institute for Biomedical Research and three more
EP1550719 B1	European	EP20050002454	December 24, 2008	double-stranded RNA (dsRNA) for inhibition of the expression of a defined gene	Roland Kreutzer and Stefan Limmer	Alnylam Europe AG
US7056704 B2	USA	US 10/832,432	June 6, 2006	RNAi mediating small RNA molecules	Thomas Tuschl et al.	Max-Planck-Gesellschaft zur Foerderung der Wissenschaften E.V.
US 7750144 B2	USA	US 10/912,440	July 6, 2010	mediates silencing of a target gene; lessening the base pair strength between the 5′ end of the first strand and the 3′ end of the second strand of the duplex as compared with the base pair strength	Phillip D. Zamore et al.	University of Massachusetts
EP1633890 B1	European	EP20040753972	October 20, 2010	methods and compositions for enhancing the efficacy and specificity of RNAi	Phillip D. Zamore et al.	University of Massachusetts
EP1309726 B1	European	EP20010922870	December 2, 2009	RNA sequence-specific mediators of RNAi	Thomas Tuschl et al.	Whitehead Institute for Biomedical Research and three more
US7825230 B2	USA	US 11/545,280	November 2, 2010	human microRNA targets in HIV genome and a method of identification thereof	Samir Kumar Brahmachari et al.	Council of Scientific & Industrial Research
US7683036 B2	USA	US 10/909,125	March 23, 2010	oligomeric compounds and compositions for use in modulation of small non-coding RNAs	Christine Esau et al.	Regulus Therapeutics
US7582744 B2	USA	US 11/200,703	September 1, 2009	chemically modified oligonucleotides	Muthiah Manoharan et al.	Alnylam Pharmaceuticals
US 7592441 B2	USA	US 11/418,875	September 22, 2009	miRNA for diagnosis, prognosis, and treatment of liver cancer	Itzhak Bentwich et al.	Rosetta Genomics
US7642348 B2	USA	US 11/429,720	January 5, 2010	miRNA for diagnosis, prognosis, and treatment of prostate cancer; linear amplification and labeling for hybridization techniques like Luminex and microarray analysis	Itzhak Bentwich et al.	Rosetta Genomics
US 7825229 B2	USA	US 11/418,870	November 2, 2010	miRNAs; diagnosis, prognosis, and treatments; drug screening; linear amplification and labeling for hybridization techniques like Luminex and microarray analysis; gene expression inhibition	Itzhak Bentwich et al.	Rosetta Genomics
US7635563 B2	USA	US 11/171,175	December 22, 2009	method for identifying miRNA expression (microarrays; RT-PCR)	H. Robert Horvitz et al.	Massachusetts Institute of Technology

virus (RSV) infection during lung transplantation. ALN-RSV01 is a naked and unchanged siRNA that targets the conserved N protein in the RSV genome. This molecule completed its phase IIb trial. The results documented the safety and tolerability of inhaled ALN-RSV01 in naturally infected patients (http://www.alnylam.com/capella/presentations/complete-results-of-our-aln-rsv01-Phase-IIb-study/). The safety and tolerability study involved 101 healthy adults (65 active, 36

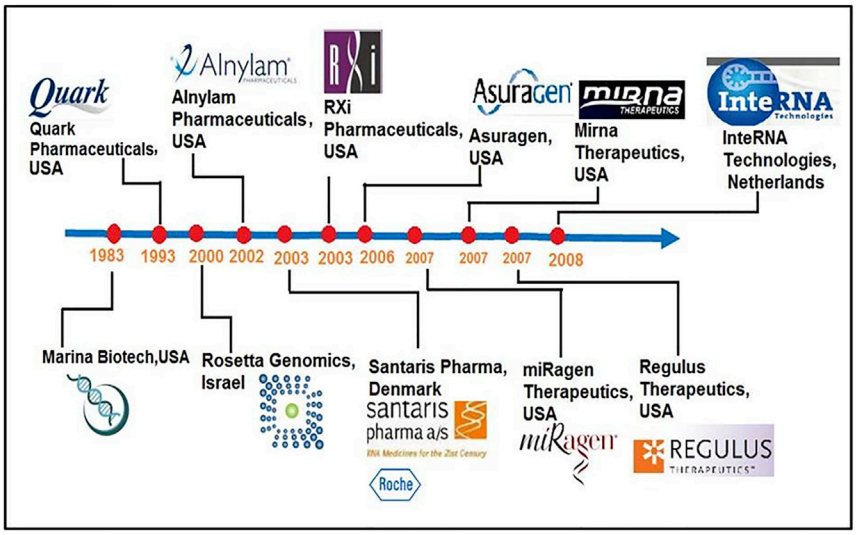

Figure 2. List of Biopharmaceutical Companies Involved in miRNA and siRNA Therapeutics
Different significant biopharmaceutical companies with their year of establishment since 1983 to 2017 that are involved in the development of the therapeutic miRNA and siRNA molecules.

placebo-controlled) with doses ranging up to 150 mg as a single dose with five daily doses.[29]

ALN-AAT is another molecule developed against alpha-1-antitrypsin (AAT)-mediated liver diseases that is currently in phase 1/2 of clinical trials. AAT insufficiency is frequently assessed as liver disease in patients.[30] This pharmaceutical company is developing another siRNA-based therapeutic called ALN-TTR. ALN-TTR is a targeting molecule for transthyretin-mediated amyloidosis for polyneuropathy. This molecule is in clinical trials sponsored by Alnylam Pharmaceuticals. The licensed program includes ALN-TTR02, which is in a phase III APOLLO clinical trial. Alnylam will retain rights to develop a program in the United States, Europe, and the rest of the world.[31]

PF-04523655 from Quark Pharmaceuticals

Quark Pharmaceuticals made strategic alliances with Pfizer for the further development of PF-04523655. Pfizer invested $145 million in the formulation of the compound. The companies have completed a phase I dose-related study for this molecule in human subjects with choroidal neovascularization (CNV), as well as minor age-related macular degeneration (wet AMD).[32] They are currently performing phase II clinical trial as an open-label multi-center CNV study. These companies are also conducting a clinical trial for a different indication for diabetic macular edema. In this clinical trial, they are performing a multi-center, randomized, comparator study evaluating the efficacy and safety of the molecule versus laser treatments in subjects with diabetic macular edema.[33]

QPI-1002 and Quark Pharmaceuticals

QPI-1002, a synthetic siRNA, can inhibit the expression of the pro-apoptotic protein p53. This drug is being developed to prevent acute kidney injury (AKI) following primary cardiovascular surgery, as well as for prophylaxis of the delayed graft function (DGF) after deceased donor renal transplantation.[34] It has been noted that AKI is a clinically overwhelming disease that leads to approximately 5% of hospital admissions, and within 30 days, the mortality rate has been recorded to be more than 50% after the onset of AKI after surgery.[35] DGF is also one of the most universal complications in the immediate period after renal transplantation, affecting 25%–40% of deceased donor renal transplant patients.[36] It is currently in a phase II clinical trial by Quark Pharmaceuticals. The QPI-1002 molecule has been granted orphan drug designation for prophylaxis of DGF in kidney transplantation by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA). However, in August 2010, Quark signed an agreement with Novartis for the exclusive license of this molecule for all indications.

Excellair and Zabecor Pharmaceuticals

Excellair is a designed siRNA that has anti-inflammatory properties. This siRNA targets and silences the Syk kinase gene, a critical gene linked to inflammation. This drug has shown potential and security in preclinical studies for the management of asthma and other inflammatory disorders.[37] This molecule has completed a phase II clinical trial.

ALN-VSP and Alnylam Pharmaceuticals

The molecule ALN-VSP comprises two siRNAs. This molecule is a systemically delivered RNAi therapeutic that targets two genes, *VEGF* and *KSP*. It is used for the management of advanced solid liver tumors (mainly primary and secondary liver cancers).[38] Lipid nanoparticles (LNPs) can efficiently deliver siRNAs to cellular targets. Alnylam Pharmaceuticals, an important RNAi therapeutics company, has completed a phase I clinical trial with this drug.

ALN-RSV is currently one of the most advanced siRNA programs in the world that uses an original siRNA formulated in a saline environment to target the RSV N gene. It completed a phase I trial (two intranasal and one inhalational) to prove its safety and tolerability at doses up to 3 mg/kg. A double-blind, randomized, placebo-controlled study using 88 patients was shown to reduce the occurrence of upper respiratory tract infection with RSV upon intranasal ALN-RSV treatment, thus showing its safety and efficacy.[29]

CALAA-01 and Calando Pharmaceuticals

The targeted therapeutic molecule CALAA-01 is a tumor inhibitor that targets the M2 subunit of ribonucleotide reductase (RRM2). RRM2 protein is involved in DNA replication and is an essential

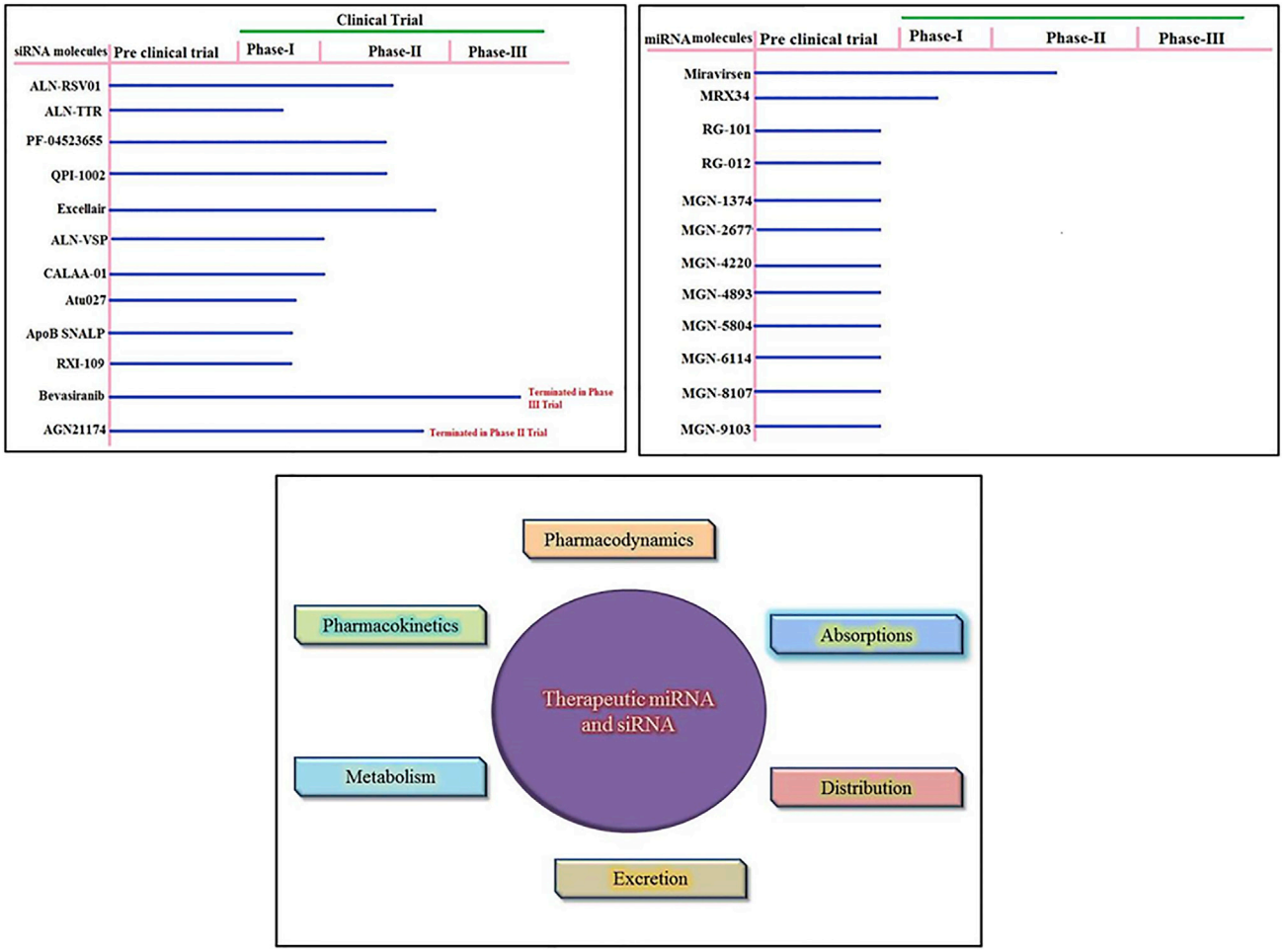

Figure 3. Status of Various miRNA and siRNA Therapeutics under Clinical Trials

(Top left panel) Different siRNA molecules that are in a clinical trial and their status of clinical trial. (Top right panel) Different miRNAs molecules that are in preclinical and/or clinical trial and their status of clinical trial. (Bottom panel) Different important issues related to ADME during development of miRNA- and siRNA-based therapeutics molecules.

protein for completing cell division. It has been noted that RRM2 regulates Bcl-2 in different cancers, especially head, neck, and lung cancers, and plays an active role in tumor progression. Therefore, RRM2 is a potential target for cancer therapy.[39] An anti-RRM2 siRNA sequence was developed that exhibits significant anti-proliferative activity in human, mouse, rat, and monkey cancer cells.[40] Nanoparticles containing anti-RRM2 siRNA sequence were evaluated in vivo for targeted delivery. The nanoparticles contain a synthetic delivery arrangement that uses a linear, cyclodextrin-containing polycation, transferrin, and siRNA.[41] The Investigational New Drug (IND) application was submitted to the US FDA, and Calando Pharmaceuticals received approval in 2008 to initiate a phase I clinical trial for this siRNA-based drug in patients with solid tumors. The provisional phase I clinical trial study data were published in 2010.[42,43] This siRNA therapeutic is currently a significant drug in cancer programs.

Atu-027 and Silence Therapeutics

Atu027 contains siRNA combined with a lipoplex delivery system and displays RNAi-mediated suppression of protein kinase N3 (PKN3) gene expression in vascular endothelial cells. Silence Therapeutics is conducting a human phase I study for therapeutic Atu027 in patients with advanced cancer.[44] The PKN3 target gene is a critical factor for cancer progression and metastasis. A phase Ib/IIa study for Atu027 in combination with gemcitabine was completed after the lead-in safety period.[45]

ApoB SNALP and Tekmira Pharmaceuticals

Apolipoprotein B (ApoB) stable nucleic acid lipid particles (SNALPs) are designed for the treatment of elevated low-density lipoprotein (LDL) cholesterol or "bad" cholesterol (hypercholesterolemia). ApoB-specific siRNAs were encoded in SNALPs.[46] Tekmira Pharmaceuticals initiated a phase 1 clinical trial by enrolling 23 adult

Table 2. Significant Therapeutics siRNAs That Are in the Development Phase, Their Indication, and Their Biopharmaceutical Company

Therapeutic siRNAs	Indication	Target	Pharmaceutical Company	Remarks
ALN-RSV01	treatment of respiratory syncytial virus (RSV) infection during lung transplantation	RSV nucleocapsid	Alnylam Pharmaceuticals	phase IIb clinical trial
ALN-TTR02	treatment of transthyretin-mediated amyloidosis	transthyretin (TTR)	Alnylam Pharmaceuticals	phase III APOLLO study
PF-04523655 (formerly known as RTP-801i)	treatment of age-related macular degeneration and diabetic macular edema	HIF-1-responsive gene, RTP801	Quark Pharmaceuticals	phase II wet AMD with 0.5 mg of Lucentis given by intravitreal injection with 3 mg of PF-04523655 given every 2 weeks from week 4
QPI-1002	for the avoidance of AKI following primary cardiovascular surgery as well as for the prophylaxis of delayed graft function (DGF) following deceased donor renal transplantation	p53	Quark Pharmaceuticals	granted Orphan drug designation; in phase II study with randomized 1:1 to receive 10 mg/kg single-bolus intravenous (i.v.) dose of QPI-1002
Excellair	for the treatment of inflammatory disorders like asthma	spleen tyrosine kinase (Syk) gene	Zabecor Pharmaceuticals	phase II clinical trial for asthma
ALN-VSP	for the treatment of liver cancer	*VEGF* gene, kinesin spindle (KSP) protein gene	Alnylam Pharmaceuticals	completed phase I extension study
CALAA-01	to inhibit tumor and cancer therapy	M2 subunit of ribonucleotide reductase (RRM2) gene	Calando Pharmaceuticals	phase 1b clinical trial; delivery system is into a nanoparticle <100 nm in diameter
Atu-027	for the treatment of advanced solid tumors	protein kinase N3 gene	Silence Therapeutics	phase I clinical trial
PF-655 (formerly REDD14NP and RTP801i)	for the treatment of age-related macular degeneration	RTP801 gene	Quark Pharm	phase II clinical trial
QPI-1007	treatment of nonarteritic anterior ischemic optic neuropathy	caspase-2 gene	Quark Pharm	
AGN211745	treatment of age-related macular degeneration	VEGFRI gene	Sirna Therapeutics	phase II clinical trial with 164 subjects
ApoB SNALP	for the treatment of hypercholesterolemia	apolipoprotein B gene	Tekmira Pharmaceuticals	phase I clinical trial concluded
RXI-109	for the treatment of fibrosis or scarring of the skin at a post-surgical wound site or the prevention of dermal scarring for the management of proliferative vitro retinopathy (PVR) and other ocular disorders	connective tissue growth factor (CTGF) gene	RXi Pharmaceuticals	phase 1 clinical trial
SYL040012	ocular hypertension	β_2-adrenergic receptor gene	Sirna Therapeutics	phase II trial completed
Bevasiranib	treatment of AMD or diabetic macular edema	VEGF gene	OPKO Health	phase III clinical trial, bevasiranib's clinical trial was terminated
AGN21174	age-related macular degeneration	VEGFR1 gene	Allergan	terminated in phase II
SPC2996	chronic lymphocytic leukemia	Bcl-2 gene	Santaris Pharma	completed phase II trial

volunteers with mild hypercholesterolemia. Among them, 17 were exposed to ApoB SNALP, while the rest received a placebo.[28] They further concluded the program in 2010 and mentioned that ApoB SNALP were well tolerated among the majority of patients with no liver toxicity; however, in some cases, immune stimulation was observed.

RXI-109 and RXi Pharmaceuticals

RXI-109 is an siRNA (self-delivering RNAi compound [sd-rxRNA]) that acquired FDA clearance to start clinical trials. Its phase I clinical trial started in June 2012 for the treatment of fibrosis or scarring of the skin at post-surgical wound sites or for dermal scarring prevention.[47] It is used to manage proliferative vitro retinopathy (PVR) and other ocular disorders.[48]

Setbacks to siRNA Therapeutics Development

There has been a delay in clinical trials with siRNA therapeutics, because two clinical trials have been withdrawn because of poor performance.

Bevasiranib and OPKO Health

Bevasiranib is a so-called siRNA that can control vascular endothelial growth factor (VEGF) gene expression; this molecule is a naked

Table 3. Significant Therapeutics miRNA that Are in the Development Phase, Their Indication, and Their Biopharmaceutical Company

Therapeutic miRNAs	Indication	Biopharmaceutical Company	Remarks
Miravirsen	the indication of the hepatitis C virus (HCV) infection	Santaris Pharma	phase IIa clinical trial
MRX34	for the treatment of a variety of cancers such as colon cancer, non-small-cell lung cancer (NSCLC), hepatocellular carcinoma, cervical cancer, ovarian cancer, etc.	Mirna Therapeutics	phase 1 clinical trial halted because of immune responses
RG-101	for the treatment of HCV	Regulus Therapeutics	an owned GalNAc-conjugated anti-miR
RG-012	for the treatment of Alport syndrome	Regulus Therapeutics	in the pipeline to initiate clinical trial phase II
MGN-1374	for the treatment of post-myocardial infarction remodeling	miRagen Therapeutics	targets miR-15 and miR-195; it is in the preclinical stage
MGN-2677	for the treatment of vascular disease	miRagen Therapeutics	targets miR-143/145; it is in the pipeline
MGN-4220	for the treatment of cardiac fibrosis	miRagen Therapeutics	targets miR-29; it is in the pipeline
MGN-4893	for the treatment of disorders like abnormal red blood cell production such as polycythemia vera	miRagen Therapeutics	targets miR-451; it is in the pipeline
MGN-5804	for the treatment of cardiometabolic disease	miRagen Therapeutics	targets miR-378; it is in the pipeline
MGN-6114	for the treatment of peripheral arterial disease	miRagen Therapeutics	targets miR-92; it is in the pipeline
MGN-9103	for the treatment of chronic heart failure	miRagen Therapeutics	targets miR-208; it is in the pipeline

siRNA. OPKO Health is a multi-national pharmaceutical company that has selected bevasiranib for the treatment of AMD or diabetic macular edema.[49,50]

At first, it was expected that the drug would enter the market very quickly. The bevasiranib phase III clinical trial was terminated in 2009 because of poor performance. In the initial phase III trial called the COBALT study, a combination therapy study was examined. This phase III trial was also canceled because of unsatisfactory performance. However, further phase III clinical studies with other regimens using bevasiranib are in preparation.

AGN21174 and Allergan

During the development of AGN211745, a clinical study program was conducted by Allergan in collaboration with Sirna Therapeutics. The molecule targets VEGF receptor I (VEGFRI) for treating AMD.[51] Unfortunately, AGN211745 was also terminated in phase II for those specific indications.

After reports from the independent data monitoring committee, these two studies were terminated ahead of time before reaching their endpoints. Even though the safety of the molecules was acceptable, the exiting of these molecules from their clinical trials was a major setback for the siRNA-based therapeutic industry.

Significant miRNA Therapeutics in Preclinical and/or Clinical Trials and Their Biopharmaceutical Companies

miRNA-based therapeutics have entered in the past few years and are currently entering clinical trials (Figure 3, top right). Newer companies have established miRNA therapeutics for particular indications. These companies include miRagen Therapeutics, Regulus Therapeutics, and Mirna Therapeutics. Snapshots of these therapeutics are presented in Table 3.

Miravirsen and Santaris Pharma

Miravirsen is an LNA-modified DNA phosphorothioate antisense oligonucleotide (ASO) that inhibits miR-122.[52,53] This is the first miRNA-targeted drug to enter phase II clinical trials to understand its safety and tolerability in the patients; this drug is sponsored by Santaris Pharma (Roche Innovation Centre). The clinical trials were conducted for its use against HCV infection. In addition to the United States, the phase IIa clinical trial is also being undertaken in several other countries, including the Netherlands, Germany, Poland, Romania, and Slovakia. The study is investigating miravirsen in combination with telaprevir and ribavirin in null responders to pegylated interferon and ribavirin.

MRX34 and miRNA Therapeutics

Several miRNAs can perform as tumor-suppressor genes in human cancers, including miR-34.[54,55] MRX34 delivers an imitation miRNA to miR-34 that performs as a tumor suppressor. This miRNA is lost or repressed in patient tumors.[56] This drug can be used with an extensive variety of cancers, such as colon cancer, non-small-cell lung cancer (NSCLC), hepatocellular carcinoma, cervical cancer, and ovarian cancer. Mirna Therapeutics is the biopharmaceutical company that is performing the MRX34 phase 1 clinical trial on liver-based cancers. This clinical study was halted in 2016 because of multiple immune-related severe adverse events (SAEs).

RG-101, RG-012, and Regulus Therapeutics

It has been noted that miRNA-122 is a liver-related miRNA.[57] It has also been shown that miR-122 is an important target for HCV infection therapy.[58] Regulus Therapeutics is developing RG-101, an N-acetylgalactosamine (GalNAc)-conjugated anti-miR that targets miRNA-122 to treat HCV.

miR-21 plays an important role in fibrogenic diseases in different organs, including the kidneys. Therefore, anti-miRNA-21

oligonucleotides can prevent Alport nephropathy.[59] RG-012, an anti-miR targeting miRNA-21, is a Regulus Therapeutics molecule in the clinical trial pipeline for the treatment of Alport syndrome. This disease is a life-threatening genetic kidney disease that does not currently have an approved therapy.[60]

Anti-miRs and miRagen Therapeutics

miRagen Therapeutics is a USA-based biopharmaceutical company that is developing innovative RNA-targeting therapies with a particular emphasis on miRNAs for unmet human health needs. miRagen Therapeutics is developing several miRNA-based therapeutics, including MGN-1374, MGN-2677, MGN-4220, MGN-4893, MGN-5804, MGN-6114, MGN-8107, and MGN-9103.

MGN-9103 was suggested to play useful roles in diabetes and obesity. Obesity, type 2 diabetes, and heart failure are associated with aberrant cardiac metabolism. It has been noted that the cardiac-specific miR-208 target gene *MED13* provides resistance to high-fat-diet-induced obesity and produces insulin sensitivity and glucose tolerance in mice.[61] MGN-9103 is an LNA-modified ASOs.[62,63] DNA-like nucleotides lacking contiguous stretch of DNA are also called ASOs.[64] Such ASOs are capable of blocking gene expression by binding to RNA and are also known as "steric blockers." miR-208 is used for the treatment of persistent heart failure and is located in an alphaMHC gene intron. It is a heart-specific miRNA that is required for cardiac hypertrophy, fibrosis, and myosin switching.[63,65]

Another miRagen Therapeutics lead drug is MGN-4893, which targets miR-451. miR-451 is required for red blood cell expansion. Inhibition of miR-451 in mice, using anti-miR-451, blocked erythrocyte differentiation, which was helpful for the treatment of disorders relevant to abnormal red blood cell production.[66–68] Another significant miRagen Therapeutics drug is MGN-1374, an LNA-modified ASO for myocardial infarction. It is an 8-mer LNA oligonucleotide that targets the miR-15 family seed region.

ADME for Therapeutic miRNAs and siRNAs

ADME denotes the absorption, distribution, metabolism, and excretion of a new chemical entity (NCE) molecule.[69,70] Any new pharmaceutical compound that is administered in the human body undergoes pharmacokinetics and pharmacodynamics. Pharmacokinetics illustrates how the body affects a particular drug after taking it through the method of absorption and distribution. The ADME of drugs occurs through drug-metabolizing enzymes, as well as different drug transporters expressed in various tissues such as the small intestine, liver, and kidney. Drug-metabolizing enzymes, such as cytochrome P450 (CYP or P450) isoforms, play crucial roles in drug metabolism.[71] Drug transporters, including ABC and SLC transporters, also play key roles in drug absorption, distribution, and excretion.[72] However, several important issues related to ADME have been observed by biopharmaceutical companies during the development of miRNA- and siRNA-based therapeutics (Figure 3, bottom).

In the majority of the cases, polyanionic molecules are used to develop anti-miRs because they are extremely water-soluble. Another important point is that these molecules have small molecular mass ranging from 2 to 6 kDa. These miRNA-based NCEs are weak candidates for oral administration, and their intestinal absorption is low.[73] Even with the application of oral enhancers, intestinal absorption of anti-miRs is not up to the mark. Therefore, anti-miR oligonucleotides are currently administered using a parenteral route. The two types of parenteral administration routes that are currently being used are intravenous and subcutaneous injections, as well as infusions.[20,74] It is currently easier to make an anti-miR aqueous solution because of its water solubility. However, more studies focused on ADME intravenous and subcutaneous injections, as well as infusions, are necessary. A better delivery system that can provide long biological half-lives is required; thus, more research is needed in these directions. With longer biological half-lives, it may be possible to reduce the frequency of injections so that anti-miRs can be administered more frequently.

Levin[75] described that LNA-modified DNA/phosphorothioate (PS) oligonucleotide backbone modifications improve the pharmacokinetic properties of antisense LNA-modified DNA/PS oligonucleotides. Different studies have reported efficient LNA-modified anti-miRs that harbor complete PS backbones. miR-122 silencing uses a high-affinity 15-nt LNA/DNA PS oligonucleotide.[76,77] Saline-formulated LNA-anti-miR-122 delivery uses intraperitoneal (i.p.) or intravenous (i.v.) injection methods to treat mice. This compound has been efficiently taken up by the liver.[78,79]

In other study, Elmén et al.[76,77] used single LNA-anti-miR i.p. injections with doses ranging from 1 to 200 mg/kg in a dose-dependent study to show decreased serum cholesterol in mice. In the same survey, administration of PBS-formulated LNA-anti-miR to African green monkeys with doses ranging from 1 to 10 mg/kg shows accumulation of the LNA-anti-miR compound in the liver.[76,77] The same research group also showed that PCSK9 LNA ASOs induced a significant reduction in LDL cholesterol in nonhuman primates.[80] These results show that i.p. injections of LNA-anti-miR compounds were well accepted in both mice and primates. Therefore, the parenteral route of administration for miRNA-based new chemical entities (NCEs) may provide better pharmacokinetics and pharmacodynamics.

Efficient Delivery Systems for Therapeutic miRNAs and siRNAs Can Reduce the Effective Dose

Delivering a therapeutic miRNA and siRNA to its target tissue is a challenging task. The drug molecule enters the cell through the membrane and eventually comes into the cytoplasm.[81] RNA-based therapeutics show poor pharmacological properties, such as off-targeting, low serum stability, and innate immune responses, which cause significant challenges for clinical applications.[82,83]

Studies have shown that local or topical delivery (eye, skin) of therapeutic RNAs displayed better bioavailability in target tissues than non-target tumor tissues.

Different delivery systems are used for better bioavailability, including PEGylated liposomes, lipidoids, and biodegradable polymers. Vesicles with diameters between 50 and 500 nm have been used to deliver therapeutic miRNAs and siRNAs. These vesicles prevent the drugs from being filtered by the kidneys and improve intracellular delivery.[81,82,84]

Liposomes contain lipid bilayers with an aqueous core that can contain therapeutic miRNA and siRNA molecules.[16] Lipoplexes are a liposome-based delivery system that contains cationic lipids; this delivery system allows negatively charged therapeutic miRNA and siRNA molecules to traverse the lipid bilayer. The negatively charged miRNA and siRNA molecules and their hydrophilicity are balanced by the cationic lipids.[81,85,86]

The resulting net positive charge facilitates the liposomes to bind to anionic cell surface molecules. The composition of these lipid particles is modified to promote fusion with cytoplasmic, nuclear, and endosomal membranes to support endosomal release within the cell. This can also solve the problem of pH sensitivity for the therapeutic molecules. In the end, liposomes intermingled with anionic phospholipids in the endosome produce non-bilayer structures that disrupt the endosomal membrane and liberate the miRNA and siRNA molecules.

It is extremely important to develop new in vivo delivery systems for miRNA and siRNA molecules that can target specific cells and tissues. Liposomal encapsulation technology has improved the half-life of these therapeutic RNAs in human blood. Diverse miRNA and siRNA molecule carriers have been suggested and developed differently that may have better stabilities and efficiencies with therapeutic RNA delivery systems. However, pharmaceutical companies need to apply these delivery systems to promote more efficient delivery of commercial therapeutic RNAs.

Future Prospects

RNA-based therapeutics has demonstrated great promise for the treatment of different diseases and is still evolving. The main obstruction linked with the clinical application of RNA-based therapeutics targeting strategies is determining how to accurately deliver the therapeutic agents into the targeted cells. Recent efficient delivery systems such as nanoparticle-based delivery systems may reduce doses, which will be beneficial for treatment of different diseases including cancer. Engineered nanoparticles are specially used for delivery to specific cells, which will help to achieve this goal. It will also help the co-delivery approach of RNA-based therapeutics with anticancer drugs. In addition, synchronized delivery of RNA-based therapeutics and chemotherapy agents to the tumor cells is highly challenging. RNA-based therapeutics combined with conventional chemotherapy agents might offer a new approach to treat malignant tumors in the near future and will ultimately help to bring the RNA-based therapeutics amalgamation therapy to the clinic for the treatment of patients.

Conclusions

The discovery of therapeutic miRNA and siRNA is considered one of the most exhilarating and significant therapeutic breakthroughs in pharmaceutical development, IP rights (IPRs), and business points of view. The development of therapeutic miRNAs and siRNAs is progressing at a quick pace. However, pharmaceutical companies working with therapeutic miRNAs and siRNAs are somewhat different from those focusing on NCE molecules. This is primarily due to the technical differences between these two kinds of molecules. RNA-based drug molecules are more similar to traditional gene therapy. Soon, there will be no specific applications or efficacy guidelines for miRNAs and siRNAs, which will allow researchers and companies to utilize this important technology to solve real-world problems.

It is likely that there may be product failure during the development of drug molecules, which can be attributed to a number of factors including safety, efficacy, target selection, and delivery technologies. These and other factors, such as clinical trial design and commercial considerations, will require optimization to produce successful drugs.

Nevertheless, in the near future, RNA-based therapeutics will overcome these obstacles, and therapeutic miRNAs and siRNAs will enter into the clinic as next generation drugs. This group of therapeutics definitely has the potential to contribute significantly to the future of medicine.

AUTHOR CONTRIBUTIONS

C.C. and S.-S.L. designed the manuscript, and C.C. and A.R.S. wrote the manuscript. G.S. assisted in the design of the manuscript and prepared the figures. C.G.P.D. and S.-S.L. edited the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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REFERENCES

1. Lee, R.C., Feinbaum, R.L., and Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell *75*, 843–854.

2. Pasquinelli, A.E., Reinhart, B.J., Slack, F., Martindale, M.Q., Kuroda, M.I., Maller, B., Hayward, D.C., Ball, E.E., Degnan, B., Müller, P., et al. (2000). Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature *408*, 86–89.

3. Reinhart, B.J., Slack, F.J., Basson, M., Pasquinelli, A.E., Bettinger, J.C., Rougvie, A.E., Horvitz, H.R., and Ruvkun, G. (2000). The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature *403*, 901–906.

4. Calin, G.A., Dumitru, C.D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., et al. (2002). Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. USA *99*, 15524–15529.

5. Kozomara, A., and Griffiths-Jones, S. (2011). miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res. *39*, D152–D157.

6. Lindow, M., and Kauppinen, S. (2012). Discovering the first microRNA-targeted drug. J. Cell Biol. *199*, 407–412.

7. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature *391*, 806–811.

8. Montgomery, M.K. (2006). RNA interference: unraveling a mystery. Nat. Struct. Mol. Biol. *13*, 1039–1041.

9. Lares, M.R., Rossi, J.J., and Ouellet, D.L. (2010). RNAi and small interfering RNAs in human disease therapeutic applications. Trends Biotechnol. *28*, 570–579.

10. Hydbring, P., and Badalian-Very, G. (2013). Clinical applications of microRNAs. F1000Res. *2*, 136.

11. Kim, V.N., Han, J., and Siomi, M.C. (2009). Biogenesis of small RNAs in animals. Nat. Rev. Mol. Cell Biol. *10*, 126–139.

12. Axtell, M.J., Westholm, J.O., and Lai, E.C. (2011). Vive la différence: biogenesis and evolution of microRNAs in plants and animals. Genome Biol. *12*, 221.

13. Ambros, V. (2004). The functions of animal microRNAs. Nature *431*, 350–355.

14. Meister, G., and Tuschl, T. (2004). Mechanisms of gene silencing by double-stranded RNA. Nature *431*, 343–349.

15. Hammond, S.M., Bernstein, E., Beach, D., and Hannon, G.J. (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature *404*, 293–296.

16. Wang, J., Lu, Z., Wientjes, M.G., and Au, J.L.-S. (2010). Delivery of siRNA therapeutics: barriers and carriers. AAPS J. *12*, 492–503.

17. Stevens, A.J., Jensen, J.J., Wyller, K., Kilgore, P.C., Chatterjee, S., and Rohrbaugh, M.L. (2011). The role of public-sector research in the discovery of drugs and vaccines. N. Engl. J. Med. *364*, 535–541.

18. McMillan, G.S., Narin, F., and Deeds, D.L. (2000). An analysis of the critical role of public science in innovation: the case of biotechnology. Res. Policy *29*, 1–8.

19. Laitala-Leinonen, T. (2010). Update on the development of microRNA and siRNA molecules as regulators of cell physiology. Recent Pat. DNA Gene Seq. *4*, 113–121.

20. van Rooij, E., Purcell, A.L., and Levin, A.A. (2012). Developing microRNA therapeutics. Circ. Res. *110*, 496–507.

21. McLeod, B.W., Hayman, M.L., Purcell, A.L., Marcus, J.S., and Veitenheimer, E. (2011). The 'real world' utility of miRNA patents: lessons learned from expressed sequence tags. Nat. Biotechnol. *29*, 129–133.

22. Davis, E.A. (2001). Overview of comments in "Utility Examination Guidelines". Fed. Regist. 1092–1099.

23. Reynolds, T. (2000). Pricing human genes: the patent rush pushes on. J. Natl. Cancer Inst. *92*, 96–97.

24. Lazonick, W., and Tulum, Ö. (2011). US biopharmaceutical finance and the sustainability of the biotech business model. Res. Policy *40*, 1170–1187.

25. Haussecker, D. (2008). The business of RNAi therapeutics. Hum. Gene Ther. *19*, 451–462.

26. Haussecker, D. (2012). The business of RNAi therapeutics in 2012. Mol. Ther. Nucleic Acids *1*, e8.

27. Jana, S., Chakraborty, C., Nandi, S., and Deb, J.K. (2004). RNA interference: potential therapeutic targets. Appl. Microbiol. Biotechnol. *65*, 649–657.

28. Vaishnaw, A.K., Gollob, J., Gamba-Vitalo, C., Hutabarat, R., Sah, D., Meyers, R., de Fougerolles, T., and Maraganore, J. (2010). A status report on RNAi therapeutics. Silence *1*, 14.

29. DeVincenzo, J., Cehelsky, J.E., Alvarez, R., Elbashir, S., Harborth, J., Toudjarska, I., Nechev, L., Murugaiah, V., Van Vliet, A., Vaishnaw, A.K., and Meyers, R. (2008). Evaluation of the safety, tolerability and pharmacokinetics of ALN-RSV01, a novel RNAi antiviral therapeutic directed against respiratory syncytial virus (RSV). Antiviral Res. *77*, 225–231.

30. Tapper, E.B., Patwardhan, V.R., and Curry, M. (2015). Low yield and utilization of confirmatory testing in a cohort of patients with liver disease assessed for alpha-1 antitrypsin deficiency. Dig. Dis. Sci. *60*, 1589–1594.

31. Cartwright, H. (2012). Alnylam partners with Sanofi's Genzyme to advance its ALN-TTR programme in Asia-Pacific. PharmaDeals Review *2012*, Published online November 12, 2012. http://dx.doi.org/10.3833/pdr.v2012.i11.183.

32. Hanout, M., Ferraz, D., Ansari, M., Maqsood, N., Kherani, S., Sepah, Y.J., Rajagopalan, N., Ibrahim, M., Do, D.V., and Nguyen, Q.D. (2013). Therapies for neovascular age-related macular degeneration: current approaches and pharmacologic agents in development. BioMed Res. Int. *2013*, 830837.

33. Nguyen, Q.D., Schachar, R.A., Nduaka, C.I., Sperling, M., Basile, A.S., Klamerus, K.J., Chi-Burris, K., Yan, E., Paggiarino, D.A., Rosenblatt, I., et al.; DEGAS Clinical Study Group (2012). Dose-ranging evaluation of intravitreal siRNA PF-04523655 for diabetic macular edema (the DEGAS study). Invest. Ophthalmol. Vis. Sci. *53*, 7666–7674.

34. Burnett, J.C., and Rossi, J.J. (2012). RNA-based therapeutics: current progress and future prospects. Chem. Biol. *19*, 60–71.

35. Chertow, G.M., Burdick, E., Honour, M., Bonventre, J.V., and Bates, D.W. (2005). Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J. Am. Soc. Nephrol. *16*, 3365–3370.

36. Troppmann, C., Gillingham, K.J., Benedetti, E., Almond, P.S., Gruessner, R.W., Najarian, J.S., and Matas, A.J. (1995). Delayed graft function, acute rejection, and outcome after cadaver renal transplantation. The multivariate analysis. Transplantation *59*, 962–968.

37. Watts, J.K., and Corey, D.R. (2010). Clinical status of duplex RNA. Bioorg. Med. Chem. Lett. *20*, 3203–3207.

38. 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. (2013). First-in-humans trial of an RNA interference therapeutic targeting VEGF and KSP in cancer patients with liver involvement. Cancer Discov. *3*, 406–417.

39. Rahman, M.A., Amin, A.R., Wang, D., Koenig, L., Nannapaneni, S., Chen, Z., Wang, Z., Sica, G., Deng, X., Chen, Z.G., and Shin, D.M. (2013). RRM2 regulates Bcl-2 in head and neck and lung cancers: a potential target for cancer therapy. Clin. Cancer Res. *19*, 3416–3428.

40. Heidel, J.D., Liu, J.Y.-C., Yen, Y., Zhou, B., Heale, B.S., Rossi, J.J., Bartlett, D.W., and Davis, M.E. (2007). Potent siRNA inhibitors of ribonucleotide reductase subunit RRM2 reduce cell proliferation in vitro and in vivo. Clin. Cancer Res. *13*, 2207–2215.

41. Heidel, J.D., Yu, Z., Liu, J.Y.-C., Rele, S.M., Liang, Y., Zeidan, R.K., Kornbrust, D.J., and Davis, M.E. (2007). Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA. Proc. Natl. Acad. Sci. USA *104*, 5715–5721.

42. Davis, M.E., Zuckerman, J.E., Choi, C.H.J., Seligson, D., Tolcher, A., Alabi, C.A., Yen, Y., Heidel, J.D., and Ribas, A. (2010). Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature *464*, 1067–1070.

43. Ribas, A., Kalinoski, L., Heidel, J.D., Peterkin, J., Seligson, D.B., Zuckerman, J.E., Choi, C., Yen, Y., Davis, M.E., and Tolcher, A.W. (2010). Systemic delivery of siRNA via targeted nanoparticles in patients with cancer: results from a first-in-class phase I clinical trial. J. Clin. Oncol. *28* (*Suppl. 15*), 3022.

44. Schultheis, B., Strumberg, D., Santel, A., Vank, C., Gebhardt, F., Keil, O., Lange, C., Giese, K., Kaufmann, J., Khan, M., and Drevs, J. (2014). First-in-human phase I study of the liposomal RNA interference therapeutic Atu027 in patients with advanced solid tumors. J. Clin. Oncol. *32*, 4141–4148.

45. Santel, A., Aleku, M., Röder, N., Möpert, K., Durieux, B., Janke, O., Keil, O., Endruschat, J., Dames, S., Lange, C., et al. (2010). Atu027 prevents pulmonary metastasis in experimental and spontaneous mouse metastasis models. Clin. Cancer Res. *16*, 5469–5480.

46. Zimmermann, T.S., Lee, A.C., Akinc, A., Bramlage, B., Bumcrot, D., Fedoruk, M.N., Harborth, J., Heyes, J.A., Jeffs, L.B., John, M., et al. (2006). RNAi-mediated gene silencing in non-human primates. Nature *441*, 111–114.

47. Cartwright, H. (2011). RXi pharmaceuticals looks beyond RNAi therapeutics with Apthera acquisition. PharmaDeals Review, *2011*, Published online April 21, 2011. http://dx.doi.org/10.3833/pdr.v2011.i4.1461.

48. Libertine, L., Lewis, G.P., Luna, G., Fisher, S.K., Cardia, J., Panderarinathan, L., Bulock, K., Pavco, P.A., and Byrne, M. (2014). RXI-109 treatment for proliferative vitreoretinopathy (PVR) and other ocular disorders. Invest. Ophthalmol. Vis. Sci. *55*, 1120.

49. Garba, A.O., and Mousa, S.A. (2010). Bevasiranib for the treatment of wet, age-related macular degeneration. Ophthalmol. Eye Dis. *2*, 75–83.

50. Singerman, L. (2009). Combination therapy using the small interfering RNA bevasiranib. Retina *29* (6 Suppl.), S49–S50.

51. Zhang, K., Zhang, L., and Weinreb, R.N. (2012). Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. Nat. Rev. Drug Discov. *11*, 541–559.

52. Gebert, L.F., Rebhan, M.A., Crivelli, S.E., Denzler, R., Stoffel, M., and Hall, J. (2014). Miravirsen (SPC3649) can inhibit the biogenesis of miR-122. Nucleic Acids Res. *42*, 609–621.

53. Janssen, H.L., Reesink, H.W., Lawitz, E.J., Zeuzem, S., Rodriguez-Torres, M., Patel, K., van der Meer, A.J., Patick, A.K., Chen, A., Zhou, Y., et al. (2013). Treatment of HCV infection by targeting microRNA. N. Engl. J. Med. *368*, 1685–1694.

54. Okada, N., Lin, C.-P., Ribeiro, M.C., Biton, A., Lai, G., He, X., Bu, P., Vogel, H., Jablons, D.M., Keller, A.C., et al. (2014). A positive feedback between p53 and miR-34 miRNAs mediates tumor suppression. Genes Dev. *28*, 438–450.

55. Xie, K., Liu, J., Chen, J., Dong, J., Ma, H., Liu, Y., and Hu, Z. (2014). Methylation-associated silencing of microRNA-34b in hepatocellular carcinoma cancer. Gene *543*, 101–107.

56. Bouchie, A. (2013). First microRNA mimic enters clinic. Nat. Biotechnol. *31*, 577.

57. Dubin, P.H., Yuan, H., Devine, R.K., Hynan, L.S., Jain, M.K., and Lee, W.M.; Acute Liver Failure Study Group (2014). Micro-RNA-122 levels in acute liver failure and chronic hepatitis C. J. Med. Virol. *86*, 1507–1514.

58. Motavaf, M., Safari, S., and Alavian, S.M. (2014). Targeting microRNA-122: walking on cutting edge of hepatitis C virus infection therapy. Acta Virol. *58*, 301–308.

59. Gomez, I.G., MacKenna, D.A., Johnson, B.G., Kaimal, V., Roach, A.M., Ren, S., Nakagawa, N., Xin, C., Newitt, R., Pandya, S., et al. (2015). Anti-microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. J. Clin. Invest. *125*, 141–156.

60. Chau, B.N., Xin, C., Hartner, J., Ren, S., Castano, A.P., Linn, G., Li, J., Tran, P.T., Kaimal, V., Huang, X., et al. (2012). MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. Sci. Transl. Med. *4*, 121ra118.

61. Grueter, C.E., van Rooij, E., Johnson, B.A., DeLeon, S.M., Sutherland, L.B., Qi, X., Gautron, L., Elmquist, J.K., Bassel-Duby, R., and Olson, E.N. (2012). A cardiac microRNA governs systemic energy homeostasis by regulation of MED13. Cell *149*, 671–683.

62. Hennessy, E.J., and Moore, K.J. (2013). Using microRNA as an alternative treatment for hyperlipidemia and cardiovascular disease: cardio-miRs in the pipeline. J. Cardiovasc. Pharmacol. *62*, 247–254.

63. Montgomery, R.L., Hullinger, T.G., Semus, H.M., Dickinson, B.A., Seto, A.G., Lynch, J.M., Stack, C., Latimer, P.A., Olson, E.N., and van Rooij, E. (2011). Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. Circulation *124*, 1537–1547.

64. Aartsma-Rus, A., Van Vliet, L., Hirschi, M., Janson, A.A., Heemskerk, H., De Winter, C.L., de Kimpe, S., van Deutekom, J.C., 't Hoen, P.A., and van Ommen, G.B. (2009). Guidelines for antisense oligonucleotide design and insight into splice-modulating mechanisms. Mol. Ther. *17*, 548–553.

65. van Rooij, E., Sutherland, L.B., Qi, X., Richardson, J.A., Hill, J., and Olson, E.N. (2007). Control of stress-dependent cardiac growth and gene expression by a microRNA. Science *316*, 575–579.

66. Rasmussen, K.D., and O'Carroll, D. (2011). The miR-144/451eGFP allele, a novel tool for resolving the erythroid potential of hematopoietic precursors. Blood *118*, 2988–2992.

67. Rasmussen, K.D., Simmini, S., Abreu-Goodger, C., Bartonicek, N., Di Giacomo, M., Bilbao-Cortes, D., Horos, R., Von Lindern, M., Enright, A.J., and O'Carroll, D. (2010). The miR-144/451 locus is required for erythroid homeostasis. J. Exp. Med. *207*, 1351–1358.

68. Patrick, D.M., Zhang, C.C., Tao, Y., Yao, H., Qi, X., Schwartz, R.J., Jun-Shen Huang, L., and Olson, E.N. (2010). Defective erythroid differentiation in miR-451 mutant mice mediated by 14-3-3ζ. Genes Dev. *24*, 1614–1619.

69. Caldwell, G.W. (2000). Compound optimization in early- and late-phase drug discovery: acceptable pharmacokinetic properties utilizing combined physicochemical, in vitro and in vivo screens. Curr. Opin. Drug Discov. Devel. *3*, 30–41.

70. Ruiz-Garcia, A., Bermejo, M., Moss, A., and Casabo, V.G. (2008). Pharmacokinetics in drug discovery. J. Pharm. Sci. *97*, 654–690.

71. Guengerich, F.P. (2006). Cytochrome P450s and other enzymes in drug metabolism and toxicity. AAPS J. *8*, E101–E111.

72. Giacomini, K.M., Huang, S.-M., Tweedie, D.J., Benet, L.Z., Brouwer, K.L., Chu, X., Dahlin, A., Evers, R., Fischer, V., Hillgren, K.M., et al.; International Transporter Consortium (2010). Membrane transporters in drug development. Nat. Rev. Drug Discov. *9*, 215–236.

73. Khatsenko, O., Morgan, R., Truong, L., York-Defalco, C., Sasmor, H., Conklin, B., and Geary, R.S. (2000). Absorption of antisense oligonucleotides in rat intestine: effect of chemistry and length. Antisense Nucleic Acid Drug Dev. *10*, 35–44.

74. Stenvang, J., Petri, A., Lindow, M., Obad, S., and Kauppinen, S. (2012). Inhibition of microRNA function by antimiR oligonucleotides. Silence *3*, 1.

75. Levin, A.A. (1999). A review of the issues in the pharmacokinetics and toxicology of phosphorothioate antisense oligonucleotides. Biochim. et Biophys. Acta *1489*, 69–84.

76. Elmén, J., Lindow, M., Schütz, S., Lawrence, M., Petri, A., Obad, S., Lindholm, M., Hedtjärn, M., Hansen, H.F., Berger, U., et al. (2008). LNA-mediated microRNA silencing in non-human primates. Nature *452*, 896–899.

77. Elmén, J., Lindow, M., Silahtaroglu, A., Bak, M., Christensen, M., Lind-Thomsen, A., Hedtjärn, M., Hansen, J.B., Hansen, H.F., Straarup, E.M., et al. (2008). Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. Nucleic Acids Res. *36*, 1153–1162.

78. Krützfeldt, J., Kuwajima, S., Braich, R., Rajeev, K.G., Pena, J., Tuschl, T., Manoharan, M., and Stoffel, M. (2007). Specificity, duplex degradation and subcellular localization of antagomirs. Nucleic Acids Res. *35*, 2885–2892.

79. Krützfeldt, J., Rajewsky, N., Braich, R., Rajeev, K.G., Tuschl, T., Manoharan, M., and Stoffel, M. (2005). Silencing of microRNAs in vivo with 'antagomirs'. Nature *438*, 685–689.

80. Lindholm, M.W., Elmén, J., Fisker, N., Hansen, H.F., Persson, R., Møller, M.R., Rosenbohm, C., Ørum, H., Straarup, E.M., and Koch, T. (2012). PCSK9 LNA antisense oligonucleotides induce sustained reduction of LDL cholesterol in nonhuman primates. Mol. Ther. *20*, 376–381.

81. Broderick, J.A., and Zamore, P.D. (2011). MicroRNA therapeutics. Gene Ther. *18*, 1104–1110.

82. Hong, C.A., and Nam, Y.S. (2014). Functional nanostructures for effective delivery of small interfering RNA therapeutics. Theranostics *4*, 1211–1232.

83. Miller, A.D. (2013). Delivery of RNAi therapeutics: work in progress. Expert Rev. Med. Devices *10*, 781–811.

84. Love, K.T., Mahon, K.P., Levins, C.G., Whitehead, K.A., Querbes, W., Dorkin, J.R., Qin, J., Cantley, W., Qin, L.L., Racie, T., et al. (2010). Lipid-like materials for low-dose, in vivo gene silencing. Proc. Natl. Acad. Sci. USA *107*, 1864–1869.

85. Sioud, M. (2015). RNA interference: mechanisms, technical challenges, and therapeutic opportunities (Springer).

86. Wang, X., Yan, F., Du, Q., and Liang, Z. (2010). siRNAs as potential drugs. Drug Discov. Today. Technol. *7*, e95–e146.