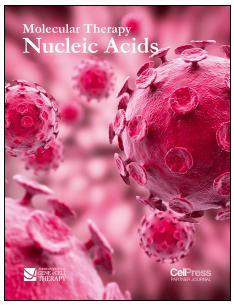
Accepted Manuscript



Therapeutic miRNA and siRNA: moving from bench to clinic as next generation medicine

Chiranjib Chakraborty, AshishRanjan Sharma, Garima Sharma, C. George Priya Doss, Sang-Soo Lee

PII: S2162-2531(17)30190-7

DOI: 10.1016/j.omtn.2017.06.005

Reference: OMTN 84

To appear in: *Molecular Therapy: Nucleic Acid*

Please cite this article as: Chakraborty C, Sharma A, Sharma G, Priya Doss CG, Lee S-S, Therapeutic miRNA and siRNA: moving from bench to clinic as next generation medicine, *Molecular Therapy: Nucleic Acid* (2017), doi: 10.1016/j.omtn.2017.06.005.

Therapeutic miRNA and siRNA: moving from bench to clinic as next generation medicine

Chiranjib Chakraborty[1,2,*,#], Ashish Ranjan Sharma[2,#], Garima Sharma[2], C. George Priya Doss[3], Sang-Soo Lee[2,*]

[1]Department of Bioinformatics and Biochemistry, Galgotias University, Greater Noida 201306, Uttar Pradesh, India

[2]Institute for Skeletal Aging & Orthopedic Surgery, Hallym University-Chuncheon Sacred Heart Hospital, Chuncheon 24252, Republic of Korea

[3]Department of Integrative Biology, VIT University, Vellore 632014, Tamil Nadu, India,

 # Authors contributed equally.

**Correspondence to*: Sang-Soo Lee, Department of Orthopedic Surgery, College of Medicine, Hallym University Hospital, Chuncheon-si, Gangwon-do 24252, Republic of Korea. E-mail: totalhip@hallym.ac.kr, 123sslee@gmail.com;

Chiranjib Chakraborty, Department of Bioinformatics and Biochemistry, Galgotias University, Greater Noida 201306, Uttar Pradesh, India. E-mail: drchiranjib@yahoo.com.

Email ID:

Chiranjib Chakraborty: drchiranjib@yahoo.com

Ashish Ranjan Sharma: boneresearch@hallym.ac.kr

Garima Sharma: microbio.garima@gmail.com

George Priya Doss: georgecp77@yahoo.co.in

Sang-Soo Lee: 123sslee@gmail.com

Abstract

In this decade, therapeutic microRNA (miRNA) and small interfering RNA (siRNA) are one of the most important biopharmaceuticals which are in commercial space as future medicine. This review summarizes the patents of miRNA and siRNA based new drugs, and also provides a snapshot about significant biopharmaceutical companies which are investing for the therapeutic development of miRNA and siRNA molecules. An insight view about individual siRNAs and miRNAs drugs have been depicted with their present status which are gaining attention in therapeutic landscape. The efforts of the biopharmaceutical are discussed with the status of their preclinical/clinical trials. Here, some of the setbacks have been highlighted during the biopharmaceuticals 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 about miRNA and siRNA based drugs.

INTRODUCTION

Victor Ambros and his groups from the Harvard University led to a new milestone in research by discovering 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; this was published in 2002. In this work, Calin et al. describe two human miRNA genes, mir-15a and mir-16-1, that are down-regulated or frequently deleted CLL diseases [4]. Presently, thousands of miRNAs have been found in plants and animals including humans. In 2011, Kozomara and Griffiths-Jones recorded that 18,226 miRNAs have been noted in animals, plants, and viruses and that 1,921 miRNAs were encrypted in the human genome [5]. Significantly, the world's first microRNA therapeutic, miravirsen, a short LNA for miR-122, is currently moving towards the market. This drug is currently in Phase-II clinical trials for the treatment of HCV infection [6].

Small interfering RNA (siRNA) is a potent tool for target-specific gene silencing through RNAi. Gene silencing by RNA interference (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 miRNAs 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 others miRNA therapeutics are in the pre-clinical 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] that are recognized as RISCs [15]. However, there are differences between miRNA and siRNA modes of action, as 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 bio-pharmaceutical companies and the status of their preclinical/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 has reviewed recent patent applications regarding micro-RNA biology [19, 20] describe the patent search through the Delphion patent database where they have found 1661 American miRNA-related patent papers as documented by International Patent classification codes. miRNA-related patent documents cover approximately 60 IPC code categories. Nearly 50% of US 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 illnesses or disorders. In the case of other diseases, less than 10% of the patents were issued for each other disorder. The authors also show that 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); USA (University of Massachusetts, USA; Massachusetts Institute of Technology, USA; Rockefeller University, New York, USA); Asia (College of Medicine, Pochon Cha University Industry-Academic Cooperation Foundation, Gyeonggi-do, South Korea; 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 (Carlsbad, USA), Stanford University, USA, etc. [21]. The 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 US patent search database (www.patft.uspto.gov) and European Patent Office database (www.epo.org/searching.html) and found that more patents have been granted for siRNA Compared to miRNAs in both databases (Fig-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 patents were granted for "siRNA and cancer" (6560 patents), while the lowest number of patents were 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 due problems with nucleic acid delivery systems in eye-related diseases.

We performed an analysis on the US patents for different cancer and found that the highest number of patents was granted for "siRNA and breast cancer" (3284 patents). These finding 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 IP properties 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 or European patents related to "microRNA" 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 R&D expenditures in industry increased 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, Inc., Rosetta Genomics Ltd, and Regulus Therapeutics Inc., were

established during the last 25 years (Fig-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 as 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 programmes have entered clinical trials in the past decade (Fig. 3A). The NIH in the USA has developed a database regarding the molecules that have completed clinical trials or are in ongoing clinical trials (http://www.clinicaltrials.gov). Seven out of fourteen RNAi therapeutics are for localized and topical use (Four for the eye, two for the respiratory tract and one for the skin). Approximately 1500 patients and healthy volunteers have been enrolled in RNAi clinical programmes that use siRNA therapeutics [28]. We will discuss some of the important therapeutic siRNAs with on-going clinical trials below. Snapshots of these therapeutics are presented in Table-2.

ALN-RSV01 and ALN-TTR from Alnylam Pharmaceuticals

Alnylam Pharmaceuticals (Alnylam Pharmaceuticals Inc., Cambridge, MA, USA) 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 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 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 which is currently in phase ½ 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 programme includes ALN-TTR02, is in phase III APOLLO clinical trial. Alnylam will retain rights to develop a programme in the US, 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 USD 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-centre CNV study. These companies are also conducting a clinical trial for a different indication for diabetic macular oedema. 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 phase II clinical trial by Quark Pharmaceuticals Inc., (Fremont, CA, USA). The QPI-1002 molecule has been granted orphan drug designation for prophylaxis of DGF in kidney transplantation by the US-FDA and 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

This molecule comprises two siRNAs and is referred to as ALN-VSP. 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 tumours (mainly primary and secondary liver cancers) [38]. Lipid nanoparticles (LNP) can efficiently deliver siRNAs to cellular targets.

Alnylam Pharmaceuticals, Inc., an important RNAi therapeutics company, has completed Phase I clinical trial with this drug.

ALN-RSV is currently one of the most advanced siRNA programmes in the world that uses an original siRNA formulated in a saline environment to target the Respiratory Syncytial Virus (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 tumour inhibitor that targets the M2 subunit of ribonucleotide reductase (RRM2). RRM2 protein is involved in DNA replication and is an essential 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 tumour 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 IND application was submitted to the USFDA and Calando Pharmaceuticals received approval in 2008 to initiate a Phase I clinical trial for this siRNA-based drug in patients with solid tumours. The provisional phase I clinical trial study data were published in 2010 [42, 43]. This siRNA therapeutic is currently a significant drug in cancer programmes.

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 (London, UK) 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

ApoB SNALP is designed for the treatment elevated LDL cholesterol or "bad" cholesterol (hypercholesterolemia). APOB-specific siRNAs were encoded in stable nucleic acid lipid particles (SNALP)[46]. Tekmira Pharmaceuticals initiated a Phase 1 clinical trial by enrolling 23 adult volunteers with mild hypercholesterolemia. Among them, 17 were exposed to Apo-B SNALP, while the rest received a placebo [28] . They further concluded the program in 2010 and mentioned that ApoB SNALP were well tolerated among majority of patients with no liver toxicity, however, in some cases immune stimulation was observed.

RXI-109 and RXi Pharmaceuticals

RXI-109 is a siRNA (self-delivering RNAi compound (sd-rxRNA)) that acquired FDA clearance to start clinical trials. Its Phase 1 clinical trial started in June 2012 for the treatment of fibrosis or scarring of the skin at a post-surgical wound sites or 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, as two clinical trials have been withdrawn due to poor performance.

Bevasiranib and OPKO Health Inc.

Bevasiranib is a so-called siRNA that can control VEGF gene expression; this molecule is a naked siRNA. OPKO Health, Inc. 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 the terminated in 2009 due to poor performance. In the initial Phase III trial called the COBALT study, a combination therapy study was examined. This Phase III trial was also cancelled due to unsatisfactory performance. However, further Phase III clinical studies with other regimens using Bevasiranib are in preparation.

AGN21174 and Allergan Inc.

During the development of AGN211745, a clinical study programme was conducted by Allergan Inc., (Irvine, CA, USA) in collaboration with Sirna Therapeutics, Inc. The molecule targets 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 were major setbacks for the siRNA-based therapeutic industry.

Significant miRNA therapeutics in preclinical/clinical trials and their biopharmaceutical companies

miRNA- based therapeutics have entered and are entering clinical trials in the past few years and currently, respectively (Fig. 3B). 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 a locked nucleic acid (LNA)-modified DNA phosphorothioate antisense oligonucleotide 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 drug patients; this drug is sponsored by Santaris Pharma (Roche Innovation Centre, Copenhagen A/S, Denmark). The clinical trials were conducted for its use against Hepatitis C virus (HCV) infection. In addition to the USA, 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 tumour-suppressor genes in human cancers, including miR-34 [54, 55]. MRX34 delivers an imitation microRNA to miR-34 that performs as a tumour suppressor. This miRNA is lost or repressed in patient tumours [56]. This drug can be used with an extensive variety of cancers, such as colon cancer, NSCLC, hepatocellular carcinoma, cervical cancer, and ovarian cancer. Mirna Therapeutics (Austin, TX 78744, United States) is the biopharmaceutical company that is performing the MRX34 phase 1 clinical trial on liver-based cancers. This clinical study was halted in 2016 due to multiple immune-related severe adverse events (SAE)

Anti-miRs (RG-101 and RG-012) and Regulus Therapeutics

It has been noted that miRNA-122 is a liver-related micro-RNA [57]. It has also been shown that miR-122 is an important target for hepatitis C virus infection therapy [58]. Regulus

Therapeutics (San Diego, USA) is developing RG-101, a 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-microRNA-21 oligonucleotides can prevent Alport nephropathy [59]. RG-012, an anti-miR targeting microRNA-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 (MGN-1374, MGN-2677, MGN-4220, MGN-4893, MGN-5804, MGN-6114, MGN-8107, and MGN-9103) and miRagen Therapeutics

miRagen Therapeutics is a USA-based biopharmaceutical company that is developing innovative RNA-targeting therapies with a particular emphasis on microRNAs 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/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 antisense oligos (ASO) [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 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 antimiR-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. Hullinger et al. (2012) showed that miR-15 inhibition provides safeguards against cardiac ischaemic injury.

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 (Fig. 3C).

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 weights ranging from 2–6 kD. 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 due to their 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 described that LNA-modified DNA/PS oligonucleotide backbone modifications improve the pharmacokinetic properties of antisense LNA-modified DNA/PS oligonucleotides [75] (Levin, 1999). Different studies have reported efficient LNA-modified anti-miRs that h arbour complete PS backbones. miR-122 silencing uses a high-affinity 15 nucleotide LNA/DNA PS oligonucleotide [76, 77]. Saline-formulated LNA-antimiR-122 delivery uses intraperitoneal (i.p.) or intravenous injection (i.v.) methods to treat mice. This compound has been efficiently up taken by the liver [78, 79].

In other study, Elmén et al. 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-antimiR to African green monkeys with doses ranging from 1 to 10 mg/kg show accumulation of the LNA-antimiR compound in the liver [76, 77]. The same research group also showed that PCSK9 LNA antisense oligonucleotides 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 tumour 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 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 is 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 RNA in human blood. Diverse miRNA and siRNA molecule carriers have been suggested and developed different 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 nano-particle based delivery systems may reduce doses which will be beneficial for different diseases treatment including cancer. Engineered nanoparticles are specially used for delivery to specific cells which will help with to achieve this goal. It will also help to 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 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, IPR 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 new chemical entity (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.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) under the Ministry of Education [NRF-2014R1A1A4A03009388] and by a grant of the Korea Health Technology R&D project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI12C1265). We acknowledge our colleagues for useful discussion and comments on this manuscript.

REFERENCES

1. Lee, RC, Feinbaum, RL, 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, AE, Reinhart, BJ, Slack, F, Martindale, MQ, Kuroda, MI, Maller, B, et al. (2000). Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature *408*: 86-89.
3. Reinhart, BJ, Slack, FJ, Basson, M, Pasquinelli, AE, Bettinger, JC, Rougvie, AE, et al. (2000). The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature *403*: 901-906.
4. Calin, GA, Dumitru, CD, Shimizu, M, Bichi, R, Zupo, S, Noch, E, et al. (2002). Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences *99*: 15524-15529.
5. Kozomara, A, and Griffiths-Jones, S (2010). miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic acids research: gkq1027.

6. Lindow, M, and Kauppinen, S (2012). Discovering the first microRNA-targeted drug. The Journal of cell biology *199*: 407-412.

7. Fire, A, Xu, S, Montgomery, MK, Kostas, SA, Driver, SE, and Mello, CC (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature *391:* 806-811.

8. Montgomery, MK (2006). RNA interference: unraveling a mystery. Nature Structural and Molecular Biology *13:* 1039.

9. Lares, MR, Rossi, JJ, and Ouellet, DL (2010). RNAi and small interfering RNAs in human disease therapeutic applications. Trends in biotechnology *28*: 570-579.

10. Hydbring, P, and Badalian-Very, G (2013). Clinical applications of microRNAs. F1000Research *2.*

11. Kim, VN, Han, J, and Siomi, MC (2009). Biogenesis of small RNAs in animals. Nature reviews Molecular cell biology *10*: 126-139.

12. Axtell, MJ, Westholm, JO, and Lai, EC (2011). Vive la différence: biogenesis and evolution of microRNAs in plants and animals. Genome biology *12*: 1.

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 **4***31*: 343-349.

15. Hammond, SM, Bernstein, E, Beach, D, and Hannon, GJ (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature *404*: 293-296.

16. Wang, J, Lu, Z, Wientjes, MG, and Au, JL-S (2010). Delivery of siRNA therapeutics: barriers and carriers. The AAPS journal *12*: 492-503.

17. Stevens, AJ, Jensen, JJ, Wyller, K, Kilgore, PC, Chatterjee, S, and Rohrbaugh, ML (2011). The role of public-sector research in the discovery of drugs and vaccines. New England Journal of Medicine *364*: 535-541.

18. McMillan, GS, Narin, F, and Deeds, DL (2000). An analysis of the critical role of public science in innovation: the case of biotechnology. Research policy *29*: 1-8.

19. Laitala-Leinonen, T (2010). Update on the development of microRNA and siRNA molecules as regulators of cell physiology. Recent patents on DNA & gene sequences *4*: 113-121.

20. van Rooij, E, Purcell, AL, and Levin, AA (2012). Developing microRNA therapeutics. Circulation research *110*: 496-507.

21. McLeod, BW, Hayman, ML, Purcell, AL, Marcus, JS, and Veitenheimer, E (2011). The'real world'utility of miRNA patents: lessons learned from expressed sequence tags. Nature biotechnology *29*: 129-133.

22. Davis, ea (2001). Overview of Comments in "Utility Examination Guidelines". Fed Regist: 1092-1099.

23. Reynolds, T (2000). Pricing human genes: the patent rush pushes on. Journal of the National Cancer Institute *92:* 96-97.

24. Lazonick, W, and Tulum, Ö (2011). US biopharmaceutical finance and the sustainability of the biotech business model. Research policy *40:* 1170-1187.

25. Haussecker, D (2008). The business of RNAi therapeutics. Human gene therapy *19*: 451-462.

26. Haussecker, D (2012). The business of RNAi therapeutics in 2012. Molecular therapy Nucleic acids *1*: e8.

27. Jana, S, Chakraborty, C, Nandi, S, and Deb, J (2004). RNA interference: potential therapeutic targets. Applied microbiology and biotechnology *65:* 649-657.

28. Vaishnaw, AK, Gollob, J, Gamba-Vitalo, C, Hutabarat, R, Sah, D, Meyers, R, et al. (2010). A status report on RNAi therapeutics. Silence *1*: 1.

29. DeVincenzo, J, Cehelsky, JE, Alvarez, R, Elbashir, S, Harborth, J, Toudjarska, I, et al. (2008). Evaluation of the safety, tolerability and pharmacokinetics of ALN-RSV01, a novel RNAi antiviral therapeutic directed against respiratory syncytial virus (RSV). Antiviral research *77:* 225-231.

30. Tapper, EB, Patwardhan, VR, 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. Digestive diseases and sciences *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.*

32. Hanout, M, Ferraz, D, Ansari, M, Maqsood, N, Kherani, S, Sepah, YJ, et al. (2013). Therapies for neovascular age-related macular degeneration: current approaches and pharmacologic agents in development. BioMed research international *2013.*

33. Nguyen, QD, Schachar, RA, Nduaka, CI, Sperling, M, Basile, AS, Klamerus, KJ, et al. (2012). Dose-Ranging Evaluation of Intravitreal siRNA PF-04523655 for Diabetic Macular Edema (the DEGAS Study) PF-04523655 a Small-Interfering RNA for DME. Investigative ophthalmology & visual science *53*: 7666-7674.

34. Burnett, JC, and Rossi, JJ (2012). RNA-based therapeutics: current progress and future prospects. Chemistry & biology *19*: 60-71.

35. Chertow, GM, Burdick, E, Honour, M, Bonventre, JV, and Bates, DW (2005). Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. Journal of the American Society of Nephrology *16:* 3365-3370.

36. Troppmann, C, Gillingham, KJ, Benedetti, E, Almond, PS, Gruessner, RW, Najarian, JS, et al. (1995). Delayed graft function, acute rejection, and outcome after cadaver renal transplantation: a multivariate analysis. Transplantation *59:* 962-968.

37. Watts, JK, and Corey, DR (2010). Clinical status of duplex RNA. Bioorganic & medicinal chemistry letters *20*: 3203-3207.

38. Tabernero, J, Shapiro, GI, LoRusso, PM, Cervantes, A, Schwartz, GK, Weiss, GJ, et al. (2013). First-in-humans trial of an RNA interference therapeutic targeting VEGF and KSP in cancer patients with liver involvement. Cancer discovery *3*: 406-417.

39. Rahman, MA, Amin, AR, Wang, D, Koenig, L, Nannapaneni, S, Chen, Z, et al. (2013). RRM2 regulates Bcl-2 in head and neck and lung cancers: a potential target for cancer therapy. Clinical Cancer Research *19*: 3416-3428.

40. Heidel, JD, Liu, JY-C, Yen, Y, Zhou, B, Heale, BS, Rossi, JJ, et al. (2007). Potent siRNA inhibitors of ribonucleotide reductase subunit RRM2 reduce cell proliferation in vitro and in vivo. Clinical Cancer Research *13*: 2207-2215.

41. Heidel, JD, Yu, Z, Liu, JY-C, Rele, SM, Liang, Y, Zeidan, RK, et al. (2007). Administration in non-human primates of escalating intravenous doses of targeted nanoparticles containing ribonucleotide reductase subunit M2 siRNA. Proceedings of the National Academy of Sciences *104:* 5715-5721.

42. Davis, ME, Zuckerman, JE, Choi, CHJ, Seligson, D, Tolcher, A, Alabi, CA, et al. (2010). Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. Nature *464*: 1067-1070.

43. Ribas, A, Kalinoski, L, Heidel, J, Peterkin, J, Seligson, D, Zuckerman, J, et al. (2010). Systemic delivery of siRNA via targeted nanoparticles in patients with cancer: Results from a first-in-class phase I clinical trial. ASCO Annual Meeting Proceedings, vol. *28*. p 3022.

44. Schultheis, B, Strumberg, D, Santel, A, Vank, C, Gebhardt, F, Keil, O, et al. (2014). First-in-human phase I study of the liposomal RNA interference therapeutic Atu027 in patients with advanced solid tumors. Journal of Clinical Oncology *32:* 4141-4148.

45. Santel, A, Aleku, M, Röder, N, Möpert, K, Durieux, B, Janke, O, et al. (2010). Atu027 prevents pulmonary metastasis in experimental and spontaneous mouse metastasis models. Clinical Cancer Research *16*: 5469-5480.

46. Zimmermann, TS, Lee, AC, Akinc, A, Bramlage, B, Bumcrot, D, Fedoruk, MN, 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.

48. Libertine, L, Lewis, GP, Luna, G, Fisher, SK, Cardia, J, Panderarinathan, L, et al. (2014). RXI-109 Treatment for Proliferative Vitreoretinopathy (PVR) and other Ocular Disorders. Investigative ophthalmology & visual science *55:* 1120-1120.

49. Garba, AO, and Mousa, SA (2010). Bevasiranib for the treatment of wet, age-related macular degeneration. Ophthalmology and eye diseases *2*: 75.

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

51. Zhang, K, Zhang, L, and Weinreb, RN (2012). Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. Nature reviews Drug discovery *11:* 541-559.

52. Gebert, LF, Rebhan, MA, Crivelli, SE, Denzler, R, Stoffel, M, and Hall, J (2014). Miravirsen (SPC3649) can inhibit the biogenesis of miR-122. Nucleic acids research *42*: 609-621.

53. Janssen, HL, Reesink, HW, Lawitz, EJ, Zeuzem, S, Rodriguez-Torres, M, Patel, K, et al. (2013). Treatment of HCV infection by targeting microRNA. New England Journal of Medicine *368*: 1685-1694.

54. Okada, N, Lin, C-P, Ribeiro, MC, Biton, A, Lai, G, He, X, et al. (2014). A positive feedback between p53 and miR-34 miRNAs mediates tumor suppression. Genes & development *28*: 438-450.

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

56. Bouchie, A (2013). First microRNA mimic enters clinic. Nature biotechnology *31*: 577-577.

57. Dubin, PH, Yuan, H, Devine, RK, Hynan, LS, Jain, MK, and Lee, WM (2014). Micro□RNA□122 levels in acute liver failure and chronic hepatitis C. Journal of medical virology *86*: 1507-1514.

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

59. Gomez, IG, MacKenna, DA, Johnson, BG, Kaimal, V, Roach, AM, Ren, S, et al. (2015). Anti–microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. The Journal of clinical investigation *125*: 141-156.

60. Chau, BN, Xin, C, Hartner, J, Ren, S, Castano, AP, Linn, G, et al. (2012). MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. Science translational medicine *4*: 121ra118-121ra118.

61. Grueter, CE, van Rooij, E, Johnson, BA, DeLeon, SM, Sutherland, LB, Qi, X, et al. (2012). A cardiac microRNA governs systemic energy homeostasis by regulation of MED13. Cell *149*: 671-683.

62. Hennessy, EJ, and Moore, KJ (2013). Using microRNA as an alternative treatment for hyperlipidemia and cardiovascular disease: cardio-miRs in the pipeline. Journal of cardiovascular pharmacology *62*: 247.

63. Montgomery, RL, Hullinger, TG, Semus, HM, Dickinson, BA, Seto, AG, Lynch, JM, et al. (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, AA, Heemskerk, H, De Winter, CL, et al. (2009). Guidelines for antisense oligonucleotide design and insight into splice-modulating mechanisms. Molecular Therapy *17:* 548-553.

65. van Rooij, E, Sutherland, LB, Qi, X, Richardson, JA, Hill, J, and Olson, EN (2007). Control of stress-dependent cardiac growth and gene expression by a microRNA. Science *316*: 575-579.

66. Rasmussen, KD, 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, KD, Simmini, S, Abreu-Goodger, C, Bartonicek, N, Di Giacomo, M, Bilbao-Cortes, D, et al. (2010). The miR-144/451 locus is required for erythroid homeostasis. The Journal of experimental medicine *207*: 1351-1358.

68. Patrick, DM, Zhang, CC, Tao, Y, Yao, H, Qi, X, Schwartz, RJ, et al. (2010). Defective erythroid differentiation in miR-451 mutant mice mediated by 14-3-3ζ. Genes & development *24*: 1614-1619.

69. Caldwell, GW (2000). Compound optimization in early-and late-phase drug discovery: acceptable pharmacokinetic properties utilizing combined physicochemical, in vitro and in vivo screens. Current Opinion in Drug Discovery and Development *3*: 30-41.

70. Ruiz□Garcia, A, Bermejo, M, Moss, A, and Casabo, VG (2008). Pharmacokinetics in drug discovery. Journal of pharmaceutical sciences *97*: 654-690.

71. Guengerich, FP (2006). Cytochrome P450s and other enzymes in drug metabolism and toxicity. The AAPS journal *8:* E101-E111.

72. Giacomini, KM, Huang, S-M, Tweedie, DJ, Benet, LZ, Brouwer, KL, Chu, X, et al. (2010). Membrane transporters in drug development. Nature reviews Drug discovery *9*: 215-236.

73. KHATSENKO, O, MORGAN, R, TRUONG, L, YORK-DEFALCO, C, SASMOR, H, CONKLIN, B, et al. (2000). Absorption of antisense oligonucleotides in rat intestine: effect of chemistry and length. Antisense and Nucleic Acid Drug Development *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, AA (1999). A review of issues in the pharmacokinetics and toxicology of phosphorothioate antisense oligonucleotides. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression *1489*: 69-84.

76. Elmén, J, Lindow, M, Schütz, S, Lawrence, M, Petri, A, Obad, S, et al. (2008). LNA-mediated microRNA silencing in non-human primates. Nature *452*: 896-899.

77. Elmen, J, Lindow, M, Silahtaroglu, A, Bak, M, Christensen, M, Lind-Thomsen, A, 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 research **36**: 1153-1162.

78. Krützfeldt, J, Kuwajima, S, Braich, R, Rajeev, KG, Pena, J, Tuschl, T, et al. (2007). Specificity, duplex degradation and subcellular localization of antagomirs. Nucleic acids research *35*: 2885-2892.

79. Krützfeldt, J, Rajewsky, N, Braich, R, Rajeev, KG, Tuschl, T, Manoharan, M, et al. (2005). Silencing of microRNAs in vivo with 'antagomirs'. Nature *438*: 685-689.

80. Lindholm, MW, Elmén, J, Fisker, N, Hansen, HF, Persson, R, Møller, MR, et al. (2012). PCSK9 LNA antisense oligonucleotides induce sustained reduction of LDL cholesterol in nonhuman primates. Molecular Therapy *20*: 376-381.

81. Broderick, JA, and Zamore, PD (2011). MicroRNA therapeutics. Gene therapy *18*: 1104-1110.

82. Hong, CA, and Nam, YS (2014). Functional nanostructures for effective delivery of small interfering RNA therapeutics. Theranostics *4*: 1211-1232.

83. Miller, AD (2013). Delivery of RNAi therapeutics: work in progress. Expert review of medical devices *10:* 781-811.

84. Love, KT, Mahon, KP, Levins, CG, Whitehead, KA, Querbes, W, Dorkin, JR, et al. (2010). Lipid-like materials for low-dose, in vivo gene silencing. Proceedings of the National Academy of Sciences *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 Discovery Today: Technologies *7*: e125-e130.

Figure legends:

Figure 1 (A) Total number of keyword searches ("microRNA" and "siRNA") were performed on the in both US patent search database and the European patent office database (B) Number of patents in "microRNA" and "siRNA" in case of the different disease (C) Number of patents in "microRNA" and "siRNA" in event of the different type of cancers.

Figure 2 Different significant biopharmaceutical companies with their year of establishment since 1983 to till today which are involved in the development of the therapeutic miRNA and siRNA molecules.

Figure 3 (A) Different siRNAs molecules which are in a clinical trial and their status of clinical trial, (B) Different miRNAs molecules which are in preclinical/clinical trial and their status of clinical trial, (C) Different important issues related to ADME during development of miRNAs and siRNAs based therapeutics molecules

Table 1. Some relevant patent issued patents related to "microRNA" and "siRNA"

Patent number	Patent Type	Application number	Publication date	Content of the patent statement	Inventors	Applicant
CA2404890 C	USA Patent	PCT/US2001/010188	19 Nov 2013	RNA sequence-specific mediators of RNA interference	Thomas Tuschl et al.	Whitehead Institute For Biomedical Research & 7 More
EP 1309726 B1	European Patent	EP20010922870	2 Dec 2009	RNA sequence-specific mediators of RNA interference	Thomas Tuschl et al.	Whitehead Institute For Biomedical Research & 3 More
EP1550719 B1	European Patent	EP20050002454	24 Dec 2008	Double-stranded RNA (dsRNA) for inhibition the expression of a defined gene	Roland Kreutzer and Stefan Limmer	Alnylam Europe AG
US7056704 B2	USA Patent	US 10/832,432	6 Jun 2006	RNA interference mediating small RNA molecules	Thomas Tuschl et al.	Max-Planck-Gesellschaft Zur Foerderung Der Wissenschaften E.V.
US 7750144 B2	USA Patent	US 10/912,440	6 Jul 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 to the base pair strength	Phillip D. Zamore et al.	University of Massachusetts
EP1633890 B1	European Patent	EP20040753972	20 Oct 2010	methods and compositions for enhancing the efficacy and specificity of RNAi	Phillip D. Zamore et al.	University of Massachusetts
EP1309726 B1	European Patent	EP20010922870	2 Dec 2009	RNA sequence-specific mediators of RNA interference	Thomas Tuschl et al.	Whitehead Institute For Biomedical Research & 3 More
US7825230 B2	USA Patent	US 11/545,280	2 Nov 2010	Human microRNA targets in HIV genome and a method of identification thereof	Samir Kumar Brahmachari et al.	Council of Scientific & Industrial Research
US7683	USA	US 10/909,125	Mar 23,	Oligomeric	Christine	Regulus

036 B2	Patent		2010	compounds and compositions for use in modulation of small non-coding RNAs	Esau et al.	Therapeutics Inc.
US7582744 B2	USA Patent	US 11/200,703	Sep 1, 2009	Chemically modified oligonucleotides	Muthiah Manoharan et al.	Alnylam Pharmaceuticals, Inc.
US 7592441 B2	USA Patent	US 11/418,875	Sep 22, 2009	miRNA for diagnosis, prognosis, and treatment of liver cancer	Itzhak Bentwich et al.	Rosetta Genomics Ltd
US7642348 B2	USA Patent	US 11/429,720	5 Jan 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 Ltd
US 7825229 B2	USA Patent	US 11/418,870	2 Nov 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 Ltd.
US7635563 B2	USA Patent	US 11/171,175	22 Dec 2009	Method for identifying miRNA expression(Microarrays; reverse transcriptase polymerase chain reaction)	H. Robert Horvitz et al.	Massachusetts Institute of Technology

Table 2. Significant therapeutics siRNA which 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	It is in phase IIb clinical trial.
ALN-TTR02	treatment of Transthyretin mediated amyloidosis	Transthyretin (TTR)	Alnylam Pharmaceuticals	It is in 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 IV *dose* of *QPI-1002*
Excellair	For the treatment of inflammatory disorders like asthama	Spleen tyrosine kinase (Syk) gene	Zabecor Pharmaceuticals	Phase II clinical trial for asthma
ALN-VSP	For the treatment of liver cancer	*Vascular endothelial growth factor* (*VEGF*) gene, kinesin spindle(KSP)	Alnylam Pharmaceuticals	Completed Phase I extension study

		protein gene		
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 with advanced solid tumors or	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., Inc.	Phase II clinical trial
QPI-1007	treatment of nonarteritic anterior ischemic optic neuropathy	Caspase 2 Gene	Quark Pharm., Inc.	
AGN211745	treatment of age-related macular degeneration	vascular endothelial growth factor receptor I Gene	Sirna Therapeutics, Inc	phase II clinical trial with 164 subject
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	β2 adrenergic receptor gene	Sirna Therapeutics, Inc.	Phase II trial Completed
Bevasiranib	treatment of AMD or diabetic macular edema	VEGF gene	OPKO Health Inc	Phase III clinical trial, Bevasiranib's clinical was the

				terminated
AGN21174	Age-related macular degeneration	VEGFR1 gene	Allergan Inc	terminated in Phase II
SPC2996	Chronic lymphocytic leukemia	Bcl-2 gene	Santaris Pharm.	**Completed Phase II trial**

Table 3. Significant therapeutics miRNA which 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	It is in Phase IIa clinical trial
MRX34	For the treatment of a variety of cancers such as colon cancer, NSCLC, hepatocellular carcinoma, cervical cancer, ovarian cancer, etc.	Mirna Therapeutics	Phase 1 clinical trial halted due to immune responses
RG-101	For the treatment of HCV.	Regulus Therapeutics	It is a owned GalNAc-conjugated anti-miR
RG-012	For the treatment of Alport syndrome	Regulus Therapeutics	It is in the pipeline to entire in the clinical trial
MGN-1374	For the treatment of post-myocardial infarction remodeling	miRagen Therapeutics	It targets miR-15 and miR-195. It is in the preclinical stage.
MGN-2677	For the treatment of vascular disease	miRagen Therapeutics	It targets miR-143/145. It is in the pipeline
MGN-4220	For the treatment of Cardiac fibrosis	miRagen Therapeutics	It targets miR-29. It is in pipeline
MGN-4893	for the treatment of disorders like abnormal red blood cell production such as polycythemia vera	miRagen Therapeutics	It 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	It targets miR-92 . It is in the pipeline
MGN-9103	For the treatment of chronic heart failure.	miRagen Therapeutics	It targets miR-208. It is in the pipeline