Delivery of therapeutic small interfering RNA: The current patent-based landscape

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Implementing small interfering RNA (siRNA) is a promising therapy because it silences disease-related genes theoretically. However, the efficient delivery of siRNA is challenging, which limits its therapeutic applications. Various pharmaceutical delivery systems containing key technologies have been developed and patented, which are of great concern to developers in the field. Despite numerous studies devoted to siRNA-delivery technologies, few researchers have systematically examined relevant patents. Patents, as bridges connecting academic progress with applicable innovation, encapsulate cumulative technological innovations and provide valuable information for academic research and commercial development. This study aims to analyze advances in therapeutic siRNA delivery technology from a patent perspective. A total of 11,509 patent documents from 3,309 patent families were collected, classified into 10 technological categories, and comprehensively analyzed. An overall patent landscape of siRNA delivery was presented from the temporal, spatial, organizational, and technological dimensions. This work is expected to help researchers and developers in the field of siRNA delivery form a basis for decision-making by combining our findings with supplementary data.

INTRODUCTION

Small interfering RNA (siRNA) is a double-stranded RNA that incorporates itself into the RNA-induced silencing complex (RISC), where it directs Argonaute-2 to cleave sequence-specific target mRNA, thereby blocking the translation of the target mRNA and silencing the target gene.1–3 siRNA therapy has many advantages, such as ease of synthesis, low production cost (compared with protein or antibody therapies), accessible targets, high specificity, and favorable pharmacokinetic properties.4–6 Designing and synthesizing siRNA drugs are not difficult; the challenge arises with how to achieve successful delivery.7 Several obstacles prevent siRNA from attaching to the correct site, such as easy degradation by serum nucleases and rapid clearance by the immune system,8,9 difficult target site accumulation of the appropriate therapeutic dosage,10,11 low cellular uptake efficiency,12,13 and inefficient endosomal escape.14–16 Some approaches have been shown to be effective; for example, studies have shown that chemical modifications can significantly reduce siRNA’s probability of being broken down by endonucleases. Furthermore, siRNA has been successfully applied in the treatment of the polyneuropathy of hereditary TTR-mediated amyloidosis,17 acute hepatic porphyria,18 primary hyperoxaluria type 1,19 and primary hypercholesterolaemia (heterozygous familial and non-familial) or mixed dyslipidaemia.20 Therefore, because siRNA offers such a promising therapeutic modality, it is not surprising that the search for successful potential delivery methods has intensified in recent years. Interest has led to numerous patents for siRNA delivery. Patents—important elements of research and development (R&D) and vital bridges between applicable research activities and commercial development21—encapsulate cumulative technological innovations22,23 and provide fruitful sources of up-to-date information.24 Moreover, patent analysis can be used to underpin important decisions made by academics, industry leaders, and governments.25 It has been successfully applied in many biological science fields, such as anti-Parkinson’s drugs26 and chimeric antigen receptor T cell therapy,27 to discover technological position, determine technical landmarks, and forecast technology development tendencies in a sector, technology domain, or company.

However, although many studies and reviews exist on therapeutic siRNA-delivery technologies, the patents involved have received little attention. To address this gap in the literature, we reviewed global research on siRNA delivery based on multiple dimensions of patent data to examine state-of-the-art technology and development prospects. This research is expected to show an overall patent landscape of siRNA delivery in the temporal, spatial, and technological dimensions, which may provide a reference for relevant decision-making by researchers, investors, and policymakers.
RESULTS

A dataset comprising 11,509 patent documents and 3,309 Derwent World Patents Index (DWPI) patent families was generated for the analysis. As shown in Figure 1, 10 categories were technologically classified. Two broad strategies are used for siRNA delivery: one incorporates siRNA into some form of carrier, mainly consisting of viral and non-viral vectors. The other delivers siRNA on the molecular scale, primarily with chemical modification, ligand-siRNA conjugates, and physical means. In this study, physical means is a generalization of delivery by acoustic, electrical, and other external force methods, such as ultrasonic pulses, electroporation, microneedles, hydrodynamic injections, gene guns, etc. Viral vectors are mainly divided into adenoviruses and lentiviruses. Non-viral vectors primarily include lipid-based carriers, polymer carriers, inorganic carriers, and biocarriers. Biocarriers are bioengineered, bioinspired, biogenic, biomimicking, or biohybrid nanocarriers, cells, or vesicles that employ biological, biomimetic, bioinspired, or bioderived moieties as vectors. For example, biocarriers can be cell-penetrating peptides, DNA nanocages, extracellular vesicles, and so on. For patents that mention viral or non-viral vectors without going into specifics (e.g., WO2009033027A2, which simply states that nucleic acid molecules were delivered via viral or non-viral vectors [Claims #5]), an "others" category was used. The patents included in this study are listed in the Table S1.

Temporal trends and technological distribution

Figure 2A shows patent activity in terms of publication year. The ascending curve indicates a surge of interest in therapeutic siRNA delivery technology. Figure 2B shows the technological distribution at the patent-family level. The sectors represent categories: for example, polymer and lipid-based carriers were the most widely used, both accounting for about 20%. Biocarriers, ligand-siRNA conjugates, and adenoviruses accounted for 18%, 10%, and 6%, respectively. Of note, in 9 families that were labeled as lipid-based carriers and 6 as polymer carriers, the siRNAs were chemically modified or conjugated with ligands before delivery via carriers. For example, patent WO2013123996A1 describes siRNA being delivered by liposomes after modification of the 2'-hydroxyl group of ribose. We determined that the patent was related more to the carrier, so it was put into the lipid-based carrier family. Moreover, 111 patent families took two labels, as two technologies were involved. Such families with two labels are indicated by the orange bands in Figure 2B. For example, patent WO2010059253A9 provides nanoparticles with a lipid bilayer surface and a poly(lactide-co-glycolide) core; thus, it was labeled as both a lipid-based carrier and a polymer carrier. Of the families with dual labels, lipid-based/polymer carriers were the most common, accounting for about one-third. Polymer/inorganic carriers accounted for 20%, and 12% were conjugate-modified siRNA/ligands.

Technological trends over the past 20 years were also analyzed. For better visualization, Figure 3A contains families with only one label. Families with two labels, together with those that delivered modified siRNA by carriers, were defined as technology combinations. The temporal trends of the combinations are shown in Figure 3B. Information about these types of patents can be found in Table S1.

As Figure 3A shows, lipid-based carriers have always been popular, and polymer carriers have undergone substantial development since their initial implementation. While methods using inorganic carriers and
ligand-siRNA conjugates have developed slowly, the proportion of bio-carriers has declined in recent years, as has chemical modifications. Overall, technology combinations have increased in a fluctuating manner, but in the past 5 years, research has developed rapidly, indicating the field’s potential.

Considering the importance of targeted delivery to tissues and organs, we would like to highlight some specific information based on our results. In particular, we noticed that the liver was studied most often. For example, patents WO2016086227A2, WO2014193716A2, WO2019104658A1, CN110721320A, and WO2019022586A2 all relate to liver targeting. Other targeted areas included the kidneys (WO2011158933A1, US10010532B2, and EP2583691B1), the central nervous system (WO20071145659A1 and GB2563964A), and eyes (WO20160444478A1 and EP2266656B1).

**Geographic and organizational perspectives**

Over the past 20 years, 10 countries and regions have been responsible for 95% of patents published: the United States (51%), Japan (9%), mainland China (7%), Canada (6%), Korea, Germany, and Switzerland (5% each), the United Kingdom (3%), and France and Israel (2% each) (see Figure 4A). Figure 4B displays the technological origins and market destinations. More than 90% of the patents filed originated in the 10 patent offices and organizations listed. For example, the United States ranked first in terms of both origin and destination. Patents were filed multilaterally in each of the top 10 origins except mainland China; its proportion of patent families is the lowest, accounting for only 5%, while other places of origin achieved 42%–92%. For more information, see Table S2.

Aside from individuals, three organizational types were recognized as assignees for siRNA-delivery patents: private companies, universities, and research institutions (comprising government-owned research centers, hospitals, and public organizations). Fifty-four percent of the patents were owned by private companies, 29% were owned by universities, and 13% were owned by research institutions. Figure 5A shows the change in organizational types of patent assignees from 2000 to 2020. For example, universities have played an increasingly important role compared with their early years.

A collaboration chord diagram was constructed to further investigate patterns among patent assignees (Figure 5B), which can be ranked by cooperation frequency, as follows: I-I, U-R, C-U, I-C, U-U, C-R, R-R, C-C, I-U, and I-R (I: individuals; C: private companies; U: universities; R: research institutions). Obviously, it takes a lot of collaboration for individuals to make progress in the field compared with organizations.

Table 1 lists the top 20 assignees by the number of patent families held. Interestingly, of the four approved siRNA drugs, three originated from Alnylam Pharmaceuticals and one originated from Novartis. It is also worth noting that Novartis possessed a small number of patent families but filed a large number of patent documents. Conversely, Chinese groups claimed an obviously small patent family size, implying that China pays less attention to market protection worldwide.

In terms of organizational specialization, Figure 6 shows the technological distribution of the top 20 assignees. All filed patents for lipid-based carriers: Arbutus Biopharma was the most focused on developing these carriers (92% of its patents were related to this category), followed by Merck Sharp & Dohm (62%), Nitto Denko (53%), Alnylam Pharmaceuticals (43%), and Novartis (34%). The University of Tokyo preferred using polymer carriers (70%), as did Mass General Brigham (54%), the University of Washington (48%), and the Massachusetts Institute of Technology (40%). Compared with other organizations, the two
research institutions from France (INSERM and CNRS) and the University of California showed more interest in adenoviruses, while the Chinese Academy of Science was more interested in lentiviruses, inorganic carriers, and biocarriers. Chemical modification was widely used in patents owned by Alnylam Pharmaceuticals, Suzhou Ribo Life Science, and the University of Massachusetts. Finally, regarding ligand-siRNA conjugates, the organizations that patented these the most (i.e., more than 30%) were Arrowhead Pharmaceuticals, Roche Holding, and Marina Biotech.

Technological landscape and milestone patents

Figure 7 objectively and comprehensively shows the state of the art in the field of siRNA delivery, which is helpful for understanding the technological landscape and its milestones while also analyzing interrelationships between patents, clarifying the stability of patent rights and infringement risks, and identifying the technological gap and white space in commercial development.

A global patent-citation network was constructed that involved 1,587 patent families with 8,340 internal citations to investigate the internal citation relationships between patents (see Figure 7A). The nodes represent patent families, the directed edges correspond to citation relationships, and the arrows point to cited patents. Patents with the same technological attributes are likelier to link closely together to form network clusters; clearly, the entire network presents several prominent ones. In this network, lipid-based patents occupy the core position (24.70%), followed by polymer carriers (23.44%), biocarriers (13.23%), and ligand modifications (12.48%).

The citation network was also time sliced according to priority year to clearly trace the technological trajectories of siRNA delivery R&D, and the most-cited patent family in each layer was regarded as its milestone patent. In Figure 7B, these patents are displayed on a timeline with their patent numbers and English titles shown. Influential patents were cited a great deal by subsequent patents, and this process formed a route of technological development.

The route starts with WO2002044321A2, which concerns chemical modification, specifically backbone modification containing a phosphorothioate group or ribose 2’-OH group modification. Next is WO2004029212A2, which is also related to chemical modification. Its backbone modification, like WO2002044321A2’s, also contains a phosphorothioate group or ribose modification, but in this case, the ribose was modified by a 2’-fluoro group. Patents WO2005007196A2, WO2007086881A3, WO2010042877A1, WO2010088537A2, WO201170889A1, WO2014210356A1, and WO2017218704A1 all describe discoveries about lipid-based carriers, such as improved lipid formulation, structures of cationic lipids, or multitali ed lipids. Patent WO2019173787A1 specifies bacterial-toxin-derived delivery constructs that are coupled with siRNA to form a conjugate for oral delivery.

DISCUSSION

In this section, we will discuss the technical and clinical aspects of siRNA delivery. We include the strengths and weaknesses of the current methods and clinical advances, as well as discuss possible future developments, including novel but promising technologies, technological combinations, and extrahepatic delivery.

Pros and cons of the current methods

Chemical modification has the ability to improve the stability and nuclease resistance of siRNA. However, probably because RISC-compatible chemical modifications are very limited compared with other oligonucleotides, many extensive modifications to siRNA have, unfortunately, increased toxicity and reduced gene
silencing. This may explain why this method has rarely been used in recent years.

Lipid-based carriers are generally regarded as extremely versatile nanocarriers due to their excellent biodegradability and biocompatibility. Subsequent generations of lipid-based carriers display even more complex architectures and enhanced physical stabilities. Lipid-based carriers are still not perfect, however. For example, neutral liposomes confer low transfection efficiency because of low siRNA-loading efficiency. Cationic liposomes, whether mono- or polyvalent, are not appropriate for in vivo applications due to their short blood-circulation time and poor tumor penetration. Furthermore, PEGylation is not conducive to cellular uptake or endosomal escape due to steric hindrance. Lipid nanoparticles (LNPs) include multiple types of lipids to maximize delivery efficiency, but due to their large size, they can exit the blood stream.

Figure 4. Types and cooperation of patent assignees
(A) Changes in assignee types. (B) Collaboration patterns. The number represents cooperation frequency of different types of assignees (C, private companies; U, universities; R, research institutions; I, individuals).

Figure 5. Geographical distribution
(A) The patent publication global trend (US, United States; JP, Japan; CN, China mainland; CA, Canada; KR, South Korea; DE, Germany; CH, Switzerland; GB, United Kingdom; FR, France; IL, Israel). (B) Technological origination and market destination. The number in brackets on the left is the amount of patent documents, and the percentage on the right is each patent office’s share. (USPTO, United States Patent and Trademark Office; WIPO, World Intellectual Property Organization; EPO, European Patent Office; CNIPA, China National Intellectual Property Administration; JPO, Japan Patent Office; CIPO, Canadian Intellectual Property Office; IP AU, Intellectual Property Australia; KIPO, Korean Intellectual Property Office; CGPTM, India Controller General of Patents, Designs, and Trade Marks; HK IPD, Intellectual Property Department of Hong Kong).
only at sites where the endothelial barrier is fenestrated. Thus, most siRNA-LNPs are focused on liver diseases because of limited biodistribution.\textsuperscript{28}

Polymers offer an attractive avenue for improving stability, pharmacokinetics, and cellular uptake. For example, cationic polymers are able to interact with negatively charged siRNA through electrostatic interactions. They can also protect siRNA from enzymatic degradation as well as facilitate endosomal escape via the proton sponge effect.\textsuperscript{40,41} Non-specific cytotoxicity still remains a major issue, however: it worsens as the molecular weight and degree of branching of polymers increase.\textsuperscript{42}

Biocarriers are of interest due to their unique advantages like high biocompatibility and biodegradability.\textsuperscript{33} However, the clinical transformation of these formulations remains a challenge because of the difficulties associated with their active targeting ability.

Ligand-siRNA conjugates offer selective delivery while reducing the toxicity normally associated with carriers.\textsuperscript{28} However, RNAi-mediated, off-target effects from N-acetylglalactosamine (GaINAc)-siRNA conjugates leading to liver toxicity have been observed.\textsuperscript{43} The mechanism of ligand-siRNA conjugates also determines the dependence of the efficiency and specificity of active targeting on the number of receptors. Due to the limited number of receptors, receptor-mediated endocytosis is prone to saturation and limits delivery efficiency.\textsuperscript{44}

Viral vectors exhibit efficiency advantages. However, immunogenicity is inevitable. In addition to safety concerns, production is also an issue because of its complexity. Lentiviral vectors are less cytotoxic than adenoviral vectors, but they still pose risks, for example, of insertional mutagenesis.\textsuperscript{45}

Inorganic carriers are valuable because of their unique properties, which can be far greater than traditional organic materials. They have good resistance to microbial attacks, excellent storage stability, and favorable physical functionalities. However, biocompatibility, biodegradation, and site application are still issues that must be addressed.\textsuperscript{32,46}

Many physical means of delivering siRNA into cells exist. We focus on electroporation, microinjection, and ultrasound-mediated targeted delivery (UMTD) because they are the top three methods used at the family level. Electroporation is efficient and easy to perform, but it requires a large amount of siRNA. Microinjection allows for the exact injection of siRNA into a single cell, but it is a slow, sequential process. Ultrasound’s cavitation effect can penetrate a cell membrane and create a reversible pore, thus facilitating the entry of therapeutic substances. Thus, UMTD is appropriate for deep tissue penetration and high-concentration aggregation. However, this technology faces challenges in developing a new generation of drug-loaded nanoparticles that have a suitable size range and excellent drug release at modest acoustic pressures and energies.

### Table 1. Top 20 assignees of siRNA delivery patents

| Rank | Assignee | Location       | No. of patent families | No. of patent documents | Type |
|------|----------|----------------|------------------------|-------------------------|------|
| 1    | Alnylam Pharmaceuticals | United States | 132                    | 437                     | C    |
| 2    | United States Health & Human Services | United States | 91                     | 146                     | R    |
| 3    | Massachusetts Institute of Technology | United States | 63                     | 251                     | U    |
| 4    | Arbutus Biopharma | Canada | 61                     | 439                     | C    |
| 5    | University of California | United States | 59                     | 243                     | U    |
| 6    | Roche Holding | Switzerland | 55                     | 215                     | C    |
| 7    | Chinese Academy of Science | China | 43                     | 46                      | R    |
| 8    | The University of Texas System | United States | 42                     | 122                     | U    |
| 9    | Nitto Denko Corporation | Japan | 40                     | 348                     | C    |
| 10   | Marina Biotech | United States | 36                     | 183                     | C    |
| 11   | Suzhou Ribo Life Sciences | China | 36                     | 73                      | C    |
| 12   | The French National Center for Scientific Research (CNRS) | France | 34                     | 130                     | R    |
| 13   | Merck Sharp & Dohm | United States | 34                     | 89                      | C    |
| 14   | Arrowhead Pharmaceuticals | United States | 33                     | 181                     | C    |
| 15   | Novartis | Switzerland | 29                     | 207                     | C    |
| 16   | Mass General Brigham | United States | 28                     | 96                      | R    |
| 17   | The French National Institute of Health and Medical Research (INSERM) | France | 27                     | 91                      | R    |
| 18   | University of Tokyo | Japan | 26                     | 75                      | U    |
| 19   | University of Massachusetts | United States | 25                     | 108                     | U    |
| 20   | University of Washington | United States | 25                     | 105                     | U    |
Emergent promising technologies

Many emerging technologies have been developed to overcome previous barriers and facilitate the efficiency of siRNA delivery. Generally, these technologies are associated with biocompatible and biodegradable materials, thus reducing toxicity and immunological rejection. They offer the possibility of a variety of functionalization and induced release strategies, which is beneficial for achieving target accumulation of siRNAs at therapeutic doses. Low cellular uptake efficiency and inefficient endosomal escape during delivery can also be enhanced. The following is a detailed discussion of several emerging technologies. We also list representative patents to illustrate how they can help facilitate delivery.

Since the toxicity of synthetic polymers is appreciable, naturally derived polymers are desirable in siRNA-delivery applications because they are biocompatible, biodegradable, and non-toxic by nature. Among them, chitosan (CS) nanoparticles have attracted much attention. CS is a linear alkaline polysaccharide derived from chitin, whose amine group is protonated at a pH below 6.6; this results in electrostatic interactions between siRNA and CS. For example, patent WO2014036649A1 indicates a gelling composition comprising CS and guanosine 5’-diphosphate (GDP), wherein the composition formed a gel when the CS was mixed with the GDP at a pH range of 5–6. The gel and siRNA were formulated for injection. It is worth noting that CS nanoparticles suffer from low stability, but that issue can be mitigated by cross-linkers. Surface modification can improve targeted delivery. Therefore, the development of novel materials and agents, such as cross-linkers or ligands, is required. Moreover, smart CS-based nanoparticles, such as pH-sensitive and thermo-responsive systems, seem to offer a promising path forward for siRNA therapeutics.

Cell-penetrating peptides (CPPs) correspond to short synthetic peptides of 30 residues. They can trigger their cargo to cross cell membranes into cytoplasm, improving the pathway within the cell and thus facilitating interaction with the target. CPPs have been extensively studied for delivery, and a variety of short polycationic CPPs have been synthesized. In siRNA delivery, CPPs have been reported to form non-covalent complexes with biomolecules. Generally, the nanocomplexes have a diameter of 100–200 nm. For instance, US9303076B2, held by Roche Holding, documents several amino acid sequences of CPPs. The CPPs that formed alpha-helical secondary structures were used for the translocation of molecules through the cell membrane, thereby improving internalization efficacy. In addition, CPPs are believed to favor endosomal escape, but their cellular uptake mechanism remains unclear. Thus, it is essential to address this issue to optimize future strategies.

Extracellular vesicles (EVs) are phospholipid bilayer membrane-enclosed biological entities secreted by various types of cells. As natural endogenous transport carriers, EVs have many inherent advantages, such as high transmembrane uptake efficiency, no endosomal escape or immune origin, and the ability to cross the blood-brain barrier. Recently, EVs have become a widely used emerging technology. For example, WO2014028763A1 provides an exosome-based therapy against neurodegenerative disorders. Isolated exosomes from neural...
cells induced by cytokine interferon gamma (IFN-γ) comprise additional therapeutic siRNA. An effective amount of pharmaceutical composition was administered to patients to treat multiple sclerosis or neuropathy. Notably, advances using EVs have been enabled largely because of existing methods and apparatuses for EV isolation and characterization. However, subpopulations remain a challenge due to isolation deficiencies in high-purity homogeneous EVs. Advances in various aspects of EV research, such as isolation, analytical characterization, and standardization of methods, would greatly enhance the mechanistic understanding of their biology. These insights will further help translate the application of EVs into efficient diagnostic and therapeutic methods.

DNA has been used to construct cage-like structures ranging in size from ~10 to 100 nm with molecular weights of up to ~60 MDa. These nanocages are assembled using various strategies, including the one-pot method, modular assembly, hierarchical self-assembly, and the DNA-origami technique. DNA nanocages can encapsulate external moieties for targeted delivery and triggered release; they can also be functionalized in a variety of ways to achieve targeted attachment. Many patents have applied DNA nanocages to siRNA-delivery technology; for example, patent US9919001B2 concerns a drug carrier with a self-assembled three-dimensional (3D) L-DNA nanocage structure. With a compact architecture that embeds functional siRNA inside, this invention could improve serum stability and cellular uptake efficiency. In other words, unrestricted design prospects and superior features make DNA nanocages promising candidates for siRNA delivery.

Technological combinations
In the past 5 years, more and more patents have used multiple technologies to ameliorate continuous delivery. Lipid-polymer hybrid nanoparticles (LPNs) are core-shell nanoparticle structures. As shown in WO2014036534A1, the advantages of polymeric nanoparticles, such as physical stability, may synergize with the biocompatibility of lipids. Significantly, LPNs have recently been observed exhibiting superior in vivo cellular delivery efficacy compared with polymeric nanoparticles and liposomes. A promising prospect is that LPNs will provide the ideal platform for drug delivery in the future. The potential of combining polymeric carriers with inorganic nanoparticles is also encouraging. In US20120207682A1, a sustained-release composition was created that comprised a polymeric layer surrounding a core for the administration of one or more siRNAs. This approach exhibits the complementary characteristics of hard nanomaterials and more flexible organic polymers, particularly in terms of their precise sizes, shapes, and soft dynamic properties.

Extrahepatic delivery
Today, systemic delivery to the liver is a clinical reality because of the approval of four siRNA drugs. The next step is to develop extrahepatic delivery to expand the benefits of treatment and the management of human diseases. However, cellular uptake and cytosolic delivery are key challenges that must be addressed before the potential of siRNAs can be fully realized. Closer collaboration between chemists and biologists is required to develop solutions that make use of the subtle aspects of biological pathways. Encouragingly, several important advances
have been made relating to the targeted delivery of siRNA to renal, central nervous system (CNS), and ocular areas.\textsuperscript{59} For example, patent WO2011158933A1 describes a pharmaceutical composition for treating renal fibrosis comprising siRNA as an HSP47 inhibitor and a liposome carrier containing retinol as a targeting agent for extracellular-matrix-producing cells in the kidney. Patent WO2007145659A1 provides a synthetic low-density lipoprotein (LDL) nanoparticle, which comprises a lipid moiety and a synthetic chimeric peptide. The synthetic LDL nanoparticle is capable of incorporating siRNA into target diseases associated with the expression of the LDL receptor (e.g., brain cancer). Patent WO2016044478A1 indicates a method for treating myocilin (MYOC) glaucoma using adeno-associated viral (AAV) vectors. The AAV vectors encode RNAi that target MYOC.

Clinical advances in siRNA therapeutics

So far, four siRNA drugs have been approved for use: ONPATTRO (patisiran), GIVLAARI (givosiran), OXLUMOTM (lumasiran), and LEQVIO (incisiran). ONPATTRO (patisiran) was formulated with LNPs to achieve effective delivery; the others employ GalNAc ligands.\textsuperscript{17-20} More information is available on the Cortellis Drug Discovery Intelligence Database at https://www.cortellis.com/drugdiscovery/. Among these four drugs, there are 630 relevant patents, occupying only about 5% of the overall patent dataset in this study. In other words, most patents related to the delivery of therapeutic siRNA are still far from clinical application, and more efforts are needed to bring them to patients’ bedsides.

At least 50 clinical trials are in the pipeline of drug discovery for diseases of rare, genetic, infectious, cardiometabolic, ophthalmological, and cancerous natures. The delivery systems mainly focus on LNPs and the GalNAc-siRNA conjugate.\textsuperscript{59} For example, many liposomal siRNA-delivery formulas, such as the EphA2-targeting DOPC-encapsulated siRNA (https://ClinicalTrials.gov/show/NCT01591356), PRO-040201 for treating high cholesterol (https://ClinicalTrials.gov/show/NCT00927459), and Atu027 for treating advanced solid cancer (https://ClinicalTrials.gov/show/NCT00938574), are undergoing clinical trials. At least 13 other conjugates are in the clinical development stages.\textsuperscript{60}

Due to its mechanism, siRNA can theoretically be used to silence any disease-related genes. siRNA drugs’ R&D time is also much shorter than that of small molecules, monoclonal antibodies, and proteins.\textsuperscript{61} This also makes siRNA therapy more attractive to the academic and industrial sectors. For the past 20 years, many efforts have been made to address the delivery issues that limit therapeutic applications. Despite much progress, the application of siRNA therapeutics is still challenging. In the future, extrahepatic delivery is expected to be at the forefront of this field. New target gene candidates and novel materials and agents used for targeting strategies will continue to be developed. Led by Alnylam Pharmaceuticals, companies are devoting their time and effort to fighting liver disease, ocular issues, CNS disorders, and other illnesses. There is no doubt that siRNA regimens will perform well in the next few years.

MATERIALS AND METHODS

The Derwent Innovation platform, a worldwide provider of enhanced patent documents, was used in this study (https://clarivate.com/products/derwent-innovation/). We adopted the criteria of the
Reporting Items for Patent Landscapes (RIPL) statement and referred to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) to collect all patent documents. Patents that dealt with inventions related to siRNA-delivery technologies and were published between January 1, 2000, and December 31, 2020, were retrieved.

In the search for patents filed on siRNA-delivery technologies, siRNA, its unabridged name, and its main derivatives, abbreviations, and synonyms were input into the title, abstract, and claims (CTB) fields of the database search engine. The word delivery and its main synonyms were also added to the search terms because we found that the number of siRNA-related patents was too many to process. The patent search terms were constructed as follows: CTB = (siRNA* OR (small ADJ interfer* ADJ RNA*) OR (short ADJ interfer* ADJ RNA*) OR (silenc* ADJ RNA*)) AND CTB = (deliver* OR vehicle OR carrier) AND PY ≥ (2000) AND PY ≤ (2020).

The flow diagram of the patent sample (see Figure 8) shows the number of patent documents included at each stage and the reasons for removal. Initially, 42,414 patent documents were obtained from the Derwent Innovation database. We first excluded duplicate records (n = 13,589) and obviously irrelevant patents (n = 16,129) manually. Among the 12,696 full patents selected for detailed review, we also excluded 1,187 patents with little valuable information, meaning that the patent was related to therapeutic siRNA delivery but the information disclosed was of little value and/or vaguely described. The included patents were given labels after being checked manually to distinguish the siRNA technology used.

Each patent record consisted of detailed information, including priority year, publication year, citations, assignees, etc. The OpenRefine tool (http://openrefine.org/) was used in this study to unify the names of assignees. The processing approaches for typical cases of assignees are listed in Table S3. Moreover, we used various descriptive approaches, including line charts, 100% stacked column charts, Sankey diagrams, and chord diagrams, to illustrate patent indicators and associations. All charts and diagrams were created using Microsoft Excel and RStudio (https://www.rstudio.com/). Also, a method of software usage and the University of Macau for guidance on methods such as software usage and the University of Macau—Dr. Stanley Ho Medical Development Foundation for financial support via the project SHMDF-OIRS/2021/001.

**AUTHOR CONTRIBUTIONS**

Y.-J.H. and H.Y. organized and supervised this study. D.-F.O. and Y.Z. also supervised this study. Y.C. and S.-H.X. contributed to investigation, data curation, and writing of the original draft and the revision. F.L. contributed to the data curation. X.-J.K. checked the figures and contributed to conceptualization and review and editing of the writing.

**DECLARATION OF INTERESTS**

The authors declared that they have no conflicts of interest to this work.

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