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Demystifying RNAi for stronger patent searches

Peter Blasi and Anne Marie Clark of CAS IP Services discuss avoiding pitfalls in patentability and freedom to operate searches.

With the approval of two small interfering RNA (siRNA) biopharmaceuticals and 52 clinical trials spanning multiple therapeutic areas in progress, the value and complexity of the patent landscape around RNA interference (RNAi) is increasing. Challenges with searching siRNA include non-standardized terminology, drug delivery that is claimed separately from the molecules, and the limitations of familiar biosequence search methodologies. Due to these challenges, RNAi searches require specialized expertise in search techniques and information sources where those techniques should be implemented.

This article examines how RNAi inventions are claimed and described in the specification, which information sources ensure the most comprehensive retrieval, and the best search techniques to use. These examples and techniques aim to demystify the process for finding relevant patent and non-patent literature related to efficiently retrieving these sequences.

Résumé

Anne Marie Clark

Anne Marie is a Senior Searcher on the CAS IP Services team. Her particular subject expertise is small molecules, sequences, and formulations, and she is skilled in patent searching, analysis and landscaping, and competitive benchmarking.

Peter Blasi

Peter leads business development for the CAS IP Services team, introducing clients to the value of CAS IP Services and bringing them together with seasoned search professionals to meet their information needs.

Peter Blasi

Anne Marie Clark

RNA interference discovery and mechanism

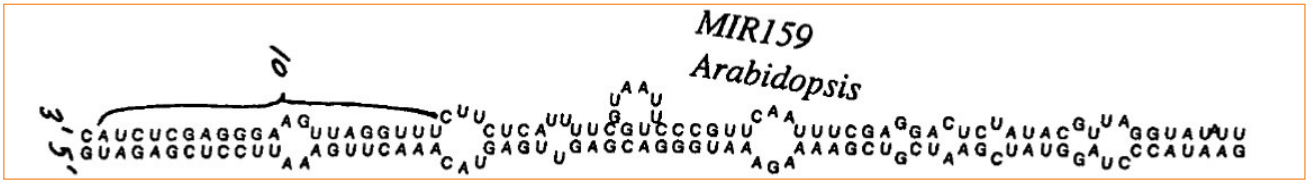
In 1998, Andrew Fire and Craig Mello discovered RNA interference (RNAi), sometimes called Post-Transcriptional Gene Silencing (PTGS), for which they received the Nobel Prize in 2006. Small interfering RNAs (siRNAs) selectively silence the expression of a target gene by degrading its mRNA. The silenced gene is specified by the small RNA component, which recognizes the target by base pairing.

RNAi allows a brand-new class of drugs. Sequence-specific gene silencing can eliminate the root cause of a disease, and can halt or reverse the disease progression, rather than slowing it or only treating the symptoms. There are two common types of RNAi molecules: micro RNA (miRNA) and small interfering RNA (siRNA). Both are non-coding RNAs, as they do not code for a protein. miRNA are small RNA molecules which silence genes by binding to target messenger RNAS (mRNAs). Mechanisms include:

1) Cleavage of the mRNA strand into two pieces,
2) Destabilization of the mRNA through shortening of its poly(A) tail, and
3) Less efficient translation of the mRNA into proteins by ribosomes.

Since their discovery in 1993, miRNA have been found in all eukaryotic cells conserved across the species. miRNAs are small non-coding RNA molecules (containing about 22 nucleotides) and often have hairpin structures as seen in Figure 1.

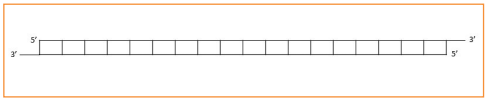
Figure 1. miRNA hairpin structure binding a target sequence.



Source: US Patent Application No. 20050144669

siRNA, sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA, typically 20-25 base pairs in length, similar to miRNA. Typically, siRNA duplexes have 21-nt sense and 21-nt antisense strands, paired with a 2-nt 3' overhang as seen in Figure 2. In therapeutic siRNAs, 2'-deoxynucleotides in the 3' overhangs are used instead of ribonucleotides since they are cheaper to synthesize and probably more nuclease resistant.

Figure 2 Structure of siRNA



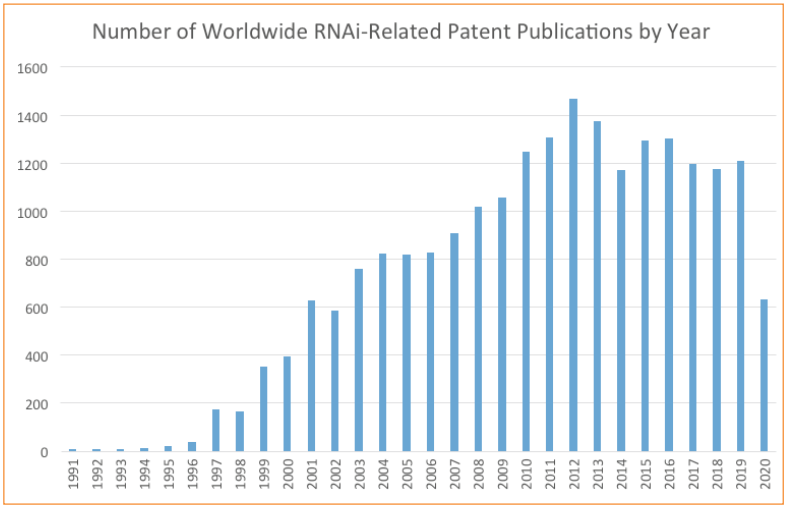
Source: US Patent No. 9260470

siRNAs are 21-23 nucleotide double stranded RNA (dsRNA) duplexes with symmetric 2-3 nucleotide 3' overhangs and 5' phosphate and 3' hydroxyl groups

Growth in RNAi Patent Publications

Since the discovery of RNAi, there has been steady growth in the volume of worldwide patent publications that disclose its different forms. The following two figures show the number of worldwide patent publications and the companies with the most RNAi-related patent publications since 1991.

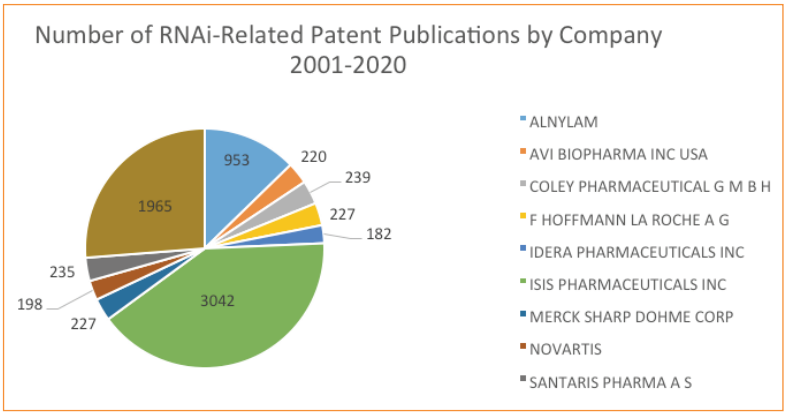
Figure 3: Number of worldwide RNAi-related patent publications by year



(Source: CAS content collection)

Note: Due to the elapsed time between patent application and publication, the last two years of data are incomplete.

Figure 4: Top 10 companies filing RNAi-related patent publications since 1991



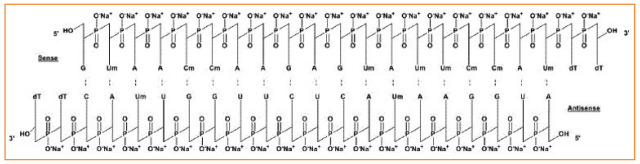
(Source: CAS content collection).

RNAi Drug Discovery

There are 52 clinical trials on RNAi-based drugs spanning multiple therapeutic areas in progress. Successful drugs must be safe and effective with relatively convenient dosing. For therapeutics, siRNA development challenges include off-target transcript silencing (causing safety concerns), delivery to target cells, and degradation by nucleases causing the drug to be degraded before achieving a therapeutic dose. Chemical modification and bioconjugation can help overcome these problems.

The US Food and Drug Administration recently approved two siRNA drugs: Onpattro (Patisiran) and Givlaari (Givosiran). Both drugs were developed by Alnylam Pharmaceutical and are shown below in Figures 5 and 6. Patisiran is a chemically modified, double-stranded small interfering ribonucleic acid (siRNA), formulated as a lipid complex for delivery to hepatocytes and is used for the treatment of transthyretin-mediated amyloidosis. Patisiran specifically binds to a genetically conserved sequence in the 3' untranslated region (3'UTR) of mutant and wild-type transthyretin (TTR) messenger RNA (mRNA).

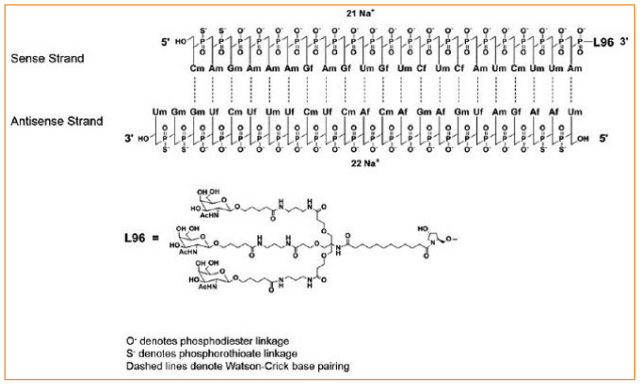
Figure 5: Chemical Structure of Patisiran



(Source: US Food and Drug Administration)

Givosiran is an aminolevulinate synthase 1-directed small interfering RNA (siRNA), covalently linked to a ligand containing three N-acetylgalactosamine (GalNAc) residues to enable delivery of the siRNA to hepatocytes. Givosiran is used to treat acute hepatic porphyria and it is both chemically modified and bioconjugated.

Figure 6: Chemical Structure of Givosiran



(Source: US Food and Drug Administration)

Abbreviations: Af = adenine 2'-F ribonucleoside; Cf = cytosine 2'-F ribonucleoside; Uf = uracil 2'-F ribonucleoside; Am = adenine 2'-OMe ribonucleoside; Cm = cytosine 2'-OMe ribonucleoside; Gf=guanine 2'F ribonucleoside; Gm = guanine 2'-OMe ribonucleoside; Um = uracil 2'-OMe ribonucleoside; L96 = triantennary GalNAc (N-acetylgalactosamine)

How RNAi is Represented in Patents

RNAi is claimed in a variety of ways within patents. siRNA or RNAi terminology is not always used, and chemical modifications can be described a variety of ways which means that there is no single strategy that can be used to efficiently retrieve this information from different databases. For example, US Patent No. 8,334,373 does not use the term siRNA or claim specific sequences. It uses double-stranded ribonucleic acid terminology and claims chemical modifications as sequence motifs making both retrieval and analysis challenging. Claim 1 is shown below.

Figure 7 Claim in Patent No. 8,334,373

We claim:

1. A double-stranded ribonucleic acid (dsRNA) having a sense strand and an antisense strand and having increased stability in a biological sample, wherein the sense strand and the antisense strand are both modified, said antisense-strand modifications consisting of:

(a) 2'-modified uridines in all occurrences of the sequence motif 5'-uridine-adenine-3' (5'-ua-3'), and

(b) 2'-modified cytidines in all occurrences of the sequence motif 5'-cytidine-adenine-3' (5'-ca-3').

Table 1: Summary of the Invention in the '373 Patent

A double-stranded ribonucleic acid (dsRNA) having increased stability in a biological sample, wherein the dsRNA comprises at least one of

(a) 2′-modified uridines in all occurrences of the sequence motif 5′-uridine-adenine-3′ (5′-ua-3′), or

(b) 2′-modified uridines in all occurrences of the sequence motif 5′-uridine-guanine-3′ (5′-ug-3′), or

(c) 2′-modified cytidines in all occurrences of the sequence motif 5′-cytidine-adenine-3′ (5′-ca-3′), or

(d) 2′-modified 5′-most uridines in all occurrences of the sequence motif 5′-uridine-uridine-3′ (5′-uu-3′), or

(e) 2′-modified 5′-most cytidines in all occurrences of the sequence motif 5′-cytidine-cytidine-3′ (5′-cc-3′), or

(f) 2′-modified cytidines in all occurrences of the sequence motif 5′-cytidine-uridine-3′ (5′-cu-3′), or

(g) 2′-modified uridines in all occurrences of the sequence motif 5′-uridine-cytidine-3′ (5′-uc-3′).”

In US Patent No. 9,101,643, lipid-formulated compositions and methods for inhibiting expression of transthyretin (ttr) are claimed, and analysis of the full patent document is required to determine the scope of the claims, as shown below.

Figure 8: Claim 1 from '643 Patent

I claim:

1. A method of reducing a TTR amyloid deposit by >90% in a subject having the TTR amyloid deposit, comprising administering to the subject an effective amount of a composition comprising a TTR dsRNA and determining TTR amyloid deposition in the subject, wherein: the TTR dsRNA comprises a sense strand and an antisense strand, the antisense strand consisting of SEQ ID NO: 1010 and the sense strand consisting of SEQ ID NO: 1009; the effective amount is 0.01-30 mg/kg TTR dsRNA; and the composition comprises a lipid formulation comprising a cationic lipid.

Table 2: SEQ ID No. 1009

<210> SEQ ID NO 1009
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide
<220> FEATURE:
<223> OTHER INFORMATION: Description of Combined DNA/RNA Molecule: Synthetic oligonucleotide

<400> SEQUENCE: 1009

guaaccaaga guauuccaut t 21

However, as seen in Figure 8 and Table 2, neither the claim nor the sequence listing describes the chemical modifications. The chemical modifications are described in Tables 3 and 4, making analysis challenging.

Table 3: Abbreviations of Nucleotide Monomers Used in Nucleic Acid Sequence Representation

"Abbreviation	Nucleotide(s)
A	adenosine-3'-phosphate
C	cytidine-3'-phosphate
G	guanosine-3'-phosphate
U	uridine-3'-phosphate
N	any nucleotide (G, A, C, or T)
a	2'-O-methyladenosine-3'-phosphate
c	2'-O-methylcytidine-3'-phosphate
g	2'-O-methylguanosine-3'-phosphate
u	2'-O-methyluridine-3'-phosphate
T, dT	2'-deoxythymidine-3v-phosphate
sT; sdT	2'-deoxy-thymidine-5'phosphate-phosphorothioate

Table 4: Sequences With Chemical Modifications

Duplex #	Strand Oligo #	Position*	Sequence 5' to 3'	SEQ ID No.
AD-18324 s	A-32337	509	GGAuuucAuGuAAccAAGAdTdT	1001
AD-18324 as	A-32338	527	UCUUGGUuAcAUGAAAUCCdTdT	1002
AD-18328 s	A-32345	518	GuAAccAAGAGuAuuccAudTdT	1009
AD-18328 as	A-32346	536	AUGGAAuACUCUUGGUuACdTdT	1010

Search Techniques to Retrieve RNAi References

A comprehensive search for RNAi prior art should include both a sequence search and a text search, because of the inconsistencies of how RNAi-related inventions are claimed. There are a variety of different human-curated and algorithmically-curated databases with information for both sequences and text. A typical workflow for a patent search is: start with sequences with human-curated sequence databases such as CAS BIOSEQUENCES™ first, then search algorithmically-curated sequence databases. After the sequence search, search text terms in human-curated databases, such as CAplus℠, and then finally search full-text patent databases. An siRNA search should start by searching the unmodified base sequence in a biosequence database.

Some common methodologies for sequence searches include:

- Exact sequence - retrieves sequences with exact match, and same length.
- Subsequence - retrieves sequences where the query sequence may be embedded.
- Motif search - retrieves sequences with. repeats, alternatives, spacers, etc.
- BLAST (Basic Local Alignment Search Tool) - searches sequence similarity (generally expressed as % of sequence identity, or %ID).

siRNA and miRNA sequences are usually first searched using a motif allowing specific variables or exact search where 100% matches are found.

The following questions should be considered by the searcher and attorney:

- Should overhanging nucleotides be trimmed from the strategy? The overhanging nucleotides can be replaced with bioconjugates or modified nucleotides.
- Will the strategy retrieve chemical modifications or bioconjugates?
- Will the bioconjugate molecule be searched separately?

Conducting an RNAi sequence search using human-curated databases can yield precise results. When searching text, the strategy should include such human-curated databases like CAplus which offer the increased power and precision of controlled vocabulary as well as full-text patent databases. When developing a search strategy, consider multiple ways the siRNA can be described such as modified dsRNA, oligonucleotides, oligomers, RNA interference, RNAi. For chemical modifications, the modification pattern, not just a specific sequence, can be claimed and should be searched. Claims can also be target-focused rather than substance-focused; so, that aspect may also be searched. To ensure the most reliable results, RNAi searches should cover different features, uses, and characteristics of the siRNA/miRNA.

> **"These examples and techniques aim to demystify the process."**

Conclusion

A high-quality, professional search performed using multiple information sources and search techniques specific to RNAi is extremely valuable to support drafting the strongest possible RNAi claims. A search performed by a reliable professional with database and strategy knowledge is the key first-step to assist attorneys who are advising their clients on patentability and freedom-to-operate.

Contact

CAS IP Services
Tel: 866-360-0814
www.scienceip.org
www.cas.org