US 20130109091A1

(19) **United States**

(12) **Patent Application Publication**

Baker et al.

(10) **Pub. No.: US 2013/0109091 A1**

(43) **Pub. Date: May 2, 2013**

(54) **COMPOSITIONS AND METHODS FOR MODULATION OF SMN2 SPLICING**

(71) Applicants: **Isis Pharmaceuticals, Inc.**, Carlsbad, CA (US); **Cold Spring Harbor Laboratory**, Cold Spring Harbor, CA (US)

(72) Inventors: **Brenda F. Baker**, Carlsbad, CA (US); **Adrian R. Krainer**, Huntington Station, NY (US); **Yimin Hua**, Jericho, NY (US)

(73) Assignees: **Cold Spring Harbor Laboratory**, Cold Spring Harbor, CA (US); **Isis Pharmaceuticals, Inc.**, Carlsbad, CA (US)

(21) Appl. No.: **13/720,474**

(22) Filed: **Dec. 19, 2012**

Related U.S. Application Data

(63) Continuation of application No. 11/993,609, filed on May 6, 2010, now Pat. No. 8,361,977, filed as application No. PCT/US06/24469 on Jun. 23, 2006.

(60) Provisional application No. 60/693,542, filed on Jun. 23, 2005.

Publication Classification

(51) **Int. Cl.**
C12N 15/113 (2006.01)

(52) **U.S. Cl.**
CPC ***C12N 15/113*** (2013.01)
USPC **435/375**; 536/24.5

(57) **ABSTRACT**

Disclosed herein are compounds, compositions and methods for modulating splicing of SMN2 mRNA in a cell, tissue or animal. Also provided are uses of disclosed compounds and compositions in the manufacture of a medicament for treatment of diseases and disorders, including spinal muscular atrophy.

COMPOSITIONS AND METHODS FOR MODULATION OF SMN2 SPLICING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 11/993,609, filed May 6, 2010, which is the US National Phase application under 35 U.S.C. 371 of PCT Application Number PCT/US2006/024469, filed Jun. 23, 2006, which claims priority to U.S. Provisional Patent Application Ser. No. 60/693,542, filed Jun. 23, 2005, each of which is incorporated by reference herein in its entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled CORE0058USC1SEQ.txt, created on Oct. 25, 2012 which is 24 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] Newly synthesized eukaryotic mRNA molecules, also known as primary transcripts or pre-mRNA, made in the nucleus, are processed before or during transport to the cytoplasm for translation. Processing of the pre-mRNAs includes addition of a 5' methylated cap and an approximately 200-250 base poly(A) tail to the 3' end of the transcript.

[0004] The next step in mRNA processing is splicing of the pre-mRNA, which occurs in the maturation of 90-95% of mammalian mRNAs. Introns (or intervening sequences) are regions of a primary transcript (or the DNA encoding it) that are not included in the coding sequence of the mature mRNA. Exons are regions of a primary transcript that remain in the mature mRNA when it reaches the cytoplasm. The exons are spliced together to form the mature mRNA sequence. Splice junctions are also referred to as splice sites with the 5' side of the junction often called the "5' splice site," or "splice donor site" and the 3' side the "3' splice site" or "splice acceptor site." In splicing, the 3' end of an upstream exon is joined to the 5' end of the downstream exon. Thus the unspliced RNA (or pre-mRNA) has an exon/intron junction at the 5' end of an intron and an intron/exon junction at the 3' end of an intron. After the intron is removed, the exons are contiguous at what is sometimes referred to as the exon/exon junction or boundary in the mature mRNA. Cryptic splice sites are those which are less often used but may be used when the usual splice site is blocked or unavailable. Alternative splicing, defined as the splicing together of different combinations of exons, often results in multiple mRNA transcripts from a single gene.

[0005] Up to 50% of human genetic diseases resulting from a point mutation are caused by aberrant splicing. Such point mutations can either disrupt a current splice site or create a new splice site, resulting in mRNA transcripts comprised of a different combination of exons or with deletions in exons. Point mutations also can result in activation of a cryptic splice site or disrupt regulatory cis elements (i.e. splicing enhancers or silencers) (Cartegni et al., Nat. Rev. Genet., 2002, 3, 285-298; Drawczak et al., Hum. Genet., 1992, 90, 41-54).

[0006] Antisense oligonucleotides have been used to target mutations that lead to aberrant splicing in several genetic [illegible] 238). Such diseases include β-thalassemia (Dominski and Kole, *Proc. Natl. Acad. Sci*. USA, 1993, 90, 8673-8677; Sierakowska et al., Nucleosides & Nucleotides, 1997, 16, 1173-1182; Sierakowska et al., *Proc. Natl. Acad. Sci*. USA, 1996, 93, 12840-44; Lacerra et al., Proc. Natl. Acad. Sci. USA, 2000, 97, 9591-9596); dystrophin Kobe (Takeshima et al., *J. Clin. Invest.,* 1995, 95, 515-520); Duchenne muscular dystrophy (Dunckley et al. *Nucleosides & Nucleotides,* 1997, 16, 1665-1668; Dunckley et al. *Human Mol. Genetics,* 1998, 5, 1083-90); osteogenesis imperfecta (Wang and Marini, *J. Clin Invest.,* 1996, 97, 448-454); and cystic fibrosis (Friedman et al., J. Biol. Chem., 1999, 274, 36193-36199).

[0007] Antisense compounds have also been used to alter the ratio of the long and short forms of Bcl-x pre-mRNA (U.S. Pat. No. 6,172,216; U.S. Pat. No. 6,214,986; Taylor et al., Nat. Biotechnol. 1999, 17, 1097-1100) or to force skipping of specific exons containing premature termination codons (Wilton et al., Neuromuscul. Disord., 1999, 9, 330-338). U.S. Pat. No. 5,627,274 and WO 94/26887 disclose compositions and methods for combating aberrant splicing in a pre-mRNA molecule containing a mutation using antisense oligonucleotides which do not activate RNAse H.

[0008] Proximal spinal muscular atrophy (SMA) is a genetic, neurodegenerative disorder characterized by the loss of spinal motor neurons. SMA is an autosomal recessive disease of early onset and is currently the leading cause of death among infants. The severity of SMA varies among patients and has thus been classified into three types. Type I SMA is the most severe form with onset at birth or within 6 months and typically results in death within 2 years. Children with type I SMA are unable to sit or walk. Type II SMA is the intermediate form and patients are able to sit, but cannot stand or walk. Patients with type III SMA, a chronic form of the disease, typically develop SMA after 18 months of age (Lefebvre et al., Hum. Mol. Genet., 1998, 7, 1531-1536).

[0009] SMA is caused by the loss of both copies of survival of motor neuron 1 (SMN1), a protein that is part of a multiprotein complex thought to be involved in snRNP biogenesis and recycling. A nearly identical gene, SMN2, exists in a duplicated region on chromosome 5q13. Although SMN1 and SMN2 have the potential to code for the same protein, SMN2 contains a translationally silent mutation at position +6 of exon 7, which results in inefficient inclusion of exon 7 in SMN2 transcripts. Thus, the predominant form of SMN2 is a truncated version, lacking exon 7, which is unstable and inactive (Cartegni and Krainer, Nat. Genet., 2002, 30, 377-384).

[0010] Chimeric peptide nucleic acid molecules designed to modulate splicing of SMN2 have been described (WO 02/38738; Cartegni and Krainer, Nat. Struct. Biol., 2003, 10, 120-125).

[0011] Antisense technology is an effective means for modulating the expression of one or more specific gene products, including alternative splice products, and is uniquely useful in a number of therapeutic, diagnostic, and research applications. The principle behind antisense technology is that an antisense compound, which hybridizes to a target nucleic acid, modulates gene expression activities such as transcription, splicing or translation through one of a number of antisense mechanisms. The sequence specificity of antisense compounds makes them extremely attractive as tools for target validation and gene functionalization, as well as [illegible]

[0012] Disclosed herein are antisense compounds useful for modulating gene expression and associated pathways via antisense mechanisms, which may include antisense mechanisms based on target occupancy. Provided herein are antisense compounds targeting SMN2 for use in modulation of SMN2 splicing. One having skill in the art, once armed with this disclosure will be able, without undue experimentation, to identify, prepare and exploit antisense compounds for these uses.

SUMMARY OF THE INVENTION

[0013] The present invention is directed to antisense compounds targeted to and hybridizable with a nucleic acid molecule encoding SMN2. Provided are antisense compounds targeted to intron, 6, exon 7 or intron 7 of SMN2 which modulate splicing of SMN2 pre-mRNAs. In one embodiment, modulation of splicing results in an increase in exon 7 inclusion. In another embodiment, modulation of splicing results in a decrease in exon 7 inclusion. Contemplated and provided herein are antisense compounds 12 to 20 nucleotides in length targeted to intron 6, exon 7 or intron 7 of SMN2, wherein the compounds comprise 2'-O-methoxyethyl sugar modifications.

[0014] In one aspect of the invention, the antisense compounds are targeted to cis splicing regulatory elements. Regulatory elements include exonic splicing enhancers, exonic splicing silencers, intronic splicing enhancers and intronic splicing silencers. Exonic and intronic splicing silencers are preferred targets.

[0015] In one embodiment, the antisense compounds comprise at least an 8-nucleobase portion of one of the exemplary compounds provided herein.

[0016] Also provided are methods for modulating splicing of SMN2 mRNA in a cell, tissue or organ using one or more of the compounds of the invention. In one embodiment, modulation of splicing is exon inclusion. In another embodiment, modulation of splicing is exon skipping. In one aspect, the compound is targeted to an intronic splicing silencer element. In another aspect, the compound is targeted to an exonic splicing silencer element.

[0017] Further provided are antisense compounds 10 to 50, 12 to 30 or 12 to 20 nucleotides in length targeted to intron 6, exon 7 or intron 7 of SMN2 comprising 2'-O-methoxyethyl sugar modifications for use in therapy. Also provided are pharmaceutical compositions comprising one or more of the compounds of the invention. Use of an antisense oligonucleotide provided herein for the preparation of a medicament for modulating splicing of an SMN2 pre-mRNA is also provided. In one aspect, modulation of splicing results in an increase in exon 7 inclusion. Use of an antisense oligonucleotide provided herein for the preparation of a medicament for the treatment of spinal muscular atrophy is further provided.

DETAILED DESCRIPTION OF THE INVENTION

[0018] Antisense technology is an effective means for modulating the expression of one or more specific gene products and is uniquely useful in a number of therapeutic, diagnostic, and research applications. Provided herein are antisense compounds useful for modulating gene expression via antisense mechanisms of action, including antisense mechanisms based on target occupancy. In one aspect, the antisense

inclusion. Further provided herein are antisense compounds targeted to cis splicing regulatory elements present in pre-mRNA molecules, including exonic splicing enhancers, exonic splicing silencers, intronic splicing enhancers and intronic splicing silencers. Disruption of cis splicing regulatory elements is thought to alter splice site selection, which may lead to an alteration in the composition of splice products.

[0019] Processing of eukaryotic pre-mRNAs is a complex process that requires a multitude of signals and protein factors to achieve appropriate mRNA splicing. Exon definition by the spliceosome requires more than the canonical splicing signals which define intron-exon boundaries. One such additional signal is provided by cis-acting regulatory enhancer and silencer sequences. Exonic splicing enhancers (ESE), exonic splicing silencers (ESS), intronic splicing enhancers (ISE) and intron splicing silencers (ISS) have been identified which either repress or enhance usage of splice donor sites or splice acceptor sites, depending on their site and mode of action (Yeo et al. 2004, *Proc. Natl. Acad. Sci. U.S.A.* 101(44):15700-15705). Binding of specific proteins (trans factors) to these regulatory sequences directs the splicing process, either promoting or inhibiting usage of particular splice sites and thus modulating the ratio of splicing products (Scamborova et al. 2004, *Mol. Cell. Biol.* 24(5):1855-1869; Hovhannisyan and Carstens, 2005, *Mol. Cell. Biol.* 25(1):250-263; Minovitsky et al. 2005, *Nucleic Acids Res.* 33(2):714-724). Little is known about the trans factors that interact with intronic splicing elements; however, several studies have provided information on exonic splicing elements. For example, ESEs are known to be involved in both alternative and constitutive splicing by acting as binding sites for members of the SR protein family. SR proteins bind to splicing elements via their RNA-binding domain and promote splicing by recruiting spliceosomal components with protein-protein interactions mediated by their RS domain, which is comprised of several Arg-Ser dipeptides (Cartegni and Krainer, 2003, *Nat. Struct. Biol.* 10(2):120-125; Wang et al. 2005, *Nucleic Acids Res.* 33(16):5053-5062). ESEs have been found to be enriched in regions of exons that are close to splice sites, particularly 80 to 120 bases from the ends of splice acceptor sites (Wu et al. 2005, *Genomics* 86:329-336). Consensus sequences have been determined for four members of the SR protein family, SF2/ASF, SC35, SRp40 and SRp55 (Cartegni et al. 2003, *Nucleic Acids Res.* 31(13):3568-3571).

[0020] Although the trans factors that bind intronic splicing regulatory elements have not been extensively studied, SR proteins and heterogeneous ribonucleoproteins (hnRNPs) have both been suggested to interact with these elements (Yeo et al. 2004, *Proc. Natl. Acad. Sci. U.S.A.* 101(44):15700-15705). Two intronic splicing enhancer elements (ISEs) have been identified in SMN2, one in intron 6 and the other in intron 7 (Miyajima et al. 2002, *J. Biol. Chem.* 22:23271-23277). Gel shift assays using the ISE in intron 7 showed formation of RNA-protein complexes, which suggests these trans proteins may be important for regulation of splicing (Miyaso et al. 2003, *J. Biol. Chem.* 278(18):15825-15831).

[0021] The role of SMN2 in diseases such as spinal muscular atrophy (SMA) makes it an important therapeutic target. SMA is a genetic disorder characterized by degeneration of spinal motor neurons. SMA is caused by the loss of both functional copies of SMN1. However, SMN2 has the poten-

lationally silent mutation (C→T) at position +6 of exon 7 (nucleotide 66 of SEQ ID NO: 1), which results in inefficient inclusion of exon 7 in SMN2 transcripts. Therefore, the predominant form of SMN2, one which lacks exon 7, is unstable and inactive. Thus, therapeutic compounds capable of modulating SMN2 splicing such that the percentage of SMN2 transcripts containing exon 7 is increased, would be useful for the treatment of SMA.

Overview

[0022] Disclosed herein are oligomeric compounds, including antisense oligonucleotides and other antisense compounds for use in modulating the expression of nucleic acid molecules encoding SMN2. This is accomplished by providing oligomeric compounds which hybridize with one or more target nucleic acid molecules encoding SMN2. As used herein, the terms "target nucleic acid" and "nucleic acid molecule encoding SMN2" have been used for convenience to encompass DNA encoding SMN2, RNA (including pre-mRNA and mRNA or portions thereof) transcribed from such DNA, and also cDNA derived from such RNA.

[0023] Provided herein are antisense compounds for use in modulation of SMN2 pre-mRNA splicing. In one embodiment, the disclosed antisense compounds are targeted to exon 7 of SMN2 such that SMN mRNA splicing is modulated. In another embodiment, the antisense compounds are targeted to intron 6 of SMN2. In another embodiment, the antisense compounds are targeted to intron 7 of SMN2. Modulation of splicing may result in exon 7 inclusion or exon 7 skipping.

[0024] Also provided are antisense compounds targeted to cis regulatory elements. In one embodiment, the regulatory element is in an exon. In another embodiment, the regulatory element is an in intron.

Modulation of Splicing

[0025] As used herein, modulation of splicing refers to altering the processing of a pre-mRNA transcript such that the spliced mRNA molecule contains either a different combination of exons as a result of exon skipping or exon inclusion, a deletion in one or more exons, or additional sequence not normally found in the spliced mRNA (e.g., intron sequence). In the context of the present invention, modulation of splicing refers to altering splicing of SMN2 pre-mRNA to achieve exon skipping or exon inclusion. In one embodiment, exon skipping results in an SMN2 mRNA transcript lacking exon 7 and exon inclusion results in an SMN2 mRNA transcript containing exon 7.

[0026] As used herein, alternative splicing is defined as the splicing together of different combinations of exons, which may result in multiple mRNA transcripts from a single gene. In the context of the present invention, an SMN2 mRNA transcript containing exon 7 and an SMN2 mRNA transcript lacking exon 7 are two products of alternative splicing.

Compounds

[0027] The term "oligomeric compound" refers to a polymeric structure capable of hybridizing to a region of a nucleic acid molecule. This term includes oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics and chimeric combinations of these. An "antisense compound" or "antisense oligomeric compound" refers to an [illegible] and which modulates its expression. Consequently, while all antisense compounds can be said to be oligomeric compounds, not all oligomeric compounds are antisense compounds. An "antisense oligonucleotide" is an antisense compound that is a nucleic acid-based oligomer. An antisense oligonucleotide can be chemically modified. Nonlimiting examples of oligomeric compounds include primers, probes, antisense compounds, antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, and siRNAs. As such, these compounds can be introduced in the form of single-stranded, double-stranded, circular, branched or hairpins and can contain structural elements such as internal or terminal bulges or loops. Oligomeric double-stranded compounds can be two strands hybridized to form double-stranded compounds or a single strand with sufficient self complementarity to allow for hybridization and formation of a fully or partially double-stranded compound.

[0028] The oligomeric compounds in accordance with this invention may comprise a complementary oligomeric compound from about 10 to about 50 nucleobases (i.e. from about 10 to about 50 linked nucleosides). One having ordinary skill in the art will appreciate that this embodies antisense compounds of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleobases.

[0029] In one embodiment, the antisense compounds of the invention are 12 to 30 nucleobases. One having ordinary skill in the art will appreciate that this embodies antisense compounds of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleobases.

[0030] In one embodiment, the antisense compounds of the invention are 12 to 20 nucleobases. One having ordinary skill in the art will appreciate that this embodies antisense compounds of 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleobases.

[0031] In one embodiment, the antisense compounds of the invention have antisense portions of 20 nucleobases.

[0032] In one embodiment, the antisense compounds of the invention have antisense portions of 18 nucleobases.

[0033] In one embodiment, the antisense compounds of the invention have antisense portions of 15 nucleobases.

[0034] In one embodiment, the antisense compounds of the invention have antisense portions of 12 nucleobases.

[0035] Antisense compounds 10-50 nucleobases in length comprising a stretch of at least eight (8) consecutive nucleobases selected from within the illustrative antisense compounds are considered to be suitable antisense compounds as well.

[0036] Compounds of the invention include oligonucleotide sequences that comprise at least the 8 consecutive nucleobases from the 5'-terminus of one of the illustrative antisense compounds (the remaining nucleobases being a consecutive stretch of nucleobases continuing upstream of the 5'-terminus of the antisense compound until the oligonucleotide contains about 10 to about 50 nucleobases). Other compounds are represented by oligonucleotide sequences that comprise at least the 8 consecutive nucleobases from the 3'-terminus of one of the illustrative antisense compounds (the remaining nucleobases being a consecutive stretch of nucleobases continuing downstream of the 3'-terminus of the antisense compound and continuing until the oligonucleotide contains about 10 to about 50 nucleobases). It is also understood that compounds may be represented by oligonucleotide

compound, and may extend in either or both directions until the oligonucleotide contains about 10 to about 50 nucleobases. The compounds described herein are specifically hybridizable to the target nucleic acid.

[0037] One having skill in the art armed with the antisense compounds illustrated herein will be able, without undue experimentation, to identify further antisense compounds.

Hybridization

[0038] As used herein, "hybridization" means the pairing of complementary strands of antisense compounds to their target sequence. While not limited to a particular mechanism, the most common mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleobases). For example, the natural base adenine is complementary to the natural nucleobases thymidine and uracil which pair through the formation of hydrogen bonds. The natural base guanine is complementary to the natural bases cytosine and 5-methyl cytosine. Hybridization can occur under varying circumstances.

[0039] An antisense compound is specifically hybridizable when there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and under conditions in which assays are performed in the case of in vitro assays.

[0040] As used herein, "stringent hybridization conditions" or "stringent conditions" refers to conditions under which an antisense compound will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances, and "stringent conditions" under which antisense compounds hybridize to a target sequence are determined by the nature and composition of the antisense compounds and the assays in which they are being investigated.

Complementarity

[0041] "Complementarity," as used herein, refers to the capacity for precise pairing between two nucleobases on either two oligomeric compound strands or an antisense compound with its target nucleic acid. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be a complementary position.

[0042] "Complementarity" can also be viewed in the context of an antisense compound and its target, rather than in a base by base manner. The antisense compound and the further DNA or RNA are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleobases which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleobases such that stable and specific binding occurs between the antisense compound and a target nucleic

antisense compound. The invention is therefore directed to those antisense compounds that may contain up to about 20% nucleotides that disrupt base pairing of the antisense compound to the target. Preferably the compounds contain no more than about 15%, more preferably not more than about 10%, most preferably not more than 5% or no mismatches. The remaining nucleotides do not disrupt hybridization (e.g., universal bases).

[0043] It is understood in the art that incorporation of nucleotide affinity modifications may allow for a greater number of mismatches compared to an unmodified compound. Similarly, certain oligonucleotide sequences may be more tolerant to mismatches than other oligonucleotide sequences. One of the skill in the art is capable of determining an appropriate number of mismatches between oligonucleotides, or between an oligonucleotide and a target nucleic acid, such as by determining melting temperature.

Identity

[0044] Antisense compounds, or a portion thereof, may have a defined percent identity to a SEQ ID NO, or a compound having a specific Isis number. As used herein, a sequence is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in the disclosed sequences of the instant invention would be considered identical as they both pair with adenine. This identity may be over the entire length of the oligomeric compound, or in a portion of the antisense compound (e.g., nucleobases 1-20 of a 27-mer may be compared to a 20-mer to determine percent identity of the oligomeric compound to the SEQ ID NO.) It is understood by those skilled in the art that an antisense compound need not have an identical sequence to those described herein to function similarly to the antisense compound described herein. Shortened versions of antisense compound taught herein, or non-identical versions of the antisense compound taught herein fall within the scope of the invention. Non-identical versions are those wherein each base does not have the same pairing activity as the antisense compounds disclosed herein. Bases do not have the same pairing activity by being shorter or having at least one abasic site. Alternatively, a non-identical version can include at least one base replaced with a different base with different pairing activity (e.g., G can be replaced by C, A, or T). Percent identity is calculated according to the number of bases that have identical base pairing corresponding to the SEQ ID NO or antisense compound to which it is being compared. The non-identical bases may be adjacent to each other, dispersed through out the oligonucleotide, or both.

[0045] For example, a 16-mer having the same sequence as nucleobases 2-17 of a 20-mer is 80% identical to the 20-mer. Alternatively, a 20-mer containing four nucleobases not identical to the 20-mer is also 80% identical to the 20-mer. A 14-mer having the same sequence as nucleobases 1-14 of an 18-mer is 78% identical to the 18-mer. Such calculations are well within the ability of those skilled in the art.

[0046] The percent identity is based on the percent of nucleobases in the original sequence present in a portion of the modified sequence. Therefore, a 30 nucleobase antisense compound comprising the full sequence of the complement of a 20 nucleobase active target segment would have a portion of 100% identity with the complement of the 20 nucleobase

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.