

**Filed On Behalf Of:**

Alnylam Pharmaceuticals, Inc.

**By:**

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212-218-2100

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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ALNYLAM PHARMACEUTICALS, INC.,  
Petitioner,

v.

SILENCE THERAPEUTICS GMBH,  
Patent Owner.

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Case PGR2018-\_\_\_\_\_  
U.S. Patent No. 9,790,501

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**DECLARATION OF JONATHAN K. WATTS, PH.D.**

ALNYLAM EXH. 1002  
PGR2018-\_\_\_\_\_

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I, Jonathan K. Watts, Ph.D., declare as follows:

**I. Engagement**

1. I have been retained by the law firm Fitzpatrick, Cella, Harper & Scinto on behalf of Alnylam Pharmaceuticals, Inc. (“Alnylam”) as an independent expert to provide my opinions on the subject matter recited in the claims of U.S. Patent No. 9,790,501 (“the ’501 patent”) (Exh. 1001).

2. I understand that Alnylam is petitioning the Patent Trial and Appeal Board (“PTAB”) to institute a post-grant review of the ’501 patent and requests that the PTAB cancel all of the claims of the ’501 patent due to lack of written description, enablement, and/or novelty.

3. I have been asked to consider and provide my opinions regarding the ’501 patent claims, including the technical subject matter of the ’501 patent and the application of various references that are prior art to the ’501 patent. In particular, I have been asked to consider what a person of ordinary skill in the art would have understood from the ’501 patent, whether the underlying application or the priority patent applications demonstrate that the inventors were in possession of the invention now claimed in the ’501 patent, whether the underlying application or the priority patent applications teach a person of ordinary skill in the art how to make and use the claimed invention without undue experimentation, and whether certain prior art references disclose the features recited in the ’501 patent claims.

4. I make this declaration based upon my own personal knowledge. I am over the age of 21 and otherwise competent to make this declaration.

## **II. Background and Qualifications**

5. I received my B.Sc. degree from Dalhousie University in 2001, winning the university medal in chemistry. I carried out my Ph.D. at McGill University (Montreal, Canada) in the chemistry of therapeutic oligonucleotides (2002-2008), working with Prof. Masad Damha. Upon finishing my Ph.D., I sought to increase the depth and breadth of my expertise by learning how to work at the interface of chemistry, biology, and pharmacology. To achieve this, I took up successive fellowships from FQRNT (Quebec funding agency) and NSERC (Canadian funding agency) in Prof. David Corey's group at the University of Texas Southwestern Medical Center. My diverse training has prepared me to lead interdisciplinary research in the chemical biology and therapeutic application of oligonucleotides. During my training, I won six competitive fellowships and over fifteen academic awards.

6. In July 2012, I began my independent career at the University of Southampton, UK, which has been ranked in the top five chemistry departments in the UK. I was promoted to associate professor after only two and a half years. In September 2015, I was recruited by the RNA Therapeutics Institute at the University of Massachusetts Medical School (Worcester, MA), and moved along

with four members of my research group. My colleagues include Craig Mello, Philip Zamore, and Victor Ambros, all pioneers in the discovery of RNA interference.

7. I have published 35 papers that have been cited over 1500 times (H-index = 20). I have filed five patents, including a 2011 patent on small (or short) interfering RNA (“siRNA”) platform technology that was licensed by Labopharm Inc. My publications and patents span the field of oligonucleotide therapeutics from basic chemistry to applications in biological research and therapeutics. I am on the Board of Directors of the Oligonucleotide Therapeutics Society and have helped organize five international scientific conferences. All of this helps me stay at the cutting edge of this field.

8. Based on my experience, I have developed considerable expertise in the field of RNA interference (“RNAi”). I have extensive education, experience, and expertise in designing, synthesizing, and analyzing nucleic acid molecules, including siRNAs. My areas of expertise include organic chemistry, nucleic acid chemical biology, and modulation of gene expression in cells and organisms with an emphasis on RNAi therapeutics. Through collaborations with industrial and academic partners, and close relationships with other experts in RNAi therapeutics, I have developed considerable expertise in designing synthetic nucleic acids, such as antisense oligonucleotides, double-stranded siRNAs, and single-stranded



siRNAs.

9. The primary focus of my research has been the design and analysis of synthetic nucleic acids with a variety of modifications to improve stability, potency and specificity. I have worked on nucleic acid modifications and gene silencing since at least 2002. Among other areas, I have conducted research aimed at gene silencing of hereditary susceptibility, such as asthma, and developing new approaches to oligonucleotide delivery.

10. As an early stage investigator, I already have an excellent track record in mentoring and teaching. I have trained three Ph.D. students to completion, with one more student set to complete his Ph.D. in the coming year. I have also trained thirteen undergraduates, six of whom have gone on to graduate studies (including at Oxford and McGill, and one who remained in my group). My first Ph.D. student to graduate, Hannah Pendergraff, was immediately hired by Roche (Copenhagen). I was nominated for four teaching awards for excellence in student support and won the Vice Chancellor's teaching award at the University of Southampton in 2014-2015.

11. A more detailed account of my work experience and other qualifications is listed in my Curriculum Vitae (Exh. 1003). Awards and distinctions I have received, organizations and committees on which I have served, teaching responsibilities I have had, graduate students and fellows I have trained,

invited lectures that I have presented, publications that I have authored or co-authored, and patents I have filed are also listed on my Curriculum Vitae.

### **III. Compensation**

12. I am being compensated for my time spent in connection with this matter at a rate of \$600 per hour. My compensation does not depend on the outcome of this proceeding or the conclusions in this declaration.

### **IV. Materials Reviewed**

13. In forming my opinions, I have reviewed material that includes the '501 patent, its prosecution history, and prior art to the '501 patent including:

- a) Allerson C.R. *et al.*, *Fully 2'-Modified Oligonucleotide Duplexes with Improved in Vitro Potency and Stability Compared to Unmodified Small Interfering RNA*, J. Med. Chem. 48:901-04 (2005) ("Allerson 2005") (Exh. 1016);
- b) Choung S. *et al.*, *Chemical Modification of siRNAs to Improve Serum Stability without Loss of Efficacy*, Biochemical and Biophysical Research Communications, 342:919-27 (2006) ("Choung 2006") (Exh. 1072);
- c) Podbevsek P. *et al.*, *Solution-State Structure of a Fully Alternately 2'-F/2'-OMe modified 42-nt dimeric siRNA Construct*, Nucleic Acids Research, 38(20):7298-307 ("Podbevsek 2010") (Exh. 1082); and

d) The other patents, publications, and materials discussed below.

14. A full list of the materials cited in my declaration is included as

## **Appendix A.**

### **V. Summary of My Opinions**

15. The '501 patent claims a vast genus of structurally diverse, chemically-modified double-stranded siRNA molecules comprising contiguous alternating 2'-O-alkyl modified ribonucleotides alternating with unmodified or differently modified ribonucleotides on both strands, and having varied duplex architectures, including blunt ends or overhangs on one or both ends.

16. However, the '501 patent specification discloses about 25 examples of blunt ended double-stranded siRNA molecules having internal ribonucleotide modifications on one or both strands, all of which are of the *same type*: those having the *same* ribonucleotide modification and pattern, 2'-O-methyl modified ribonucleotides, with the remaining ribonucleotides being *unmodified*, and the *same* duplex architecture (*i.e.*, blunt ended on both ends). The '501 patent specification fails to disclose (i) any modified siRNA molecules having a modification pattern comprising a *2'-O-alkyl* modification other than 2'-O-methyl for the modified ribonucleotides as encompassed by at least claims 1-9, 12, 14, 16, 18, 20, 23, and 24; (ii) any modified siRNA molecules having *two different* modifications, including a *2'-fluoro* modification, as encompassed by at least

claims 1-10, 12, 13, 16-20, 22, and 24-30; (iii) any modified siRNA molecules having a ***mixed*** modification pattern, *i.e.*, modified ribonucleotides alternating with both unmodified and differently modified ribonucleotides as encompassed by at least claims 1-10, 12, and 20; (iv) any modified siRNA molecules having ***overhangs*** as encompassed by claims 1 and 3-30; (v) any modified siRNA molecules having duplex stretch lengths ***greater than 21 nucleotides*** as encompassed by claims 1-30; (vi) any modified siRNA molecules having a duplex stretch length of ***17 nucleotides*** as encompassed by claims 1-30 that have RNAi activity, and which the specification states is outside the minimum structural requirement of 18-19 nucleotides for RNAi activity; and (vii) any ***structure-activity relationships*** to guide in the design of those claimed siRNA molecules.

17. Combined with the lack of predictability of the effect of chemical modifications on RNAi activity and stability of siRNA molecules, a person of ordinary skill in the art would understand that the inventors were not in possession of the genus of modified siRNA molecules encompassed by the '501 patent claims.

18. For many of the same reasons, the '501 patent specification fails to provide any guidance that would allow a person of ordinary skill in the art to practice the claimed genus of siRNA molecules without undue experimentation.

19. Like the '501 patent disclosures, the '501 patent priority applications fail to disclose or provide any guidance for, among other things, (i) any modified

siRNA molecules having contiguous alternating 2'-O-alkyl modified ribonucleotides with a modification other than a 2'-O-methyl modification; (ii) any modified siRNA molecules having two different modifications; (iii) any modified siRNA molecules having a mixed modification pattern, *i.e.*, modified ribonucleotides alternating with both unmodified and differently modified ribonucleotides; (iv) any modified siRNA molecules having a duplex length of 17 ribonucleotides that have RNAi activity; (v) any modified siRNA molecules having a duplex length greater than 21 ribonucleotides; and (vi) any modified siRNA molecules having overhangs. A person of ordinary skill in the art would understand that the priority applications fail to describe or enable the genus of modified siRNA molecules encompassed by the '501 patent claims.

20. Further, the prior art discloses siRNA molecules that meet all of the limitations of claims 1-5, 7, 9-27, and 29 of the '501 patent.

21. For example, Allerson 2005 discloses an siRNA molecule that meets all of the limitations of claims 1, 2, 9, 10, 12, 13, 16-20, 22, 24, and 25 of the '501 patent.

22. Likewise, Choung 2006 discloses an siRNA molecule that meets all of the limitations of claims 1, 3-5, 7, 9-11, 14, 15, 20, 21, and 23 of the '501 patent.

23. Further, Podbevsek 2010 discloses an siRNA molecule that meets at least the structural limitations of claims 1, 16, 19, 26, 27, and 29 of the '501 patent.

## **VI. Legal Standards**

24. The opinions given in this declaration are based on my education, training, experience, and the content of the materials I considered. My understanding of the relevant law, as discussed below, is based on discussions I had with Petitioner's counsel.

### **A. Burden of Proof**

25. I understand from counsel that a post-grant review may be instituted if Petitioner shows it is more likely than not that at least one claim of the challenged patent is unpatentable. I understand Petitioner bears the ultimate burden of proving the challenged claims are unpatentable by a preponderance of the evidence.

### **B. Prior Art**

26. I understand from counsel that the "effective filing date" of a patent claim is the actual filing date of the patent, unless the patent properly claims the benefit of an earlier filing date from a parent application that discloses the claimed invention in compliance with the written description and enablement requirements.

27. It is my understanding that patents, published patent applications, and other printed publications that were published before the effective filing date of a patent are prior art to a patent in a post-grant review proceeding.

28. It is my understanding that the effective filing date of the claims of the '501 patent is May 8, 2017, the actual filing date of the '501 patent. Therefore, it is my understanding that any patent or publication published before May 8, 2017 is

prior art to the '501 patent.

**C. Anticipation**

29. I understand from counsel that for a prior art reference to anticipate a patent claim, all of the limitations of the claim must be disclosed in a single prior art reference. I also understand that if any claim limitation is missing from the prior art reference, then the prior art reference does not anticipate that claim. I further understand that it is improper to rely upon additional prior art to supply any claim limitations that are missing from the allegedly anticipating prior art reference.

**D. Written Description**

30. I understand from counsel that a claim is supported by the written description of a patent application only when that application reasonably conveys to one of ordinary skill in the art that the inventor had possession of the full scope of the claimed subject matter as of the filing date. I understand that original claims, as filed with the application, are part of the original disclosure. I also understand that the written description requirement can be satisfied even if the application does not describe the claimed invention using the same language as the claim. However, I understand that a claim that is obvious in view of the application does not necessarily have written description support. I understand that a claim to a combination of elements must be supported by a description of that

combination in the specification, not merely a description of individual elements.

31. I understand from counsel that when claims are to a genus, applicant must disclose a representative number of species falling within the scope of the generic claim or structural features common to the members of the genus so that one of ordinary skill in the art can recognize the members of the genus. I also understand that when there is substantial variation within a genus, the applicant must describe a sufficient variety of species to reflect this variation. I understand that for inventions in an unpredictable art, adequate written description of a genus that embraces widely variant species cannot be achieved by disclosing only one species within the genus.

32. I understand from counsel that when a patent claims a genus using functional language to define a desired result, the specification must show that the applicant has invented a generic invention that achieves the claimed result, and must do so by showing that the applicant has invented species sufficient to support a claim to the functionally defined genus. I understand that mere description of the function or result achieved by an invention is not enough to show possession.

#### **E. Enablement**

33. I understand from counsel that a patent specification must teach one of ordinary skill in the art how to make and use the claimed invention without undue experimentation. Whether undue experimentation would be required is



evaluated by considering factors such as (1) the breadth of the claims; (2) the nature of the invention; (3) the state of the prior art; (4) the level of ordinary skill in the relevant art; (5) whether the relevant art is predictable; (6) the amount of direction provided by the inventor; (7) the existence of working examples; and (8) the amount of experimentation necessary to make and use the claimed invention. I understand that the fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation.

34. I understand from counsel that to meet the how-to-use requirement, the specification must establish that the claimed invention achieves its intended purpose, *i.e.*, is useful or has a practical utility.

35. I understand from counsel that in the unpredictable arts, especially those that involve new and poorly understood technology, the specification is required to give more guidance to one of ordinary skill in the art to enable the full scope of the claims.

36. I understand from counsel that it is the specification, not the knowledge of one of ordinary skill in the art that must supply the novel aspects of an invention to meet the enablement requirement.

## **VII. Person of Ordinary Skill in the Art (“POSA”)**

37. I understand from counsel that the person of ordinary skill in the art is a hypothetical person who is presumed to be familiar with the relevant scientific

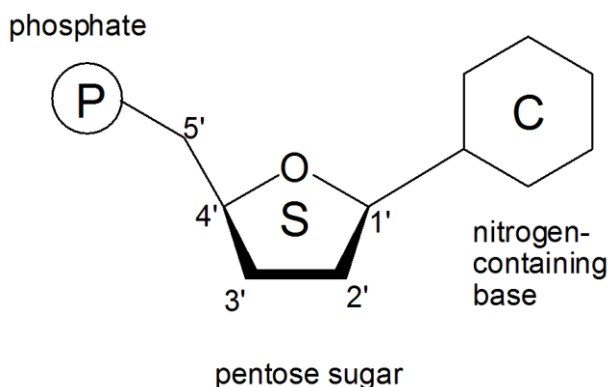
field and its literature at the time of the invention. This hypothetical person is also a person of ordinary creativity who is capable of understanding the scientific principles applicable to the pertinent field.

38. The '501 patent is generally directed to interfering ribonucleic acid molecules that are useful for inhibiting the expression of a target gene. Exh. 1001 ('501 patent) at col. 1:40-49. It is my opinion that a person of ordinary skill in the art in the field of the '501 patent, at all times between August 5, 2002 (the filing date of the European application listed on the face of the patent) and May 8, 2017 (the filing date of the '501 patent), would possess the following qualifications: (a) a Ph.D. in chemistry, medicinal chemistry, biochemistry, molecular biology, molecular pharmacology, or a closely related discipline, (b) a M.S. degree in chemistry, medicinal chemistry, biochemistry, molecular biology, molecular pharmacology, or a closely related discipline, with at least two years of practical experience in the field of RNAi, or (c) a bachelor's degree in chemistry, biochemistry, or a closely related discipline, with post-graduate work relating to RNAi. This level of education and experience would have been ordinary at all times between 2002 and 2017.

## VIII. Scientific and Technological Tutorial

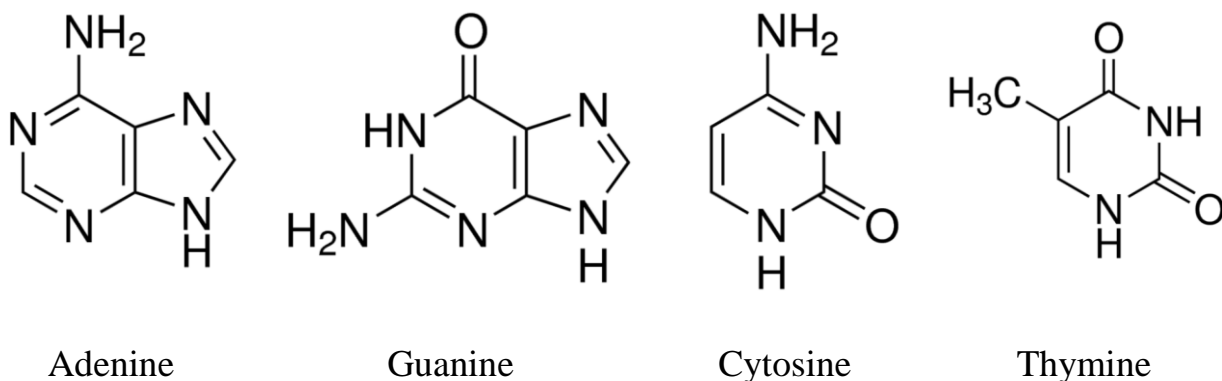
### A. Nucleotide Chemistry

39. Nucleotides are the building blocks of nucleic acids. They are molecules that chained together, form deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleotides are composed of three subunits: (i) a nitrogenous base, (ii) a five-carbon (pentose) sugar, and (iii) at least one phosphate group. Exh. 1059 (Lodish H. *et al.*, Chapter 4, Nucleic Acids, the Genetic Code and the Synthesis of Macromolecules, Molecular Cell Biology (4th ed. 2000)) (“Lodish”) at p. 101.



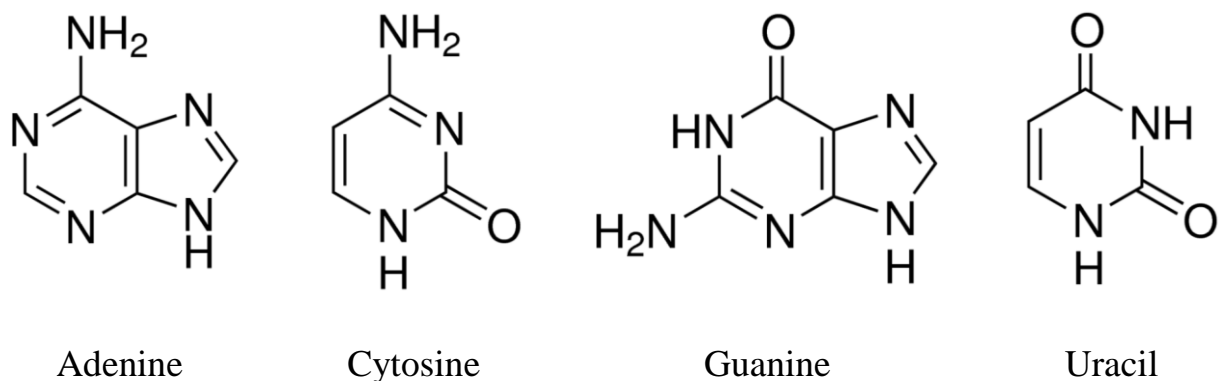
**Figure 1: A Nucleotide Unit**

40. DNA consists of the four nitrogenous bases adenine, thymine, guanine, and cytosine. The two purines—adenine (A) and guanine (G)—are double-ring systems, while the two pyrimidines—cytosine (C) and thymine (T)—are single-ring systems. Exh. 1059 (Lodish) at pp. 101-02.



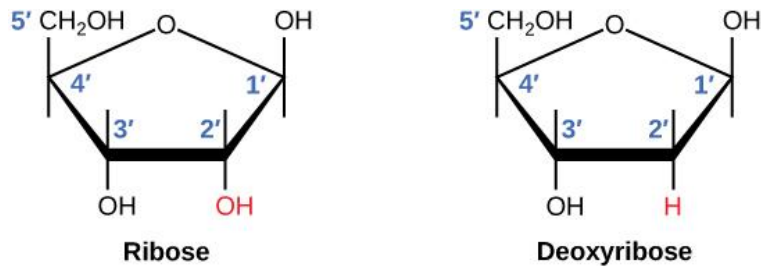
**Figure 2: Nitrogenous Bases in DNA**

41. RNA consists of three of the nitrogenous bases found in DNA—adenine (A), cytosine (C), and guanine (G)—plus uracil (U). Exh. 1059 (Lodish) at pp. 101-02.



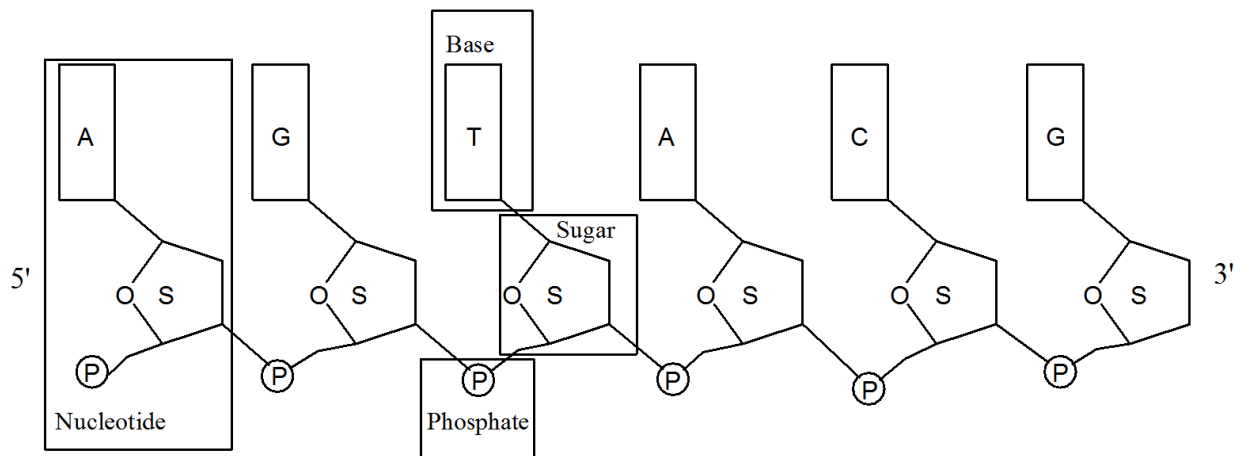
**Figure 3: Nitrogenous Bases in RNA**

42. The pentose sugar contained in RNA is ribose while the sugar contained in DNA is deoxyribose. The difference between the ribose sugar and deoxyribose sugar is a hydroxyl ( $\text{OH}$ ) group at the 2'-position of ribose as opposed to a deoxy ( $\text{H}$ ) group at the 2'-position of deoxyribose. Exh. 1059 (Lodish) at p. 101.



**Figure 4: Pentose Sugars in RNA and DNA**

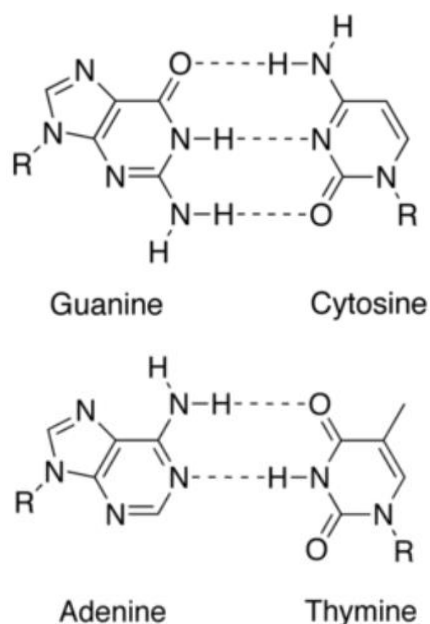
43. The strand of nucleotides comprising DNA or RNA is linked via phosphodiester bonds (also known as sugar-phosphate bonds). Strands of DNA and RNA are directional and are typically referred to as having a 5'-end and a 3'-end. The 5'-end has a terminal phosphate group or a terminal hydroxyl group, while the 3'-end typically has a terminal hydroxyl group. Exh. 1059 (Lodish) at pp. 102-03.



**Figure 5: Strand of Polynucleotides**

44. The nitrogenous bases found in one strand of nucleotides will interact with the nitrogenous bases in a complementary strand of nucleotides via hydrogen bonding. This interaction between the two nitrogenous bases of the two strands is

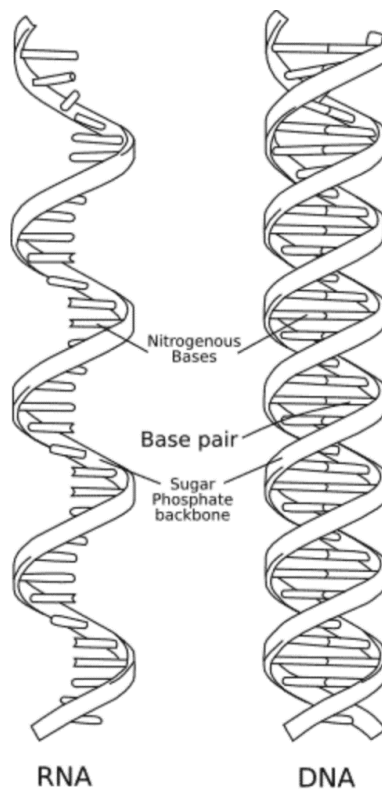
called base pairing and will only occur between two bases that are complementary: one purine and one pyrimidine. The complementary base pairs are adenine-thymine and guanine-cytosine in DNA, and adenine-uracil and guanine-cytosine in RNA (or in hybrid DNA-RNA pairing). Exh. 1059 (Lodish) at pp. 103-04 (showing a guanine-cytosine base pair with three hydrogen bonds and an adenine-thymine base pair with two hydrogen bonds).



**Figure 6: Base Pairing**

45. DNA found in the body is typically double-stranded wherein the two nucleotide strands are held together in a double helix form through hydrogen bonding between the complementary base pairs of the two strands. RNA is typically found in its single-strand form, but these single-stranded RNA molecules typically adopt complex secondary structures that often include “hairpin” or “stem loop” structures in which the RNA folds back on itself, as well as base pairing with

other RNA molecules. Exh. 1059 (Lodish) at pp. 103-05, 108-09.



**Figure 7: Typical Architecture of RNA and DNA**

46. DNA in the body serves various essential roles in the coding, decoding, regulation, and expression of genes. When a cell creates certain proteins, the portion of DNA that codes for that protein will create copies of the target gene in the form of messenger RNA (mRNA). This mRNA will then synthesize the protein through the use of ribosomes found in the cell. Exh. 1059 (Lodish) at pp. 110-13.



**Figure 8: Protein Synthesis Pathway**

## **B. RNA Interference**

47. In 1998, the first paper reporting that injection of double-stranded RNA (dsRNA) can lead to sequence-specific posttranscriptional gene silencing was published. Exh. 1058 (Fire A. *et al.*, *Potent and Specific Genetic Interference by Double-Stranded RNA in Caenorhabditis elegans*, *Nature* 391:806-11 (1998)). This effect, named RNA interference (RNAi), is a cellular mechanism for gene silencing in which double-stranded RNA (dsRNA) triggers cleavage of mRNA molecules via endogenous cellular machinery. Exh. 1057 (Harborth J. *et al.*, *Identification of Essential Genes in Cultured Mammalian Cells Using Small Interfering RNAs*, *J. Cell Sci.* 114:4557-65 (2001)) at p. 4557.

48. Shortly after, in 2001, it was discovered that the same effect could be produced in mammalian cells using synthetic short RNA duplexes. Exh. 1042 (Elbashir S.M. *et al.*, *Duplexes of 21-Nucleotide RNAs Mediate RNA Interference in Cultured Mammalian Cells*, *Nature* 411:494-98 (2001)) (“Elbashir 2001”). These first short RNAi triggers were 21-nucleotide double-stranded RNAs aligned such that each strand has a two-nucleotide overhang at the 3'-end and were referred to as siRNAs. Exh. 1042 (Elbashir 2001) at pp. 494-95.

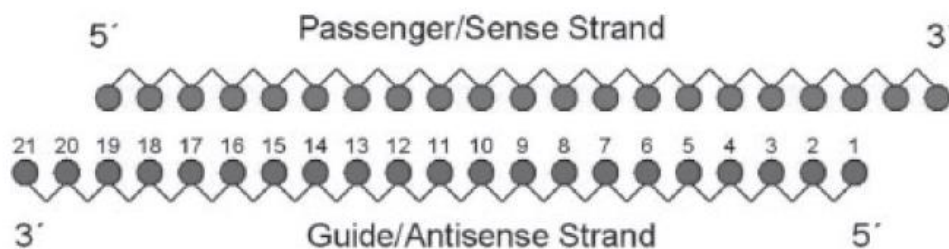
49. The discovery of RNAi and siRNA led to significant research in the field with the ultimate goal of clinical application for a wide range of conditions.



**i. siRNAs**

50. As mentioned above, siRNAs are a type of molecule that exhibits RNA interference—the ability to knock down the expression of a gene also called “gene silencing.” Exh. 1035 (Deleavey G.F. *et al.*, *Chemical Modification of siRNA*, Current Protocols in Nucleic Acid Chemistry, 39(suppl):16.3.1-22 (2009)) (“Deleavey 2009”) at p. 16.3.1. Gene silencing is a process whereby the expression of RNA is halted. The mechanism of RNAi begins with a double-stranded RNA—which can include a number of different RNA structures including siRNA, long dsRNA, and hairpin RNA—which is phosphorylated by Clp-1, a cellular kinase. The siRNA duplexes then interact with other proteins in the cell to form an RNA-induced silencing complex (RISC). Exh. 1035 (Deleavey 2009) at pp. 16.3.2-3.

51. The siRNA duplex has two strands: the antisense (guide) strand and the sense (passenger) strand:

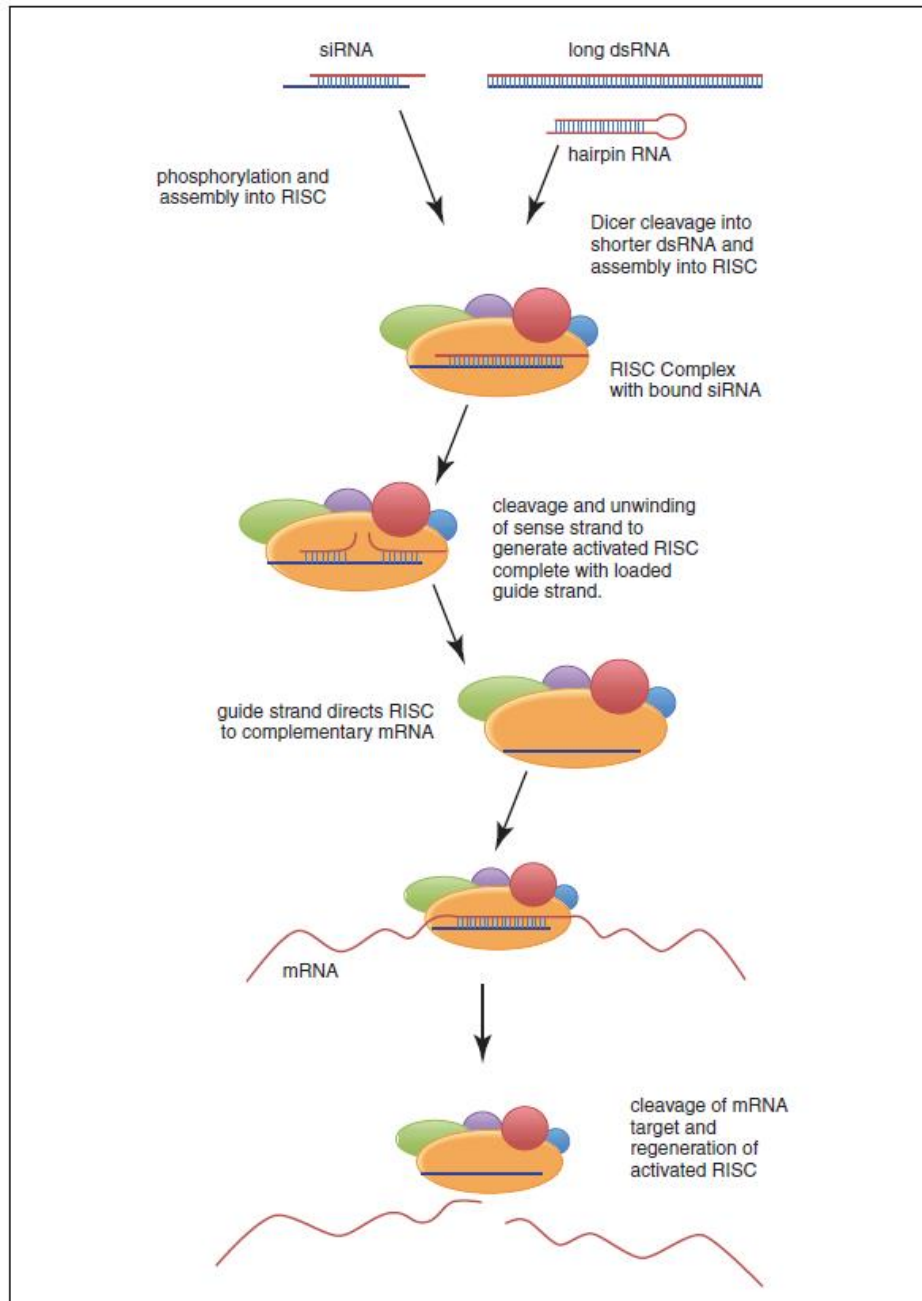


**Fig. 1.** Structure of short interfering RNA (siRNA). Both guide and passenger strands are displayed and the guide strand nucleotides are numbered in 5' to 3' direction.

Exh. 1056 (Klingelhoefer J.W. *et al.*, *Approximate Bayesian Feature Selection on a Large Meta-Dataset Offers Novel Insights on Factors That Affect siRNA Potency*, *Bioinformatics* 25(13):1594-601 (2009)) at p. 1594, Fig. 1.

52. After the siRNA duplex is loaded into the RISC complex, the sense (passenger) strand is cleaved or unwound from the antisense (guide) strand. The antisense strand, which is the strand loaded into the RISC complex, guides the RISC complex to the target mRNA. The RISC complex with the bound antisense strand then cleaves the target mRNA, typically between bases 10 and 11 counting from the 5'-end of the antisense strand. The sense strand that is not loaded into the RISC complex due to its complementary nature with the antisense strand should have the same sequence as the target mRNA. The sequence that is chosen for the sense and antisense strands is specific to the gene that is sought to be silenced.

Exh. 1035 (Deleavey 2009) at pp. 16.3.1-3; Exh. 1053 (Sharp P.A., *RNA Interference-2001*, *Genes & Development* 15:485-90 (2001)) at pp. 485-86; Exh. 1054 (Waterhouse P.M. *et al.*, *Gene Silencing as an Adaptive Defence Against Viruses*, *Nature* 411:834-42 (2001)) at pp. 834-37; Exh. 1055 (Tuschl, T. and Borkhardt, A., *Small Interfering RNAs: A Revolutionary Tool for the Analysis of Gene Function and Gene Therapy*, *Mol. Interventions* 2:158-67 (2002)) at p. 159.



**Figure 16.3.1** The mechanism of RNAi in human cells triggered by exogenously introduced dsRNAs. The largest of the ellipses represents AGO2, the catalytic engine of RISC. Adapted from Watts et al. (2008), with permission from Elsevier.

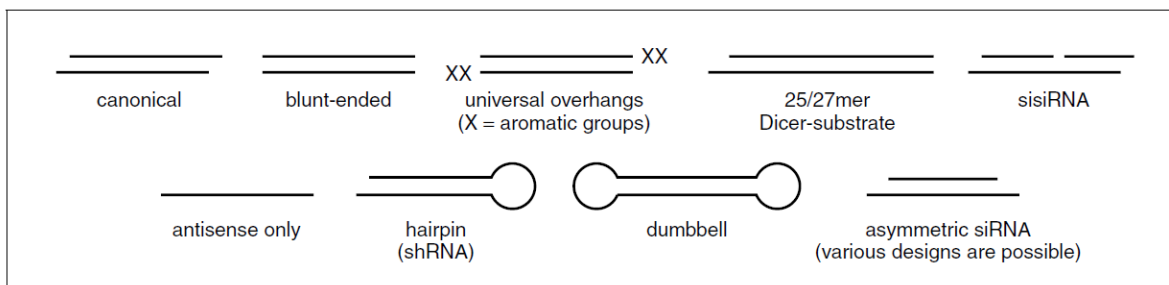
Exh. 1035 (Deleavey 2009) at Fig. 16.3.1 (depicting mechanism of RNAi in mammalian cells).

53. The use of siRNA faces a number of significant obstacles including

issues with cellular uptake, rapid degradation of RNA strands, silencing of unintended genes, and potential immune responses. To solve these problems, a wide variety of chemical modifications to the siRNA strands have been proposed. Exh. 1035 (Deleavey 2009) at p. 16.3.1.

54. The design of an effective and specific siRNA that can overcome all of the obstacles involved in the use of siRNA requires the consideration of a great number of factors. The first factor often considered is the selection of the right sequence of nucleotides, which is extremely important to ensure efficacy against the target gene and to reduce off-target effects, *i.e.*, specificity. The process will often involve testing several duplexes to find an effective RNA, oftentimes 100 to 200 sequences, even after careful selection of possible sequences. Exh. 1035 (Deleavey 2009) at pp. 16.3.3-4.

55. As mentioned above, siRNA can take on a number of different architectures. This includes considerations such as the number of nucleotides and whether to include nucleotide overhangs or use “blunt ended” RNA molecules without overhangs. Other structural options include using a single-strand RNA in a hairpin or dumbbell configuration. All of these different options can have effects on an siRNA’s efficacy, stability, and susceptibility to off-target effects. Exh. 1035 (Deleavey 2009) at pp. 16.3.4-5.



**Figure 16.3.2** Functional siRNA architectures. The sense strand is always shown on top, in the 5' to 3' direction, and the antisense strand is on the bottom, in the 3' to 5' direction. Note that three of the structures (25/27mer, hairpin, and dumbbell) require the activity of Dicer before incorporation into RISC. Adapted from Watts et al. (2008), with permission from Elsevier.

Exh. 1035 (Deleavey 2009) at Fig. 16.3.2 (depicting functional siRNA architectures).

56. Another proposed solution for addressing siRNA's shortcomings is to make chemical modifications. Exh. 1035 (Deleavey 2009) at p. 16.3.5. There is an enormous variety of chemical modifications that can be made including modifications to the sugars, backbone linkages, and nucleotide bases. Exh. 1035 (Deleavey 2009) at pp. 16.3.5-11. While these modifications have great potential to improve stability, improve cellular uptake, reduce immunostimulation, and reduce off-target effects, accurately predicting which modifications will work is extremely difficult, or downright impossible, as effective modifications exhibit strong sequence dependence and can be beneficial in some regions but detrimental in others. Exh. 1035 (Deleavey 2009) at pp. 16.3.11-16. Due to the failure to find universal chemical modifications and to elucidate rules which allow reliable predictions, the selection of appropriate modifications was, and still remains, a time-consuming and arduous task. Exh. 1035 (Deleavey 2009) at p. 16.3.16.

## **IX. Technology at Issue**

### **A. The '501 Patent**

57. The '501 patent is entitled “Interfering RNA Molecules” and names Klaus Giese, Jörg Kaufmann, and Anke Klippel-Giese as inventors. Exh. 1001 ('501 patent) at Cover. The '501 patent issued on October 17, 2017 from U.S. Application No. 15/589,968, filed on May 8, 2017 (“the '968 application”). Exh. 1001 ('501 patent) at Cover. The '501 patent is a continuation of U.S. Patent Application Nos. 14/977,710 (“the '710 application”); 14/578,636 (“the '636 application”); 13/692,178 (“the '178 application”); 12/986,389 (“the '389 application”); 12/200,296 (“the '296 application”); and 10/633,630 (“the '630 application”). Exh. 1001 ('501 patent) at Cover. The '501 patent also claims priority to U.S. Provisional Application No. 60/402,541 (“the '541 provisional”), filed on August 12, 2002 and European Application Nos. 02017601 (“EP1”) and 03008383 (“EP2”), filed on August 5, 2002 and April 10, 2003, respectively. Exh. 1001 ('501 patent) at Cover.

58. The '501 patent is generally directed to “forms of interfering ribonucleic acid molecules having a double-stranded structure” and their uses “for inhibiting expression of a target gene, and pharmaceutical compositions, cells and organisms containing these molecules.” Exh. 1001 ('501 patent) at col. 1:40-49.

59. The '501 patent states in the Background of the Invention that “[o]ne

of the problems previously encountered when using unmodified ribooligonucleotides was the rapid degradation in cells or even in the serum-containing medium.” Exh. 1001 (’501 patent) at col. 2:20-23. The specification further states that “[i]t is apparent, therefore, that synthetic interfering RNA molecules that are both stable and active in a biochemical environment such as a living cell are greatly to be desired.” Exh. 1001 (’501 patent) at col. 2:29-31.

60. The Summary of the Invention states that “[i]t is therefore an object of the present invention to provide compositions and methods using interfering RNA molecules having enhanced stability.” Exh. 1001 (’501 patent) at col. 2:35-37.<sup>1</sup>

61. In the Detailed Description, the ’501 patent states that “[t]he present inventors have surprisingly found that small interfering RNAs can be designed that are both highly specific and active as well as stable under the reaction conditions typically encountered in biological systems such as biochemical assays or cellular environments.” Exh. 1001 (’501 patent) at col. 11:19-23. The ’501 patent states:

“The various interfering RNAs previously described by Tuschl et al. (see, for example, WO01/75164) provide for a length of 21 to 23 nucleotides and a modification at the 3’ end of the double-stranded RNA. The present inventors found that stability problems of interfering RNA, including small interfering RNA (siRNA) which is generally referred to herein in the following as RNAi, actually result from attack by endonucleases rather than exonucleases as

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<sup>1</sup> The statement in col. 2:35-37 does not appear in EP1, the ’541 provisional, or EP2.

previously thought. This surprising observation permitted the present inventors to develop the methods and compositions that are the subject of the present invention.”

Exh. 1001 (’501 patent) at col. 11:23-34; *see also* col. 26:38-41.

62. The ’501 patent also states that “[t]he skilled artisan will understand that all of the ribonucleic acids of the present invention ***are suitable to cause or being involved in methods of RNA mediated interference*** such as those described, for example, in WO 99/32619, WO 00/44895 and WO 01/75164.” Exh. 1001 (’501 patent) at col. 12:41-45 (emphasis added). The ’501 patent further states that the “[t]he ribonucleic acid molecules of the present invention have general applicability and permit knockdown or knockout of any desired coding nucleotide such as an mRNA, i.e., the expression of any gene producing an RNA may be modified.” Exh. 1001 (’501 patent) at col. 19:63-67.

63. The ’501 patent describes possible uses of the ribonucleic acids of the invention based on their RNAi activity “for inhibiting the expression of a target gene” (Exh. 1001 (’501 patent) at cols. 1:46-49, 6:60-67), “for target validation” (Exh. 1001 (’501 patent) at cols. 6:27-30, 19:67-20:8), as “compositions,” including “medicaments and pharmaceutical compositions” for the treatment of various disorders (Exh. 1001 (’501 patent) at cols. 6:31-42, 6:51-59, 20:29-52, 21:31-53), for preparing a “knockdown cell” or “knockdown organism” (Exh. 1001 (’501 patent) at cols. 6:43-50, 21:12-30), as “diagnostics” (Exh. 1001 (’501



patent) at col. 20:53-63), and for “screening of pharmaceutically active compounds and/or lead optimization” (Exh. 1001 (’501 patent) at cols. 20:64-21:11).

64. Based on the patent specification, a person of ordinary skill in the art would understand that the primary focus of the ’501 patent was to provide interfering RNA molecules, including siRNAs, that were both stable and active, *i.e.*, exhibited RNAi activity in a biological system, including in a living cell.

65. The ’501 patent specification generally describes various embodiments of double-stranded RNAi molecules, including those having chemical modifications. Exh. 1001 (’501 patent) at cols. 2:38-6:67, 11:35-21:56. The specification discloses a non-limiting list of possible modifications. In particular, for end modifications, the specification states that the “modifications include, but are not limited to, inverted (deoxy) abasics, amino, fluoro, chloro, bromo, CN, CF<sub>3</sub>, methoxy, imidazole, carboxylate, thioate, C<sub>1</sub> to C<sub>10</sub> lower alkyl, substituted lower alkyl, alkaryl or aralkyl, OCF<sub>3</sub>, OCN, O-, S-, or N-alkyl; O-, S-, or N-alkenyl; SOCH<sub>3</sub>; SO<sub>2</sub>CH<sub>3</sub>; ONO<sub>2</sub>; NO<sub>2</sub>, N<sub>3</sub>; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino, polyalkylamino or substituted silyl, as, among others, described in European patents EP 0 586 520 B1 or EP 0 618 925 B1.” Exh. 1001 (’501 patent) at col. 13:35-43.

66. Further, the ’501 patent specification states that the “modification may be attached to or replace any of the OH-groups of the ribose moiety, including but

not limited to the 2'OH, 3'OH and 5'OH position, provided that the nucleotide thus modified is a terminal nucleotide.” Exh. 1001 ('501 patent) at col. 14:24-28. The specification also discusses preferred embodiments in which “at least one nucleotide of the ribonucleic acid has a modification at the 2'-position and the modification is preferably selected from the group comprising amino, fluoro, methoxy, alkoxy and alkyl.” Exh. 1001 ('501 patent) at col. 3:64-67.

67. The specification refers to “patterns” of modifications on the ribonucleic acid molecules of the invention, comprising modified and unmodified or differently modified nucleotides. Exh. 1001 ('501 patent) at col. 7:11-45, Fig. 2; *see also* cols. 15:37-43, 16:25-67, 17:24-43. The specification states that end modifications can be added to non-terminal nucleotides to form a pattern of modified nucleotides on at least one of the strands and more particularly on one of the stretches of contiguous nucleotides of the ribonucleic acid molecules. Exh. 1001 ('501 patent) at col. 15:37-59. These non-terminal modifications are also non-limiting. As the specification explains “[t]he modification of the nucleotides may be any form of modification described herein, more particularly the kind of modification as described herein as end modification, except that the so-called end modification is not necessarily located at terminal nucleotides. Rather the modification may occur at a non-terminal nucleotide.” Exh. 1001 ('501 patent) at col. 15:50-56. The specification further defines “unmodified nucleotide” to mean

either unmodified or differently modified nucleotides. Exh. 1001 ('501 patent) at col. 16:47-52. With respect to the modification of the unmodified nucleotides, the '501 patent specification states that the modification can be the same or different for the various ribonucleotides:

“It is also within the present invention that the modification of the unmodified nucleotide(s) wherein such unmodified nucleotide(s) is/are actually modified in a way different from the modification of the modified nucleotide(s), *can be the same or even different for the various nucleotides forming said unmodified nucleotides....*”

Exh. 1001 ('501 patent) at col. 16:53-58 (emphasis added).

68. In describing the scope of the invention, the inventors explained that the siRNA duplexes should have a minimum length of 18 or 19 nucleotides. Exh. 1001 ('501 patent) at cols. 12:46-53, 26:14-18. The inventors state that siRNA molecules having a duplex length of 17 or less were not functional even with overhangs. Exh. 1001 ('501 patent) at cols. 24:49-56, 25:22-27.

69. As to the duplex architecture, the '501 patent specification generally states that the ribonucleic acid molecules of the invention may be blunt ended or may have an overhang of at least one nucleotide at the 5'- or 3'-end. Exh. 1001 ('501 patent) at cols. 2:38-49, 2:65-3:22, 3:33-37, 3:46-62, 4:57-5:17. The '501 patent specification identifies a “blunt-ended” design for the RNAi molecules of the present invention and states:

“A third strategy provided by the present invention involves a ribonucleic acid comprising a double-stranded structure ... where the double-stranded structure is **blunt-ended**. As used herein the term double-stranded structure also is referred to as duplex. **This design of RNAi clearly differs** from, e.g., that described by Tuschl et al. in WO 01/75164, **which contains a 3'-overhang**.”

Exh. 1001 ('501 patent) at cols. 14:56-15:1 (emphasis added)<sup>2</sup>; *see also* col. 11:23-27 (referring to prior art providing for duplex structures having a length of 21-23 nucleotides and a modification at the 3'-end of the double-stranded RNA). The patent specification defines “overhang” as referring to “a double-stranded structure where at least one end of one strand is longer than the corresponding end of the other strand (‘the counter strand’) forming the double-stranded structure.” Exh. 1001 ('501 patent) at col. 15:1-5.

70. The patent specification additionally states that the RNAi molecules of the present invention are preferably blunt ended or have a 5'-overhang. For example, the specification states that “the interfering ribonucleic acid having a pattern of modified nucleotides may have an end modification, an end modification scheme, may be **blunt ended or may have a 5' overhang** or any combination of two or more of these elements or characteristics.” Exh. 1001 ('501 patent) at cols. 15:65-16:2 (emphasis added), 25:22-27 (noting a minimum duplex length of 18-19

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<sup>2</sup> In EP1, the '541 provisional, and EP2, the inventors state that “[t]his [blunt-ended] design of RNAi **is thus clearly different** from e.g., the one of Tuschl et al as specified in international patent application WO 01/75164 **which discloses a 3'-overhang**.” *See e.g.*, Exh. 1008 (EP2) at p. 15:17-19; *see also* Section X.A. below.

nucleotides, “independent of the further design of the RNAi molecules such as *blunt end or 5'-overhang....*” (emphasis added)), *see also* cols. 14:55-15:36.

71. The '501 patent specification includes 13 examples and 19 figures, the teachings of which are summarized below. The '501 patent specification includes a sequence listing of 182 sequences and discloses about 120 siRNA molecules, including unmodified siRNA and those having end modifications, internal 2'-O-methyl modified ribonucleotides, or loop structures as discussed below.<sup>3</sup>

72. Example 1 reports the RNAi activity of siRNA molecules having a duplex length of 21 nucleotides and a two deoxy nucleotide overhang at the 3'-ends targeting PTEN and Akt mRNA that have either no end modifications or have either NH<sub>2</sub> (amino) caps or inverted abasic groups as end protection groups (*i.e.*, end caps) at the 3'-end. Exh. 1001 ('501 patent) at cols. 21:58-24:9, Fig. 3; *see also* Fig. 19. These studies showed that the inclusion of the amino cap or inverted abasic end modifications did not result in activity loss when located at the 3'-end of the strand. Exh. 1001 ('501 patent) at col. 23:46-54. Based on these studies, a person of ordinary skill in the art would understand that the inclusion of end modifications such as amino caps or inverted abasic modifications would be

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<sup>3</sup> Figures 3-18 of the '501 patent list a total of 118 siRNA molecules, of which 49 have internal 2'-O-methyl modified ribonucleotides on one or both strands. Of those, 25 are non-duplicative 2'-O-methyl modified siRNA molecules. *See Appendix B*. EP1 and the '541 provisional disclose fewer siRNA molecules.

tolerated by the siRNA molecule when located at the 3'-end.

73. Example 2 reports the RNAi activity of siRNA molecules having a duplex length of 21 nucleotides targeting PTEN mRNA that were either blunt ended or had deoxy overhangs at the 3'- and 5'-ends. Exh. 1001 ('501 patent) at col. 24:11-29, Fig. 4. The siRNA molecules did not include any modified ribonucleotides. These studies showed that the activity of siRNA molecules having blunt ends or 5'-overhangs was comparable to those having 3'-overhangs, leading the inventors to conclude that "3'-overhangs are not required for RNAi activity."<sup>4</sup> Exh. 1001 ('501 patent) at col. 24:23-29; *see also* col. 7:58-59 ("FIGS. 4A-4B show that the 3'-overhang of RNAi molecules is not important for RNA interference."). Based on these studies, a person of ordinary skill in the art would understand that blunt ended siRNA molecules may be effective at mediating RNAi.

74. Examples 3 and 4 report the effect of siRNA duplex length and the presence of mismatches on the RNAi activity of siRNA molecules targeting Akt and PTEN mRNA, with the siRNA molecules having 3'-overhangs (Figs. 5-6, 7A)

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<sup>4</sup> In EP1, the '541 provisional, and EP2, the inventors concluded in Example 2 that "3'-overhangs are *not essential* for RNAi activity." Exh. 1006 (EP1) at p. 31 (emphasis added); Exh. 1007 ('541 provisional) at p. 31; Exh. 1008 (EP2) at p. 34.

or blunt ends (Fig. 7B). Exh. 1001 ('501 patent) at cols. 24:31-26:21, Figs. 5-7.<sup>5</sup>

The siRNA molecules did not include any modified ribonucleotides. These studies led the authors to conclude that an siRNA duplex with a stretch of less than 17 matching base pairs was not functional or specific for a single gene. Exh. 1001 ('501 patent) at cols. 24:49-56, 25:22-27, 25:44-48. Based on these studies, a person of ordinary skill in the art would understand that siRNA molecules having a duplex length of 17 nucleotides or less *would not exhibit RNAi activity, would lead to undesirable off-target effects and would not be useful for RNAi*, and that a minimum duplex length of 18-19 nucleotides is required for an siRNA molecule to be useful for RNAi. Exh. 1001 ('501 patent) at cols. 25:22-27, 26:14-18.

75. Further, the inventors reported that a single point mutation in the center of the molecule severely affected RNAi activity. Exh. 1001 ('501 patent) at col. 26:2-6. Consistent with those understandings, the specification states:

“[T]he present invention reduces this off-target problem of siRNA by two approaches: first, *by reducing the molecule length of the siRNA molecules to the minimal requirements (18-19 nt)* and thereby reducing the chance of homology to off-targets, second, by inactivation of the sense strand to prevent an unwanted RNA silencing caused by accidental complementarity of the sense strand to an unrelated target RNA (see also Example 6).”

Exh. 1001 ('501 patent) at col. 26:14-21 (emphasis added).

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<sup>5</sup> Figures 7A-B and the corresponding disclosures in Examples 3 and 4 (cols. 24:42-48, 24:62-25:21, 25:40-43, 25:64-26:13) of the '501 patent do not appear in either EP1 or the '541 provisional. See e.g., Exh. 1006 (EP1) at Examples 3 and 4.

76. Example 5 reports the serum stability of siRNA molecules having a duplex length of 21 nucleotides that are unmodified and have blunt ends, 3'- or 5'-overhangs, or end modifications, compared to an siRNA molecule that is fully modified with internal 2'-O-methyl modifications on both strands and has a 3'-overhang on both strands. Exh. 1001 ('501 patent) at col. 26:23-59, Fig. 8. The example showed that a fully 2'-O-methyl modified siRNA with 2-deoxy overhangs at the 3'-ends (79A79B) and a blunt-ended unmodified siRNA (28A28B) had greater serum stability compared to other siRNA molecules. Exh. 1001 ('501 patent) at col. 26:31-34. Based on these studies, a person of ordinary skill in the art would understand that 2'-O-methyl modifications could confer higher serum stability.

77. Additionally, based on these studies, the inventors concluded that “a blunt duplex is *more stable* than the duplex molecule with overhangs.” Exh. 1001 ('501 patent) at col. 26:34-36 (emphasis added); *see also* Fig. 8A (demonstrating higher serum stability over time of blunt ended unmodified siRNA molecule 28A28B compared to its counterpart siRNA molecules 18A18B and 30A30B having deoxy overhangs at the 3'- and 5'-ends, respectively). A person of ordinary skill in the art would understand the '501 patent examples teach that overhangs would not be useful to protect the RNAi molecules from degradation. A person of ordinary skill in the art would also understand that the patent specification teaches



the use of a blunt-ended design to confer improved stability over the prior art that taught the use of 3'-overhangs to protect against degradation by exonucleases.

78. Example 6 reports the RNAi activity of siRNA molecules having a duplex length of 21 nucleotides and deoxy overhangs at the 3'- or 5'-ends targeting PTEN mRNA having various NH<sub>2</sub> (amino) end protection groups, *i.e.*, end cap modifications. Exh. 1001 ('501 patent) at cols. 26:61-27:37, Fig. 9. These studies showed that while RNAi activity was preserved when the amino end caps were included on the sense strand, the presence of an amino end cap modification on both the 3'- and 5'-ends or the 5'-end of the antisense strand resulted in a loss of RNAi activity. Exh. 1001 ('501 patent) at cols. 27:14-20, 27:30-37. Based on these studies, a person of ordinary skill in the art would understand that amino caps are tolerated on the 3'- and 5'-ends of the sense strand but not on the 5'-end of the antisense strand. A person of ordinary skill in the art would also understand that adding an amino end cap modification (which is typically linked to the terminal phosphate via a carbon chain) is not the same as replacing the OH group of the ribose moiety of the ribonucleotide with an amino group.

79. Example 7 reports the serum stability and RNAi activity of internal 2'-O-methyl modifications of blunt ended RNAi molecules having a duplex length of 21 nucleotides targeting PTEN mRNA. Exh. 1001 ('501 patent) at cols. 27:38-28:3, Fig. 10. These studies showed that internal 2'-O-methyl modifications

reduced RNAi activity and that siRNA molecules having 2'-O-methyl modified and unmodified nucleotides retained activity, while those having fully 2'-O-methyl modified nucleotides on the antisense strand were inactive. Exh. 1001 ('501 patent) at col. 27:51-60, Fig. 10A. Based on these studies, the inventors concluded:

“2'-O alkyl modifications stabilize RNAi molecules against degradation. This clearly beneficial effect, however, is at least to a certain degree *counterbalanced* by the effect that 2'-O-alkyl modifications generally result in a reduced knockdown activity. Accordingly, the design of RNAi molecules has to balance stability against activity which makes it important to be aware of the various design principles as disclosed in the present application.”

Exh. 1001 ('501 patent) at cols. 27:62-28:3 (emphasis added); *see also* Fig. 10B.

80. Based on these studies, a person of ordinary skill in the art would understand that balancing the competing effects that 2'-O-methyl modifications have on RNAi activity and serum stability is one of the major challenges in siRNA design, and that determining how those competing effects translate into useful siRNAs is an empirical exercise.

81. Examples 8-11 report the serum stability and RNAi activity of siRNA molecules having a duplex length of 21 nucleotides (Figs. 11-15) or 19 nucleotides (Fig. 16) targeting PTEN, Akt, or p110 $\beta$  mRNA that are all blunt ended, comprising blocks of 2'-O-methyl modified and unmodified nucleotides across one or both strands, or as single 2'-O-methyl modified and unmodified nucleotides

within a strand. Exh. 1001 ('501 patent) at cols. 28:5-32:30, Figs. 11-16.<sup>6</sup> In particular, Examples 9-11 evaluated the serum stability and RNAi activity of blunt ended siRNA molecules having “alternating” single 2'-O-methyl modified and unmodified nucleotides across both strands. Exh. 1001 ('501 patent) at cols. 28:53-32:30, Figs. 12-16. Example 11 (referring to Figure 15) states that “[t]he most efficient molecules inducing PTEN mRNA had only stretches of modifications leaving the 5' end unmodified or were modified on alternating positions on both strands (FIG. 15A, molecules V10, V12).” Exh. 1001 ('501 patent) at col. 30:57-61. Example 11 also states that “[t]aken together the data shown herein demonstrate that 2'-O-methyl modifications at particularly selected positions in the siRNA duplex can increase nuclease resistance and do not necessarily abolish RNAi completely.” Exh. 1001 ('501 patent) at col. 31:23-26, Fig. 15 (comparing stability and RNAi activity of molecules V13, V14, V15, and V12).<sup>7</sup>

82. Based on these studies, a person of ordinary skill in the art would

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<sup>6</sup> Neither EP1 nor the '541 provisional includes Figures 15-16 or their corresponding disclosures in cols. 30:40-32:30 of the '501 patent. Thus, there is no disclosure in these applications of any siRNA molecules having a duplex length of 19 nucleotides that have alternating 2'-O-methyl modifications.

<sup>7</sup> The '501 patent includes two additional examples, Examples 12 and 13, which report the effect of different loop structures on RNAi activity of unmodified siRNA having a 21-nt duplex length. Exh. 1001 ('501 patent) at cols. 32:31-34:23, Figs. 17-18. These studies showed that the loop structures had no significant effect on knock-down efficiency. Exh. 1001 ('501 patent) at cols. 32:50-56, 33:4-9.

understand that 2'-O-methyl modifications at particularly selected positions along the siRNA molecule can increase stability but can also cause a reduction in RNAi activity. Exh. 1001 ('501 patent) at cols. 27:51-66, 28:23-47, 29:3-11, 29:33-37, 30:40-61, 31:3-20, 31:60-63, 32:4-18. A person of ordinary skill in the art would also understand that “highly specific interactions between the involved enzymes and precise nucleotides in the siRNA duplex” (Exh. 1001 ('501 patent) at col. 31:21-23) make predicting the competing properties of increased serum stability and decreased RNAi activity resulting from the type of nucleotide modification, the nature and extent of the modification, and the position of the modification both empirical and sequence specific.

83. Finally, a person of ordinary skill in the art would understand from the '501 patent specification, that every modification needs to be carefully considered as each modification can impact siRNA serum stability or RNAi activity or both, depending on the type of modification, the nature and extent of the modification, the position of the modification, and the sequence, and would need to be empirically tested.

## **B. The Challenged Claims of the '501 Patent**

84. As issued, the '501 patent includes 30 claims. Claim 1 is an independent claim, while the rest are dependent claims.

85. Claim 1 is reproduced below:

1. A double-stranded siRNA molecule against a target nucleic acid,

wherein the double-stranded siRNA molecule comprises a first strand and a second strand,

wherein the first strand comprises a first stretch that is complementary to the target nucleic acid,

wherein the second strand comprises a second stretch that is the same length as the first stretch and is complementary to the first stretch,

wherein the first strand and the second strand form a double-stranded structure comprising the first stretch and the second stretch,

wherein the first stretch and the second stretch each comprises contiguous alternating 2'-O-alkyl ribonucleotides,

wherein the 2'-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides,

wherein the 2'-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2'-O-alkyl ribonucleotides of the second strand, and

wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides.

Exh. 1001 ('501 patent) at claim 1.

86. Dependent claims 2-30 are described below:

87. Claim 2 is dependent on claim 1 and requires that “the double-stranded siRNA molecule is blunt ended on both ends.”

88. Claim 3 is dependent on claim 1 and requires that “at least one end of the double-stranded siRNA molecule has an overhang.”

89. Claim 4 further limits claim 3 and requires that “the overhang has one to eight nucleotides.”

90. Claim 5 further limits claim 3 and requires that “the 3’ end of the first strand has an overhang.”

91. Claim 6 further limits claim 3 and requires that “the 5’ end of the first strand has an overhang.”

92. Claim 7 further limits claim 3 and requires that “the 3’ end of the second strand has an overhang.”

93. Claim 8 further limits claim 3 and requires that “the 5’ end of the second strand has an overhang.”

94. Claim 9 is dependent on claim 1 and requires that “each of the first strand and the second strand consists of at least 18 nucleotides.”

95. Claim 10 is dependent on claim 1 and requires that “the 2’-O-alkyl ribonucleotides are 2’-O-methyl ribonucleotides.”

96. Claim 11 further limits claim 10 and requires that “the 2’-O-methyl ribonucleotides alternate with the unmodified ribonucleotides.”

97. Claim 12 is dependent on claim 1 and requires that “the differently modified ribonucleotides have an amino, fluoro, different alkoxy, or alkyl modification at the 2’-position.”

98. Claim 13 further limits claim 10 and requires that “the 2’-O-methyl

ribonucleotides alternate with 2'-fluoro ribonucleotides.”

99. Claim 14 is dependent on claim 1 and requires that “the first stretch and the second stretch each comprises contiguous alternating single 2'-O-alkyl ribonucleotides, and wherein the single 2'-O-alkyl ribonucleotides alternate with the unmodified ribonucleotides.”

100. Claim 15 further limits claim 14 and requires that “the single 2'-O-alkyl ribonucleotides are 2'-O-methyl ribonucleotides.”

101. Claim 16 is dependent on claim 1 and requires that “the first stretch and the second stretch each comprises contiguous alternating single 2'-O-alkyl ribonucleotides, and wherein the single 2'-O-alkyl ribonucleotides alternate with the differently modified ribonucleotides.”

102. Claim 17 further limits claim 16 and requires that “the single 2'-O-alkyl ribonucleotides are 2'-O-methyl ribonucleotides.”

103. Claim 18 further limits claim 16 and requires that “the differently modified ribonucleotides have an amino, fluoro, different alkoxy, or alkyl modification at the 2'-position.”

104. Claim 19 further limits claim 16 and requires that “the single 2'-O-alkyl ribonucleotides are 2'-O-methyl ribonucleotides, and the differently modified ribonucleotides are 2'-fluoro ribonucleotides.”

105. Claim 20 is dependent on claim 1 and requires that “the 5'

ribonucleotide of the first stretch is a 2'-O-alkyl modified ribonucleotide.”

106. Claim 21 further limits claim 11 and requires that “the 5' ribonucleotide of the first stretch is a 2'-O-methyl ribonucleotide.”

107. Claim 22 further limits claim 13 and requires that “the 5' ribonucleotide of the first stretch is a 2'-O-methyl ribonucleotide.”

108. Claim 23 further limits claim 14 and requires that “the 5' ribonucleotide of the first stretch is a 2'-O-alkyl modified ribonucleotide that alternates with an unmodified ribonucleotide.”

109. Claim 24 further limits claim 16 and requires that “the 5' ribonucleotide of the first stretch is a 2'-O-alkyl modified ribonucleotide that alternates with a differently modified ribonucleotide.”

110. Claim 25 further limits claim 19 and requires that “the double-stranded siRNA molecule is blunt on at least one end.”

111. Claim 26 further limits claim 19 and requires that “at least one end of the double-stranded siRNA molecule has an overhang of one to eight nucleotides.”

112. Claim 27 further limits claim 26 and requires that “the 3' end of the first strand has an overhang.”

113. Claim 28 further limits claim 26 and requires that “the 5' end of the first strand has an overhang.”

114. Claim 29 further limits claim 26 and requires that “the 3' end of the



second strand has an overhang.”

115. Claim 30 further limits claim 26 and requires that “the 5’ end of the second strand has an overhang.”

Exh. 1001 (’501 patent) at claims 2-30.

116. Upon reading the claims of the ’501 patent, a person of ordinary skill in the art would recognize the stark contrast between the ’501 patent claims and the disclosures of the examples in the patent specification.

117. As indicated above, the ’501 patent specification describes several siRNA molecules with internal 2’-O-methyl modified nucleotides in the figures, the examples, and in the sequence listing. However, all of the siRNA molecules described are of the *same type*—those having the *same* modification (2’-O-methyl) and pattern, with the remaining nucleotides being *unmodified* and the *same* duplex architecture (blunt ended on both ends). The specification also demonstrates that not all of them are stable and active, which is which is the problem the ’501 patent claims to solve. Exh. 1001 (’501 patent) at cols. 2:29-31, 2:35-37, 11:19-34. In contrast, claims 1-30 of the ’501 patent cover a vast genus of structurally diverse siRNA molecules comprising contiguous alternating 2’-O-alkyl modified ribonucleotides that alternate with unmodified or differently modified ribonucleotides. With the exception of a few claims, the ’501 patent claims place no limits on the types of modifications that could be incorporated in the siRNA

molecules or their duplex architectures and which would still be useful for RNA interference.

118. As explained in Sections X-XIII below, neither the '501 patent specification nor any of the priority applications describes or enables the vast genus of siRNA molecules encompassed by the '501 patent claims.

### **C. Claim Construction**

119. I understand from counsel that in proceedings before the PTAB, such as a post-grant review, a claim term should be given its broadest reasonable construction consistent with the specification of the patent, as understood by a person of ordinary skill in the art. I also understand that, at the same time, absent some reason to the contrary, claim terms are typically given their ordinary and accustomed meaning as would be understood by one of ordinary skill in the art.

120. I understand from counsel that dependent claims incorporate by reference each of the limitations of the claim from which they depend. I also understand that dependent claims further limit the claim from which they depend by including additional limitations.

121. I have followed these principles in my analysis of the meaning of the claims throughout this declaration. I discuss some of the terms below and what

one of ordinary skill in the art would understand those terms to mean.<sup>8</sup>

**i. “double-stranded siRNA molecule” and “double-stranded siRNA molecule against a target nucleic acid”**

122. Claims 1-30 recite or incorporate the terms “double-stranded siRNA molecule” and “a double-stranded siRNA molecule against a target nucleic acid.”

Exh. 1001 (’501 patent) at claims 1-30. I understand that these terms were included in the original claims of the ’501 patent. Exh. 1004 (’501 Patent File History, Original Claims 1-30) at claims 1-30.

123. A person of ordinary skill in the art would understand the terms “double-stranded siRNA molecule” and “double-stranded siRNA molecule against a target nucleic acid” of claims 1-30 refer to duplex small interfering RNA molecules that interfere with gene expression, *i.e.*, have RNAi activity. Such an understanding would be consistent with the specification, including the examples, which refers to the duplex siRNA molecules as having RNAi activity and their uses for RNA-mediated interference.

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<sup>8</sup> I understand from counsel that the PTAB has recently proposed adopting a standard for claim construction similar to that applied in federal district court proceedings. I understand that in federal district court proceedings, the words (*i.e.*, terms) of a claim are generally given their ordinary and customary meaning, which is the meaning that the term would have to a person of ordinary skill in the art in question at the time of the invention. I also understand in interpreting claim terms, a court looks to the words of the claims themselves, the specification, the file history, and, if required, evidence such as dictionaries, treatises, or scientific publications to determine the scope and meaning of the terms. My analysis of the claim terms would be the same even under this standard.

124. The '501 patent specification states in the Background of the Invention that “RNA-mediated interference (RNAi) is a post-transcriptional gene silencing mechanism initiated by double stranded RNA (dsRNA) homologous in sequence to the silenced gene.” Exh. 1001 ('501 patent) at col. 1:53-56. The '501 patent specification states:

“The discovery that transfection of duplexes of 21-nt into mammalian cells *interfered with gene expression* and did not induce a sequence independent interferon-driven anti-viral response usually obtained with long dsRNA led to new potential application in differentiated mammalian cells.... Interestingly *these small interfering RNAs (siRNAs)* resemble the processing products from long dsRNAs suggesting a potential bypassing mechanism in differentiated mammalian cells.”

Exh. 1001 ('501 patent) at col. 2:6-16 (emphasis added).

125. As noted above, the '501 patent specification states that a person of ordinary skill in the art “will understand that all of the ribonucleic acids of the present invention are suitable to cause or being involved in methods of RNA mediated interference....” Exh. 1001 ('501 patent) at col. 12:41-45; *see also* ¶ 62 above. The '501 patent states in the Detailed Description that “[t]ypically, the target nucleic acid sequence or *target nucleic acid is, in accordance with the mode of action of interfering ribonucleic acids, a single stranded RNA, more preferably an mRNA.*” Exh. 1001 ('501 patent) at col. 12:5-8 (emphasis added). And as noted above, the '501 patent specification describes that the focus of the

invention was to provide stable ribonucleic acid molecules that retain RNAi activity and discusses the potential uses of such molecules, which would require the ribonucleic acid molecules of the invention to cause or be involved in RNA interference, *i.e.*, have RNAi activity. *See* ¶¶ 59-64 above.

126. Additionally, as noted above, the majority of examples of the '501 patent focus on assessing the effect of end cap modifications, overhangs, mismatches, or internal 2'-O-methyl modifications on RNAi activity of siRNA molecules directed against the mRNA of PTEN or Akt. Exh. 1001 ('501 patent) at Examples 1-4, 6-13; *see also* ¶¶ 71-81 above. The siRNA molecules of the examples are described as being “Functional in Mediating RNA Interference” (Exh. 1001 ('501 patent) at col. 32:32-33 (Example 12); *see also* col. 12:22-26 (describing that certain siRNA molecules are “not functional in mediating RNAi”)), achieving “RNAi activity” (Exh. 1001 ('501 patent) at col. 27:14-17 (Example 6)), having “RNAi activity” or “mediat[ing] RNAi activity” (Exh. 1001 ('501 patent) at Examples 2-4, 6-8, 11), or being “functional” or “active” (Exh. 1001 ('501 patent) at col. 24:1-6 (Example 1)) and describe RNAi activity assays that measure target mRNA or protein expression following introduction of the siRNA molecule into a cell. Exh. 1001 ('501 patent) at Examples 1-4, 6-13.

127. Finally, I note that such a construction would also be consistent with the meaning of the term “siRNA” as it is used in the field of RNAi and as the '501

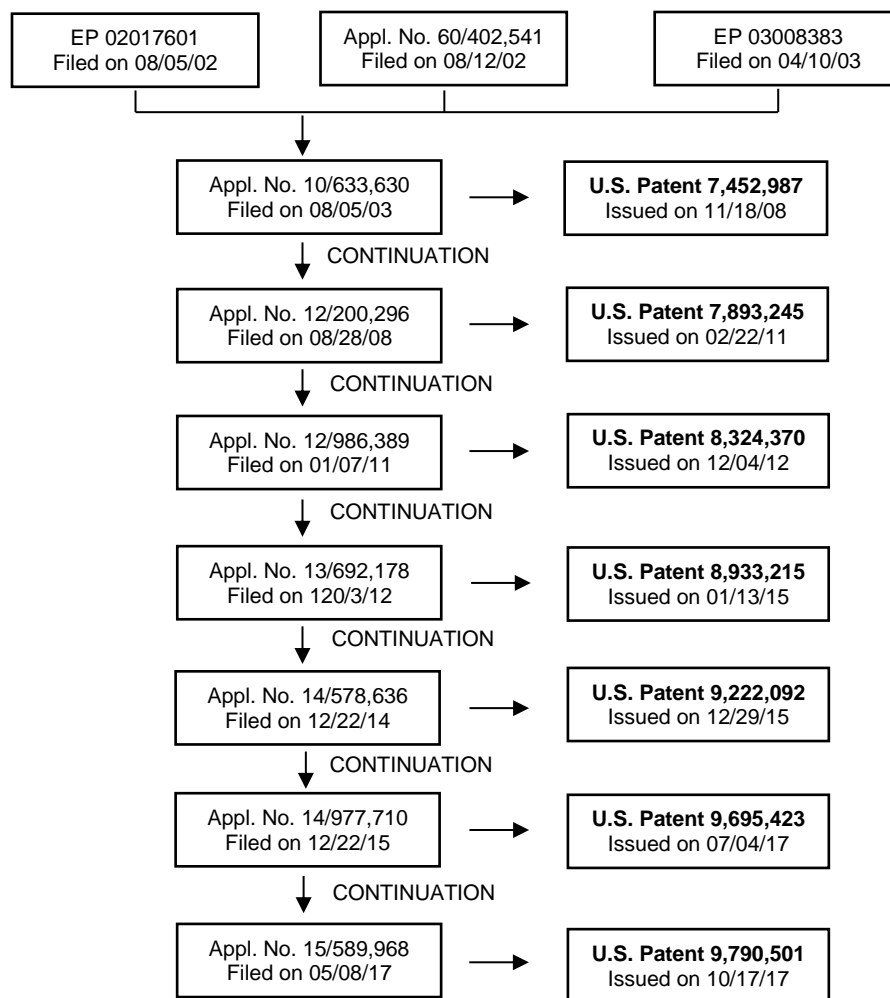
patent acknowledges. *See* ¶¶ 47-52, 124 above; Exh. 1001 ('501 patent) at col. 2:6-16; *see also* Exh. 1042 (Elbashir 2001) at p. 494 (“The mediators of sequence-specific messenger RNA degradation are 21- and 22-nucleotide small interfering RNAs (siRNAs) generated by ribonuclease III cleavage from longer dsRNAs.”); Exh. 1028 (Czauderna F. *et al.*, *Structural Variations and Stabilising Modifications of Synthetic siRNAs in Mammalian Cells*, *Nucleic Acids Res.*, 31(11):2705-16 (2003)) (“Czauderna 2003”) at p. 2705 (“Double-stranded short interfering RNAs (siRNA) induce post-transcriptional silencing in a variety of biological systems.”); Exh. 1027 (Watts J.K. *et al.*, *Chemically Modified siRNA: Tools and Applications*, *Drug Discovery Today* 13:842-55 (2008)) (“Watts 2008”) at p. 843, Glossary (“Short interfering RNAs (siRNAs): These triggers of RNAi are dsRNAs that typically contain 19–21 bp and 2-nt 3'-overhangs. siRNAs are naturally produced by Dicer-mediated cleavage of larger dsRNAs, but may also be introduced into cells exogenously.”).

#### **X. The Priority Applications Fail to Describe or Enable the '501 Claims**

128. I have been asked to analyze whether a person of ordinary skill in the art would understand that the '501 patent priority applications describe or enable the '501 patent claims. It is my opinion that a person of ordinary skill in the art would understand that neither the specifications nor the original claims of any of the priority applications demonstrate possession of the vast genus of modified

siRNA molecules encompassed by the '501 patent claims, and they fail to provide any guidance to practice the claimed invention without undue experimentation.

129. As noted in paragraph 57 above, the '501 patent claims priority to a series of continuation applications, a provisional application ('541 provisional, filed on August 12, 2002), and two European applications (EP1 and EP2) dating back to as early as August 5, 2002 (for EP1). A diagram of the '501 patent lineage is illustrated below:



130. The priority applications are substantively identical in their disclosures to the '501 patent specification. If anything, EP1 and the '541 provisional contain less disclosure than the '501 patent specification. Thus, my analysis of whether the '501 patent specification describes or enables the '501 patent claims applies equally to the specifications of the priority applications. To the extent there are any differences between the disclosures of the priority applications and the '501 patent that would affect my analysis; I have specifically discussed them below.

**A. The Provisional and European Applications Fail to Describe or Enable the Genus of siRNA Molecules Claimed**

131. The earliest applications in the '501 patent priority chain are EP1 filed on August 5, 2002 and the '541 provisional application filed on August 12, 2002.

132. I have reviewed the specifications of EP1 and the '541 provisional. Both share identical disclosures and include 55 claims, which are substantially identical to each other as well as to the eleven embodiments described in their specifications on pages 2-9. Exh. 1006 (EP1) at pp. 1-41, claims 1-55, Abstract; Exh. 1007 ('541 provisional) at pp. 1-41, claims 1-55, Abstract. The eleven embodiments of EP1 and the '541 provisional are further substantially identical to the '501 patent embodiments. Exh. 1001 ('501 patent) at cols. 2:38-6:67.<sup>9</sup> Thus,

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<sup>9</sup> The embodiments described in cols. 5:38-6:3 of the '501 patent specification do not appear in either EP1 or the '541 provisional.



none of the claims of either EP1 or the '541 provisional provides any more disclosure than their respective specifications or the '501 patent specification.

133. Both EP1 and the '541 provisional include 16 figures and 13 examples. Exh. 1006 (EP1) at Figs. 1-16, pp. 27-41; Exh. 1007 ('541 provisional) at Figs. 1-16, pp. 27-41. Neither includes a sequence listing. The claims of the two applications are otherwise identical and include minor variations, which I understand from counsel to be related to the differences in U.S. and E.P. requirements.

134. Like the '501 patent, both EP1 and the '541 provisional are directed to synthetic interfering RNA molecules having a double-stranded structure (*i.e.*, duplex), including siRNA, and their uses. Exh. 1006 (EP1) at pp. 1-2, 9; Exh. 1007 ('541 provisional) at pp. 1-2, 9. None of the claims of EP1 or the '541 provisional are directed to “a double-stranded siRNA molecule against a target nucleic acid” or “double-stranded siRNA molecule;” rather the claims are to “a ribonucleic acid comprising a double-stranded structure” or “nucleic acid.” Exh. 1006 (EP1) at claims 1-55; Exh. 1007 ('541 provisional) at claims 1-55. Additionally, none of the EP1 or the '541 provisional claims recite the terms “wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides” or “wherein the first stretch and the second stretch each comprises contiguous alternating 2'-O-alkyl ribonucleotides, wherein the 2'-

O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides” as required by the ’501 patent claims. Exh. 1006 (EP1) at claims 1-55; Exh. 1007 (’541 provisional) at claims 1-55.

135. Both EP1 and the ’541 provisional generally describe various embodiments, including those having “a certain pattern of modified nucleotides” and state in a “preferred embodiment the pattern consists of alternating single modified and unmodified nucleotides.” Exh. 1006 (EP1) at pp. 15, 18; Exh. 1007 (’541 provisional) at pp. 15, 18. EP1 and the ’541 provisional emphasize a requirement for “patterns” or particular arrangements for the modified and unmodified ribonucleotides across the first and second stretches and the first and second strands in the preferred embodiments and the examples as part of their invention. Exh. 1006 (EP1) and Exh. 1007 (’541 provisional) at pp. 16-18, 23, Examples 8-11, Figs. 2, 10-13.

136. Like the ’501 patent specification, both EP1 and the ’541 provisional include a non-limiting list of potential “end modifications” that can occur at non-terminal nucleotides. Exh. 1006 (EP1) at pp. 12-13, 15-16; Exh. 1007 (’541 provisional) at pp. 12-13, 15-16. Further, in EP1 and the ’541 provisional, the preferred modifications for the modified nucleotides are limited to “amino, fluoro, methoxy and alkyl.” Exh. 1006 (EP1) at pp. 4, 6, *see also* claims 19, 29; Exh.

1007 ('541 provisional) at pp. 4, 6, *see also* claims 19, 29.<sup>10</sup> Additionally, neither EP1 nor the '541 provisional recites “different alkoxy” for the modification of the differently modified ribonucleotides as recited in claims 12 and 18 of the '501 patent. Exh. 1006 (EP1) and Exh. 1007 ('541 provisional) at pp. 4, 6, *see also* claims 19, 29; Exh. 1001 ('501 patent) at claims 12, 18.

137. EP1 and the '541 provisional generally describe and claim ribonucleic acid molecules wherein “said first strand and/or second strand comprises a plurality of groups of modified nucleotides ... flanked on one or both sides by ... an unmodified nucleotide or a nucleotide having a modification different from the modification of the modified nucleotides” (Exh. 1006 (EP1) and Exh. 1007 ('541 provisional) at pp. 4-6, claims 20, 29), “the pattern of said first strand is shifted by one or more nucleotides relative to the pattern of the second strand,” (Exh. 1006 (EP1) and Exh. 1007 ('541 provisional) at p. 6, claim 28), the ribonucleic acid molecule is blunt ended on one or both ends (Exh. 1006 (EP1) and Exh. 1007 ('541 provisional) at pp. 2-3, 6, claims 1, 4-6, 30-33), or “at least one of the two strands has an overhang of at least one nucleotide” on the “5'-end” or the “3'-end” (Exh. 1006 (EP1) and Exh. 1007 ('541 provisional) at pp. 3-4, 6-7, 14-15, claims 7, 8, 10, 34-36, *see also* claims 13-18).

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<sup>10</sup> Claim 19 of the '541 provisional limits the 2'-modification to “fluoro, methoxy, and alkyl” only.

138. However, like the '501 patent specification, both EP1 and the '541 provisional describe several siRNA molecules with internal 2'-O-methyl modified ribonucleotides that have a *pattern of alternating unmodified and 2'-modified ribonucleotides* where the modified ribonucleotides have the *same* 2'-modification (2'-O-methyl) throughout the strand. Exh. 1006 (EP1) at Examples 7-11, Figs. 9-13; Exh. 1007 ('541 provisional) at Examples 7-11, Figs. 9-13. And like the '501 patent specification, aside from the generic disclosures identified in paragraphs 132-137 above, neither EP1 nor the '541 provisional discloses *any* siRNA molecules comprising *two differently* modified ribonucleotides in a single strand, let alone one having a “contiguous alternating” pattern of 2'-O-alkyl modified ribonucleotides alternating with differently modified ribonucleotides across both strands as encompassed by at least claims 1-10, 12, 13, 16-20, 22, and 24-30 of the '501 patent, and as required by claims 12-13, 16-19, 22, and 24-30 of the '501 patent. Exh. 1006 (EP1) at Examples 1-13, Figs. 1-16; Exh. 1007 ('541 provisional) at Examples 1-13, Figs. 1-16. Similarly, neither EP1 nor the '541 provisional discloses *any* siRNA molecules having a pattern of “contiguous alternating” 2'-O-alkyl modified ribonucleotides having a *mixed* pattern or motif, *i.e.*, where the 2'-O-alkyl modified ribonucleotides alternate with unmodified and differently modified ribonucleotides as encompassed by at least claims 1-10, 12, and 20 of the '501 patent claims; nor do they disclose *any* siRNA molecules

having a modification *other than a 2'-O-methyl* modification for the *2'-O-alkyl* modified ribonucleotides as encompassed by at least claims 1-9, 12, 14, 16, 18, 20, 23, and 24. Exh. 1006 (EP1) at Examples 1-13, Figs. 1-16; Exh. 1007 ('541 provisional) at Examples 1-13, Figs. 1-16.

139. Both EP1 and the '541 provisional application, like the '501 patent specification, also fail to describe *any* modified siRNA molecules having internal modifications that have an *overhang* on any strand as encompassed by claims 1 and 3-30 of the '501 patent and as required by claims 3-8 and 26-30 of the '501 patent. All of the examples of siRNA molecules having 2'-O-methyl modified and unmodified ribonucleotides on both strands in EP1 and the '541 provisional are *blunt ended on both ends*. Exh. 1006 (EP1) at Examples 7-11, Figs. 9-13; Exh. 1007 ('541 provisional) at Examples 7-11, Figs. 9-13. EP1 and the '541 provisional also fail to describe *any* 2'-O-alkyl modified siRNA molecules that have a double-stranded stretch length *less than 21 nucleotides* or *greater than 21 nucleotides* as encompassed by claims 1-30 of the '501 patent; all of the examples of 2'-O-methyl modified siRNA molecules have a double-stranded stretch length of 21 nucleotides (and are lacking an overhang). Exh. 1006 (EP1) and Exh. 1007 ('541 provisional) at Examples 7-11, Figs. 9-13.

140. In EP1 and the '541 provisional, like the '501 patent, the inventors distinguish their "blunt-ended" duplex design from the prior art 3'-overhang

duplex design by stating:

“A third strategy subject to the present invention is to realise a ribonucleic acid comprising a double-stranded structure ... whereby the double-stranded structure is **blunt-ended**. As used in connection with the present description, the term double-stranded structure is also referred to as duplex. ***This design of RNAi is thus clearly different*** from, e. g., the one of Tuschl et al. as specified in international patent application WO 01/75164 ***which discloses a 3'-overhang***.”

Exh. 1006 (EP1) at p. 14 (emphasis added); Exh. 1007 ('541 provisional) at p. 14.

EP1 and the '541 provisional, like the '501 patent, also ***exclude a 3'-overhang***

design from their suggested combination of design elements:

“It is also within the present invention that any ribonucleic acid designed according to this strategy may also have the features conferred to a ribonucleic acid according to the present application by any of the other design strategies disclosed herein. Accordingly, the interfering ribonucleic acid having a pattern of modified nucleotides may have an end modification, an end modification scheme, may be blunt ended or may have a **5' overhang** or any combination of two or more of these elements or characteristics.”

Exh. 1006 (EP1) at pp. 15-16 (emphasis added); Exh. 1007 ('541 provisional) at

pp. 15-16; *see also* Exh. 1006 (EP1) and Exh. 1007 ('541 provisional) at p. 14

(“Taken the effectiveness of **blunt-ended** RNAi and the advantages of an end modification... it is within the present invention to have blunt-ended RNAi carrying any end modification scheme as depicted in table 1.”) (emphasis added).

141. And similar to the '501 patent, based on experimental results, both

EP1 and the '541 provisional state that "3'-overhangs are not essential for RNAi activity." Exh. 1006 (EP1) at p. 31; Exh. 1007 ('541 provisional) at p. 31; *see also* Exh. 1006 (EP1) and Exh. 1007 ('541 provisional) at p. 24 (stating that the 3'-overhang of RNAi molecules is "not important for RNA interference"). Both EP1 and the '541 provisional, like the '501 patent, further state that "a **blunt** duplex is **more stable** than the duplex with overhangs." Exh. 1006 (EP1) at p. 33 (emphasis added); Exh. 1007 ('541 provisional) at p. 33.

142. Further, neither EP1 nor the '541 provisional discloses **any** siRNA molecules having **two differently** modified ribonucleotides that **have** RNAi activity or are **useful** for mediating RNAi; nor do they disclose **any** siRNA molecules having any modification **other than** a 2'-O-methyl modification for the modified ribonucleotides that **have** RNAi activity or are **useful** for mediating RNAi as required by the '501 patent claims.<sup>11</sup> Exh. 1006 (EP1) at Examples 1-13, Figs. 1-16; Exh. 1007 ('541 provisional) at Examples 1-13, Figs. 1-16. They also do not disclose any internally modified siRNAs having an **overhang** that **have** RNAi activity or are **useful** for mediating RNAi as required by the '501 patent claims.

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<sup>11</sup> siRNAs by definition are required to have RNAi activity as discussed in Sections IX.C.i above and XI.A.iv below. Independent of this requirement, the patent specification teaches that the purpose of the claimed invention was to provide siRNA molecules that are useful for RNAi activity as discussed in Section XII.A.i below. Thus, the claimed siRNA molecules are also required to be useful for mediating RNAi.

And, none of the claims 1-55 of EP1 or the '541 provisional provide any additional support for the claimed ribonucleic acids having RNAi activity or being useful for mediating RNAi as required by the '501 patent claims.<sup>12</sup> See Sections XI-XII below; *see also* Section IX.C.i above.

143. Thus, neither EP1 nor the '541 provisional describes or enables the vast genus of modified siRNA molecules now claimed. See Sections XI-XII below.

144. The next application in the priority chain, chronologically, is EP2, filed on April 10, 2003. I have reviewed the specification of EP2. The disclosures in EP2 are substantially similar to those in EP1 and the '541 provisional.

145. EP2 builds on the disclosures of EP1 and the '541 provisional and includes a total of 19 figures, including new Figures 7 and 15-16 (disclosed for the first time in EP2), and 13 examples (with additional disclosures in Examples 3, 4, and 11 not present in EP1 or the '541 provisional). Exh. 1008 (EP2) at pp. 6:11, 7:19-8:13, 10:29-30, 13:11-12, 17:21-22, 17:25-26, 18:10-15, 18:23, 19:22-20:19,

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<sup>12</sup> Claims 41 and 48-55 of EP1 and the '541 provisional refer to “target gene,” “knockdown cell,” “knockdown organism,” and uses of the ribonucleic acids for target validation, compositions, and “method for inhibiting the expression of a target gene in a cell or derivative thereof” that would encompass molecules having RNAi activity. However, these claims provide no more support for the '501 patent claims than the specification at least because they do not provide support for the genus of siRNA molecules comprising two different modifications that have RNAi activity or are useful in mediating RNAi as required by the '501 patent claims.



25:24-28, 25:29-26:4, 26:7-9, 26:13-14, 27:5-15, 28:25-29:22, 35:7-11, 35:21-36:7, 36:17-19, 37:3-13, 44:1-46:17, Figs. 7, 15, 16. Additionally, EP2 includes a sequence listing comprising 174 sequences. Exh. 1008 (EP2) at pp. 83-152. As filed, EP2 included 59 claims. Exh. 1008 (EP2) at claims 1-59.

146. EP2, like EP1 and the '541 provisional, describes and claims ribonucleic acids comprising a double-stranded structure and their uses. Exh. 1008 (EP2) at pp. 2-10, claims 1-59. Like EP1 and the '541 provisional, EP2 generally describes various embodiments, including those having “a certain pattern of modified nucleotides” and states in a “preferred embodiment the pattern consists of alternating single modified and unmodified nucleotides.” Exh. 1008 (EP2) at pp. 16, 19. The disclosure of the preferred nucleotide modifications in EP2 is expanded to include “alkoxy.” Exh. 1008 (EP2) at p. 4:18-21 (emphasis added), *see also* p. 6:9-11, claims 19, 29. And, like EP1 and the '541 provisional, EP2 also emphasizes a requirement for “patterns” or particular arrangements for the modified and unmodified ribonucleotides across the first and second stretches and the first and second strands in the preferred embodiments and the examples as part of their invention. Exh. 1008 (EP2) at pp. 16-20, 25-26, Examples 8-11, Figs. 2, 11-16. EP2 describes in a preferred embodiment and the examples that the 2'-O-methyl ribonucleotides are arranged at alternating nucleotide positions (*i.e.*, every second nucleotide) across both strands of the ribonucleic acid molecules. Exh.

1008 (EP2) at pp. 19-20, Examples 9-11.

147. EP2, like EP1 and the '541 provisional generally describes and claims ribonucleic acid molecules wherein “said first strand and/or second strand comprises a plurality of groups of modified nucleotides ... flanked on one or both sides by ... an unmodified nucleotide or a nucleotide having a modification different from the modification of the modified nucleotides” (Exh. 1008 (EP2) at pp. 4-7, claims 20, 29), “the pattern of said first strand is shifted by one or more nucleotides relative to the pattern of the second strand,” (Exh. 1008 (EP2) at p. 6, claim 28), “each group of modified nucleotides of the first strand being aligned with a flanking group of nucleotides in the second strand,” (Exh. 1008 (EP2) at pp. 7-8, claims 41-44), the ribonucleic acid molecule is blunt ended on one or both ends (Exh. 1008 (EP2) at pp. 2-3, 6, claims 1, 4-6, 30-33), or “at least one of the two strands has an overhang of at least one nucleotide” on the “5'-end” or the “3'-end” (Exh. 1008 (EP2) at pp. 3-4, 6-7, 15-16, claims 7, 8, 10, 13-18, 34-36).

148. Like EP1 and the '541 provisional, aside from the generic disclosures identified in paragraphs 145-147 above, EP2 does *not* disclose *any* siRNA molecules comprising *two differently* modified ribonucleotides in a single strand, let alone one having a “contiguous alternating” pattern of 2'-O-alkyl modified ribonucleotides alternating with differently modified ribonucleotides in both strands as encompassed by the '501 patent claims. Exh. 1008 (EP2) at Examples

1-13, Figs. 1-19. Similarly, EP2 does not disclose *any* siRNA molecules having a pattern of “contiguous alternating” 2’-O-alkyl modified ribonucleotides having a *mixed* pattern or motif, *i.e.*, where the modified ribonucleotides alternate with unmodified and differently modified ribonucleotides or *any* siRNA molecules having a modification *other than a 2’-O-methyl* modification as encompassed by the ’501 patent claims. Exh. 1008 (EP2) at Examples 1-13, Figs. 1-19. See *also* ¶ 138 above. The only siRNA molecules disclosed in the figures and the examples of EP2 are those comprising either end cap modifications, single internally 2’-O-methyl modified ribonucleotides, or groups of internally 2’-O-methyl modified ribonucleotides having the same modification (2’-O-methyl) throughout the strand, with the remaining ribonucleotides being *unmodified*. Exh. 1008 (EP2) at Examples 1-13, Figs. 1-19. In EP2, the examples of 2’-O-methyl modified ribonucleotides are expanded to include blunt ended siRNA molecules having a duplex length of 19 nucleotides (as opposed to a duplex length of 21 nucleotides in EP1 or the ’541 provisional). Exh. 1008 (EP2) at Example 11, Fig. 16. And like EP1, the ’541 provisional, and the ’501 patent specification, the only siRNA molecules having an *alternating* pattern of modified ribonucleotides disclosed in EP2 are those having alternating *2’-O-methyl* modified ribonucleotides that alternate with *unmodified* ribonucleotides across both strands. Exh. 1008 (EP2) at Examples 8-11; Figs. 10-13. See *also* ¶¶ 131-138 above.

149. EP2, like EP1, the '541 provisional, and the '501 patent specification, also fails to describe **any** modified siRNA molecules having internal modifications that have an **overhang** on any strand as encompassed by the '501 patent claims. All of the examples of siRNA molecules having 2'-O-methyl modified and unmodified ribonucleotides on both strands in EP2 are **blunt ended on both ends**. Exh. 1008 (EP2) at Examples 7-11, Figs. 10-16. EP2 also fails to describe **any** 2'-O-alkyl modified siRNA molecules that have a double-stranded stretch length less than 19 nucleotides or greater than 21 nucleotides as encompassed by the '501 patent claims; all of the examples of 2'-O-methyl modified siRNA molecules have a double-stranded stretch length of 19 or 21 nucleotides (and are lacking an overhang). *See also* ¶¶ 139, 148 above.

150. And like EP1 and the '541 provisional, in EP2, the inventors distinguish their “blunt-ended” duplex design from the prior art 3'-overhang duplex design, exclude a 3'-overhang design from their suggested combination of design elements, and state that “3'-overhangs are not essential for RNAi activity,” and further state that “a **blunt** duplex is **more stable** than the duplex with overhangs.” Exh. 1008 (EP2) at pp. 34 (Examples 2), 37-38 (Example 5) (emphasis added); *see also* pp. 15-17.

151. Further, similar to EP1 and the '541 provisional, EP2 does not disclose **any** siRNA molecules having **two differently** modified ribonucleotides

that *have* RNAi activity or are *useful* for mediating RNAi; nor does EP2 disclose *any* siRNA molecules having any modification *other than a 2'-O-methyl* modification for the modified ribonucleotides that *have* RNAi activity or are *useful* for mediating RNAi as required by the '501 patent claims. Exh. 1008 (EP2) at Examples 1-13, Figs. 1-19. EP2 also does not disclose *any* internally modified siRNAs having an *overhang* that *have* RNAi activity or are *useful* for mediating RNAi as required by the '501 patent claims. And, none of the claims 1-59 of EP2 provide any additional support for the claimed ribonucleic acids having RNAi activity or being useful for mediating RNAi as required by the '501 patent claims.<sup>13</sup> See Section IX.C.i above; Sections XI-XII below; *see also* ¶ 142 above.

152. Thus, EP2, like EP1, the '541 provisional, and the '501 patent specification, also fails to describe or enable the vast genus of modified siRNA molecules now claimed. See Sections XI-XII below.

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<sup>13</sup> Claims 45 and 52-59 of EP2 refer to “target gene,” “knockdown cell,” “knockdown organism,” and uses of the ribonucleic acids for target validation, compositions, and “method for inhibiting the expression of a target gene in a cell or derivative thereof” that would encompass molecules having RNAi activity. However, these claims provide no more support for the '501 patent claims than the specification at least because they do not provide support for the genus of siRNA molecules comprising two different modifications that have RNAi activity or are useful in mediating RNAi as required by the '501 patent claims.

**B. The Related U.S. Patent Applications Fail to Describe or Enable the Genus of siRNA Molecules Claimed**

153. As noted in paragraphs 57 and 129 above, the '501 patent also claims priority to six U.S. patent applications issued as the '987, '245, '370, '215, '092, and '423 patents. I have reviewed the disclosures and the claims of each of the '987, '245, '370, '215, '092, and '423 patents, including their original claims. The earliest of these is the '987 patent, which was filed on August 5, 2003 as a non-provisional U.S. application. The '987 patent is substantially identical to EP2, except for its claims and references to related applications. The '987 patent includes the disclosures of the earlier applications, with some differences, as noted in my declaration. The '987 patent, like EP2 and the '501 patent, includes a total of 19 figures and 13 examples, and includes new figures, Figures 7 and 15-16, and new disclosures in Examples 3, 4, and 11 relating to those figures. Those new disclosures relate to siRNA duplex length and mismatches (Fig. 7) and siRNA molecules with internal 2'-O-methyl modified nucleotides (Figs. 15-16). None of them disclose any siRNA molecules having *two differently* modified ribonucleotides in a single strand, let alone one having a "contiguous alternating" pattern of 2'-O-alkyl modified ribonucleotides alternating with differently modified ribonucleotides in both strands as required by the '501 patent claims. They also do not disclose any siRNA molecules having a pattern of "contiguous alternating" 2'-O-alkyl modified ribonucleotides having a *mixed* pattern or motif,

*i.e.*, where the modified ribonucleotides alternate with unmodified and differently modified ribonucleotides as encompassed by the '501 patent claims; nor do they disclose any siRNA molecules having a modification other than a 2'-O-methyl modification. They also do not disclose any siRNA molecules comprising modified and unmodified or differently modified ribonucleotides that have an ***overhang*** on any strand or a strand length ***greater than 21 nucleotides*** as encompassed by the '501 patent claims. Nor do they disclose any such siRNA molecules having RNAi activity or being useful for RNAi as required by the '501 patent claims. The '987 patent also includes a sequence listing comprising 182 sequences.

154. Each of the '245, '370, '215, '092, '423, and '501 patents are continuation applications of the '987 patent. It is my understanding that the specifications of each of the continuation applications are essentially identical in their disclosures except for their claims and references to their related U.S. applications. I am also aware of a small number of slight differences (*e.g.*, inclusion of SEQ ID Nos., corrections of typographical errors) in the specifications of these applications, none of which impacts my opinions, discussed within, that the '501 patent claims are not described or enabled by any of the continuation applications. Because each of the '987, '245, '370, '215, '092, '423, and '501 patents share the same specification, my analysis of the '501 patent specification

applies equally to the '987, '245, '370, '215, '092, and '423 patent specifications. Below I discuss the claims of each of the six related U.S. patents, including their original claims.

**i. The '987 patent and its original claims**

155. The '987 patent was filed on August 5, 2003 as U.S. Appln. No. 10/633,630 (“the '630 application”) and issued on November 18, 2008. Exh. 1020 ('987 patent) at Cover. The '987 patent as issued includes 7 claims, with one independent claim. The '987 patent claims are generally directed to a *nucleic acid molecule* comprising a double-stranded structure *consisting of* first and second stretches having the same length wherein each stretch “*consist[s] of* contiguous alternating single *2'-O-methyl* modified and single *unmodified* ribonucleotides” that form a particular phase-shifted pattern across the two strands, and each stretch consists of *15-23* ribonucleotides. Exh. 1020 ('987 patent) at claim 1 (emphasis added); *see also* claims 2-7. None of the issued claims include a limitation for any modification other than a 2'-O-methyl modification and none of the claims include a limitation for contiguous alternating ribonucleotides having *two different* modifications in both strands.

156. As originally filed, the '630 application included 32 claims directed to double-stranded *ribonucleic acids* generally. Exh. 1009 ('630 application) at claims 1-32. None of the claims of the '630 application recite “a double-stranded



siRNA molecule against a target nucleic acid,” “double-stranded siRNA molecule,” “wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides,” or “wherein the first stretch and the second stretch each comprises contiguous alternating 2’-O-alkyl ribonucleotides, wherein the 2’-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides” as required by the ’501 patent claims. Exh. 1009 (’630 application) at claims 1-32. Original claim 15 states the “pattern of modification of said first strand is shifted by one or more nucleotides relative to the pattern of modification of the second strand.” Exh. 1009 (’630 application) at claim 15.

157. Original claims 1-32 of the ’630 application are similar to the original claims of EP1, the ’541 provisional, EP2, and the embodiments listed in the ’501 patent specification, with some differences, none of which affect my analysis.<sup>14</sup> Exh. 1009 (’630 application) at claims 1-32; *see also* Exh. 1006 (EP1) at claims 1-10, 19-20, 25-34, 39-40, 42-43, 45, 51-52, 54-55; Exh. 1007 (’541 provisional) at

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<sup>14</sup> For example, original claim 2 of the ’630 application included language that the first strand “optionally comprises a second stretch that is non-complementary to said target nucleic acid” and the second strand “optionally comprises a second stretch having a sequence that differs from said target nucleic acid,” not found in any of the EP1, ’541 provisional, or EP2 claims or the eleven embodiments listed in the ’501 patent specification. Original claim 28 of the ’630 application, directed to a method of inhibiting target gene expression, does not include the language “in a cell or derivative thereof” that was included in EP1, the ’541 provisional, EP2, or the eleventh embodiment of the ’501 patent specification. Claim 49 of the ’541 provisional directed to a method of treatment required “an effective amount of a composition,” which does not appear in original claim 30 of the ’630 application.

claims 1-10, 19-20, 25-34, 39-40, 42-43, 45, 49, 51-52, 54-55; Exh. 1008 (EP2) at claims 1-10, 19-20, 25-34, 39-43, 46-47, 49, 55-56, 58, 59; Exh. 1001 ('501 patent) at cols. 2:38-67, 3:11-37, 3:63-67, 4:1-19, 4:35-5:9, 5:28-64, 6:10-15, 6:18-20, 6:36-50, 6:55-67. As such, the original claims of the '630 application do not provide any additional support for the vast genus of modified siRNA molecules now claimed. *See also* ¶¶ 131-152 above. And, none of the claims of the '630 application provide any additional support for the claimed siRNA molecules having RNAi activity or being useful for mediating RNAi.<sup>15</sup> *See* Sections XI-XII below; Section IX.C.i above.

## **ii. The '245 patent and its original claims**

158. The next patent in the priority chain, the '245 patent, was filed on August 28, 2008 as U.S. Appln. No. 12/200,296 (“the '296 application”) and issued on February 22, 2011. Exh. 1021 ('245 patent) at Cover. The '245 patent includes 11 claims, with one independent claim.

159. The '245 patent claims are generally directed to a *nucleic acid*

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<sup>15</sup> Claims 28-32 of the '630 application depend from claim 1 and refer to “method of inhibiting the expression of a target gene,” “pharmaceutical composition,” “method of treating a disease in a patient,” “a cell,” and “an organism,” respectively, which would encompass molecules having RNAi activity. However, these claims provide no more support for the '501 patent claims than the specification at least because claim 1 does not refer to any modified ribonucleic acids, let alone those having a “contiguous alternating” pattern of modified ribonucleotides that alternate with unmodified or differently modified ribonucleotides as required by the '501 patent claims.

*molecule* comprising a double-stranded structure comprising “contiguous alternating single **2’-O-methyl** modified ribonucleotides” that “alternate with **unmodified or modified** ribonucleotides” that form a particular phase-shifted pattern across the two strands. Exh. 1021 (’245 patent) at claim 1 (emphasis added); *see also* claims 2-11. None of the ’245 patent claims include a limitation for any modification other than a “2’-O-methyl” modification for the modified ribonucleotides. And, claim 5 of the ’245 patent recites “said single 2’-O-methyl modified ribonucleotides alternate with ribonucleotides that have been modified at the 2’-position with an amino, fluoro, methoxy, alkoxy or alkyl group.” Exh. 1021 (’245 patent) at claim 5.

160. As originally filed, the ’296 application included the same original claim set as the parent ’630 application. Exh. 1010 (’296 application) at claims 1-32; *see also* Exh. 1009 (’630 application) at claims 1-32. Thus, the original claims of the ’296 application do not provide any additional support for the vast genus of modified siRNA molecules now claimed. *See* ¶¶ 156-157 above.

### **iii. The ’370 patent and its original claims**

161. The next patent in the priority chain, the ’370 patent, was filed on January 7, 2011 as U.S. Appln. No. 12/986,389 (“the ’389 application”) and issued on December 4, 2012. Exh. 1022 (’370 patent) at Cover. The ’370 patent includes 23 claims, with one independent claim. The ’370 patent claims are generally

directed to a *nucleic acid molecule* comprising a double-stranded structure comprising “contiguous alternating ribonucleotides modified with an *O-alkyl* group” that “alternat[e] with *unmodified or modified* ribonucleotides” that form a particular phase-shifted pattern across the two strands. Exh. 1022 (’370 patent) at claim 1 (emphasis added); *see also* claims 2-23. Claim 5 of the ’370 patent recites “said single 2’-O-alkyl modified ribonucleotides alternate with ribonucleotides that have been modified at the 2’-position with an amino, fluoro, methoxy, alkoxy or alkyl group.” Exh. 1022 (’370 patent) at claim 5; *see also* claims 13-17.

162. As originally filed, the ’389 application included 11 claims, with one independent claim. Exh. 1011 (’389 application) at claims 1-11. The original claims were directed to a *nucleic acid molecule* comprising a double-stranded structure comprising “contiguous alternating *modified* ribonucleotides” “alternating with *unmodified or modified* ribonucleotides,” “wherein the modified ribonucleotides of said first strand are *shifted by one ribonucleotide* relative to the modified ribonucleotides of the second strand,” with each stretch consisting of “*at least 17 and fewer than 30 ribonucleotides*,” and “wherein said modified ribonucleotides are modified with *O-alkyl* groups.” Exh. 1011 (’389 application) at claim 1 (emphasis added); *see also* claims 2-11. None of the claims of the ’389 application recite “a double-stranded siRNA molecule against a target nucleic acid” or “double-stranded siRNA molecule.” Exh. 1011 (’389 application) at

claims 1-11. Claim 4 of the '389 application specified that the O-alkyl modification was a "2'-O-alkyl" modification and claim 5 recited "said single 2'-O-alkyl modified ribonucleotides alternate with ribonucleotides that have been modified at the 2'-position with an amino, fluoro, methoxy, alkoxy or alkyl group." Exh. 1011 ('389 application) at claims 4, 5. The list of modifications in claim 5 is identical to that recited in the '501 patent specification. Exh. 1001 ('501 patent) at cols. 3:63-67, 4:53-56. With the exception of claim 5, none of the claims specify the type of modification for the "modified ribonucleotides" that alternate with O-alkyl modified ribonucleotides. Claims 7-11 specify that the double-stranded structure have at least one overhang on the 3'- or 5'-end of the first or second strands, similar to the embodiments of the patent specification. Exh. 1011 ('389 application) at claims 7-11. Thus, none of the original claims 1-11 of the '389 application provide any additional support for '501 patent claims than the '501 patent specification, which as explained in Sections XI and XII below, fails to describe or enable the vast genus of modified siRNA molecules now claimed. And, none of the claims of the '389 application provide any additional support for the claimed siRNA molecules having RNAi activity or being useful for mediating RNAi as required by the '501 patent claims. *See* Sections XI-XII below; *see also* Section IX.C.i above.

#### iv. The '215 patent and its original claims

163. The next patent in the priority chain, the '215 patent, was filed on December 3, 2012 as U.S. Appln. No. 13/692,178 (“the '178 application”) and issued on January 13, 2015. Exh. 1023 ('215 patent) at Cover. The '215 patent includes 19 claims, with one independent claim. The '215 patent claims are generally directed to a ***nucleic acid molecule*** comprising a double-stranded structure that is “blunt ended on at least one end” and comprises “contiguous alternating ***modified*** ribonucleotides ... alternating with ***unmodified or differently modified*** ribonucleotides.” Exh. 1023 ('215 patent) at claim 1 (emphasis added); *see also* claims 2-19. Claim 11 of the '215 patent recites “wherein the modified ribonucleotides are selected from the group consisting of 2'-O-alkyl ribonucleotides, 2'-fluoro ribonucleotides and 2'-amino ribonucleotides.” Exh. 1023 ('215 patent) at claim 11. And, none of the claims of the '215 patent specify the modifications for the differently modified ribonucleotides. Exh. 1023 ('215 patent) at claims 1-19.

164. As originally filed, the '178 application included 20 claims with one independent claim. Exh. 1012 ('178 application) at claims 1-20. Original claim 1 is generally directed to a ***nucleic acid molecule*** comprising a first strand and a second strand that form a double-stranded structure and is blunt ended on at least one end. Exh. 1012 ('178 application) at claim 1. None of the claims of the '178

application recite “a double-stranded siRNA molecule against a target nucleic acid,” “double-stranded siRNA molecule,” or the requirements for an “overhang” as required by the ’501 patent claims. Claim 11 is directed to a nucleic acid molecule comprising “contiguous alternating *modified* ribonucleotides ... alternating with *unmodified or differently modified* ribonucleotides,” but does not specify the types of modifications for either the modified or differently modified ribonucleotides. Exh. 1012 (’178 application) at claim 11 (emphasis added). Claim 12 specifies that “the modified ribonucleotides are selected from the group consisting of 2’-O-alkyl ribonucleotides, 2’-fluoro ribonucleotides and 2’-amino ribonucleotides.” Exh. 1012 (’178 application) at claim 12. This list of modifications is a subset of the modifications recited in the ’501 patent specification (Exh. 1001 (’501 patent) at cols. 3:63-67, 4:53-56), and applies to the modified ribonucleotides only (and not the differently modified ribonucleotides). None of the original claims recite an overhang requirement either. Thus, none of the original claims of the ’178 application provide any more support for the ’501 patent claims than the ’501 patent specification, which as discussed in Sections XI and XII below, fails to describe or enable the vast genus of modified siRNA molecules now claimed. And, none of the claims of the ’178 application provide any additional support for the claimed siRNA molecules having RNAi activity or being useful for mediating RNAi as required by the ’501 patent claims. *See*

Sections XI-XII below; *see also* Section IX.C.i above.

**v. The '092 patent and its original claims**

165. The next patent in the priority chain, the '092 patent, was filed on December 22, 2014 as U.S. Appln. No. 14/578,636 (“the '636 application”) and issued on December 29, 2015. Exh. 1024 ('092 patent) at Cover. The '092 patent includes 10 claims, with one independent claim. The '092 patent claims are generally directed to a ***ribonucleic acid*** comprising a double-stranded structure that “***contains a group*** of ribonucleic acids on the ***first or second stretch/strand*** that has a modification at the 2'-position selected from an ***amino, fluoro, methoxy, alkoxy or alkyl*** modification” “flanked on one or both sides by a group of flanking ribonucleic acids having a modification at the 2'-position selected from an ***amino, fluoro, methoxy, alkoxy or alkyl*** modification that is different from the modification on said group of ribonucleic acids modified at the 2'-position,” with one to ten nucleotides in each of the modified or flanking nucleotide groups. Exh. 1024 ('092 patent) at claim 1 (emphasis added); *see also* claims 2-10.

166. As originally filed, the '636 application included a single claim. Exh. 1013 ('636 application) at claim 1. Original claim 1 is directed to a ***ribonucleic acid*** comprising a double-stranded structure that is “***blunt ended at one end or at both ends.***” Exh. 1013 ('636 application) at claim 1 (emphasis added). Original claim 1 of the '636 application is substantially identical to the first embodiment of



the '501 patent specification with minor differences. Exh. 1001 ('501 patent) at cols. 2:38-49, 3:1-10. Additionally, original claim 1 does not recite any limitations of the '501 patent claims including “a double-stranded siRNA molecule against a target nucleic acid,” “double-stranded siRNA molecule,” “wherein each of the first and the second stretch consists of at least 17 and fewer than 30 ribonucleotides,” “wherein the first stretch and the second stretch each comprises contiguous alternating 2'-O-alkyl ribonucleotides, wherein the 2'-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides,” “wherein the 2'-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2'-O-alkyl ribonucleotides of the second strand,” or any “overhang” requirement as required by the '501 patent claims. Thus, original claim 1 of the '636 application provides no more support for the '501 patent claims than the '501 patent specification, which as discussed in Sections XI and XII below, fails to describe or enable the vast genus of modified siRNA molecules now claimed. And, claim 1 of the '636 application fails to provide any additional support for the claimed siRNA molecules having RNAi activity or being useful for mediating RNAi as required by the '501 patent claims. *See* Sections XI-XII below; *see also* Section IX.C.i above.

**vi. The '423 patent and its original claims**

167. The last patent in the priority chain, the '423 patent, was filed on

December 22, 2015 as U.S. Appln. No. 14/977,710 (“the ’710 application”) and issued on July 4, 2017. Exh. 1015 (’423 patent) at Cover. The ’423 patent includes 25 claims, with two independent claims, claims 1 and 12. The claims are generally directed to a ***double-stranded siRNA molecule*** wherein at least one or both strands comprise one or more groups of 2’-modified nucleotides flanked on one or both sides by one or more groups of differently 2’-modified nucleotides, with the 2’-modifications for the modified and flanking groups being selected from an amino, fluoro, alkoxy or alkyl modification, with the number of nucleotides in each group of modified or flanking nucleotides being between one to ten, and the double-stranded siRNA molecule is “***capable of RNA interference***.” Exh. 1015 (’423 patent) at claims 1, 12 (emphasis added); *see also* claims 2-11, 13-25.

168. As originally filed, the ’710 application included a single claim directed to a ***ribonucleic acid*** comprising a double-stranded structure that was identical to the original claim of the ’636 application. Exh. 1005 (’710 application) at claim 1; Exh. 1013 (’636 application) at claim 1. Thus, original claim 1 of the ’710 application provides no more support for the ’501 patent claims than either the original claim of the ’636 application or the ’501 patent specification, which as discussed in Sections XI and XII below, fails to describe or enable the vast genus of modified siRNA molecules now claimed and fails to provide any additional support for the claimed siRNA molecules having RNAi activity or being useful for

RNAi as required by the '501 patent claims. *See* ¶ 166; Section IX.C.i above; Section XI-XII below.

169. In sum, each of the specifications and the original claims of the applications in the '501 patent priority chain fails to demonstrate possession of the vast genus of modified siRNA molecules claimed and fails to provide any guidance to practice the claimed invention without undue experimentation.

170. Finally, I note that a person of ordinary skill in the art reviewing the priority applications leading up to the '501 patent would be struck by the contrast between the claims of the earlier patents and those of the '501 patent and would see trends emerge. Between August 2003 (filing of the '987 patent) and January 2015 (issue date of the '215 patent), the Applicant pursued claims to a “nucleic acid molecule” or a “ribonucleic acid” that were double-stranded and required certain patterns of modifications. For example, the '987, '245, '370, and '215 patent claims require certain patterns of nucleotide modifications across the two strands, where the pattern of modifications is limited to ***contiguous alternating*** modified ribonucleotides that alternate with unmodified or differently modified ribonucleotides in a strand. In fact, the earliest patents ('987 and '245 patents) were limited to contiguous alternating single ***2'-O-methyl*** modified ribonucleotides that alternate with ***unmodified*** ribonucleotides or modified ribonucleotides. By contrast, in the subsequent patents ('370 and '215 patents), the claims were

directed to nucleic acid molecules comprising contiguous alternating modified ribonucleotides that alternate with “unmodified or modified ribonucleotides” that either expand the modifications for the modified ribonucleotides to “O-alkyl” (’370 patent) or place no limits on the modifications for the modified or the alternating modified ribonucleotides (’215 patent).

171. However, by December 2015 (the issue date of the ’092 patent), the claims that issued in the ’092 patent do not call for any such contiguous alternating patterns. Instead, they require the ribonucleic acid to have a different pattern of *two different 2’-modified groups of nucleotides* (the modified and flanking groups) flanking one another on the same strand. It was for the first time in the ’423 patent (issued in July 2015), that the term “a double-stranded siRNA molecule” and the requirements for RNAi activity, and additionally increased stability, and/or inhibition of the expression of the target nucleic acid were introduced into the claims. And similar to the ’092 patent, the ’423 patent claims also require the double-stranded siRNA molecules to have a pattern of *two different 2’-modified groups of nucleotides* (the modified and flanking groups) flanking one another on one or both strands. And now, based on the identical specifications from August 2003, the ’501 patent (issued in October 2017) claims “a double-stranded siRNA molecule against a target nucleic acid” and requires the “double-stranded siRNA molecule” to have a “contiguous alternating” pattern of

2'-O-alkyl modified ribonucleotides alternating with unmodified or differently modified ribonucleotides on both strands, and places no limits on the types of ribonucleotide modifications for the differently modified ribonucleotides. By claiming so broadly, the '501 patent moves the claims farther from the limited disclosures in the patent specification (and those of the priority applications) and, therefore, fails to describe or enable the vast genus of 2'-O-alkyl modified siRNA molecules now claimed. *See* Sections XI-XII below.

## **XI. The '501 Claims Lack Written Description Support**

### **A. The '501 Patent Specification Fails to Demonstrate Possession of the Genus of Modified siRNA Molecules**

172. It is my understanding that to provide written description support for generic claims like the challenged claims, the specification must describe species sufficient to demonstrate possession of the vast genus of modified siRNA molecules now claimed. *See also* ¶¶ 30-32 above. In my opinion, the '501 patent specification does not describe the claimed invention in this manner.

#### **i. The claimed genus of double-stranded siRNA molecules is vast and encompasses structurally diverse molecules**

173. Claims 1-30 are directed to genera of chemically-modified double-stranded siRNA molecules. Exh. 1001 ('501 patent) at claims 1-30. The claims require that the double-stranded siRNA molecule comprising a first and a second strand comprise complementary first and second stretches having the same length, respectively, wherein (i) the first and second *stretches* each comprise contiguous

alternating 2'-O-alkyl modified ribonucleotides alternating with unmodified or differently modified ribonucleotides (ii) the 2'-O-alkyl modified ribonucleotides of the first *strand* are shifted by one ribonucleotide relative to the 2'-O-alkyl modified ribonucleotides on the second *strand*, and (iii) each of the first and second *stretches* consists of at least 17 and fewer than 30 ribonucleotides. Exh. 1001 ('501 patent) at claim 1. In my opinion, the claims cover a potentially unlimited genus of structurally diverse double-stranded siRNA molecules for the reasons discussed below.

**a. The siRNA nucleotide sequence and target gene**

174. Neither the claimed siRNA molecules nor their target nucleic acids are defined by nucleotide sequence or structure. In fact, the claims are not limited to any particular siRNA molecules targeting any particular nucleic acid sequence or gene in any particular organism. The claimed genus of siRNA encompasses siRNA molecules targeting any nucleic acid sequences in any gene, including genes that have yet to be identified, in any organism.

175. The '501 patent specification identifies in a preferred embodiment that "the target gene is selected from the group comprising structural genes, housekeeping genes, transcription factors, motility factors, cell cycle factors, cell cycle inhibitors, enzymes, growth factors, cytokines and tumor suppressors." Exh. 1001 ('501 patent) at col. 6:4-9. Even assuming that the claimed siRNA molecules

are limited to those targeting the non-limiting list of genera of target genes (which I understand they are not), the claims would encompass hundreds of thousands of siRNA molecules.<sup>16</sup> See ¶¶ 176-177 below. But the specification only includes examples of siRNA molecules (none of which meet the requirement for two different modifications) that target one of three genes, namely PTEN, Akt, and p110 $\beta$ . Exh. 1001 ('501 patent) at Examples 1-13; *see also* ¶¶ 71-81 above. And they all target the same nucleotide sequence for PTEN, Akt, or p110 $\beta$ , respectively. Exh. 1001 ('501 patent) at Examples 1-13, Figs. 1-19.

176. Even for a particular target gene, there are thousands of options for a target site. Tiling along a transcript one nucleotide at a time would produce the same number of siRNA sequence options as the number of nucleotides in the transcript (minus the length of the siRNA). The average human mRNA length is 2,700 nucleotides, according to the NCBI human genome (build 33) as of 2003.

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<sup>16</sup> I understand from counsel that claims are generally not limited to a preferred embodiment absent some kind of disclaimer or consistent reference to the preferred embodiment as the “present invention” throughout the patent that would limit the scope of the claim.

Gene Identification Method	EST Alignment	Predicted	mRNA Alignment
Number of Genes	3,295	11,972	17,708
Exons/Gene (Avg)	4	5	12
Exon Length (Avg)	440	158	226
Intron Length (Avg)	5,605	10,512	5,478
mRNA Length (Avg)	2,127	820	2,741
Gene Length (Avg)	13,940	43,918	55,147

Exh. 1052 (NCBI News, *First Version of Human Genome Reference Sequence Debuts on DNA's 50<sup>th</sup>* (Spring 2003)) at p. 2, Table 1 (listing statistics for the first version of the human genome reference sequence, NCBI human genome build 33).

177. Assuming a length of 20 nucleotides for the siRNA, the number of siRNA sequence options would be about 2,680 for an average gene. Given that there were approximately 17,700 human genes identified as of 2003, the number of siRNA sequence options for those genes would be almost 47.5 million. As of 2017, the number of protein-coding genes in the human genome is estimated to be about 20,000. Exh. 1031 (Dolgin E., *The Greatest Hits of the Human Genome*, Nature, 551:427-31 (2017)) at p. 427. The number of siRNA sequence options for a 20-nt siRNA for those human genes would be about 53.6 million. And even for a particular siRNA sequence, the genus would encompass thousands of structurally diverse double-stranded siRNA molecules having diverse chemical and duplex architectures.



**b. Chemical architecture of the siRNA molecules**

178. First, the claims require that the first and second *stretches* of the first and second strands of the double-stranded siRNA molecule each comprise contiguous alternating *2'-O-alkyl* modified ribonucleotides alternating with *unmodified or differently modified* ribonucleotides. Exh. 1001 ('501 patent) at claim 1. The claims also require the 2'-O-alkyl modified ribonucleotides of the first strand to be shifted by one relative to the 2'-O-alkyl modified ribonucleotides of the second strand, which the patent specification refers to as a phase-shifted arrangement. Exh. 1001 ('501 patent) at claim 1; *see also* cols. 17:28-36, 17:56-18:2, 18:12-18, Fig. 2B. Based on the claim language, the claims include siRNA molecules having various patterns or motifs within each stretch of each strand, including:

- 2'-O-alkyl modified (M) ribonucleotides alternating with unmodified (U) ribonucleotides (*e.g.*, M/U/M motif);
- 2'-O-alkyl modified (M) ribonucleotides alternating with differently modified (D) ribonucleotides (*e.g.*, M/D/M motif); and
- 2'-O-alkyl modified (M) ribonucleotides alternating with unmodified (U) or differently modified (D) ribonucleotides, *i.e.*, a mixed motif (*e.g.*, M/U/M/D motif).

179. With respect to the 2'-O-alkyl modified ribonucleotides, based on the specification, a person of ordinary skill in the art would understand that a “2'-O-alkyl” group includes, but it is not limited to, C<sub>1</sub> to C<sub>12</sub>, primary, secondary, tertiary, substituted or unsubstituted alkyl groups attached to an oxygen. Exh.

1001 ('501 patent) at col. 13:44-46; *see also* ¶ 185 below. A person of ordinary skill in the art would therefore understand that the “M”s in the above exemplary motifs could be independently  $M_1, M_2, \dots M_m$  where  $m$  represents the number of types of 2'-O-alkyl modifications for the 2'-O-alkyl modified ribonucleotides.

180. With respect to the differently modified ribonucleotides, the patent specification makes clear that the modification of the differently modified ribonucleotides can be the same or different for the various ribonucleotides. Exh. 1001 ('501 patent) at col. 16:53-61; *see also* ¶ 67 above. Thus, a person of ordinary skill would understand that any of the “D”s in the above exemplary motifs could be independently  $D_1, D_2, \dots D_n$  where  $n$  represents the number of types of modifications for the differently modified ribonucleotides.

181. Second, as to the modifications themselves, the '501 patent specification provides a non-limiting list of possible nucleotide modifications for either terminal or non-terminal (*i.e.*, internal) nucleotides along the siRNA strands, many of which are sub-genera encompassing numerous species, including:

- inverted (deoxy) abasics
- fluoro
- bromo
- CF<sub>3</sub>
- imidazole
- thioate
- substituted lower alkyl
- OCF<sub>3</sub>
- O-, S-, or N-alkyl
- SOCH<sub>3</sub>
- ONO<sub>2</sub>
- N<sub>3</sub>
- heterocycloalkaryl
- polyalkylamino
- as, among others, described in European patents EP 0 586 520 B1 or EP 0 618 925 B1<sup>17</sup>
- amino
- chloro
- CN
- methoxy
- carboxylate
- C<sub>1</sub> to C<sub>10</sub> lower alkyl
- alkaryl or aralkyl
- OCN
- O-, S-, or N-alkenyl
- SO<sub>2</sub>CH<sub>3</sub>
- NO<sub>2</sub>
- heterocycloalkyl
- aminoalkylamino
- or substituted silyl

Exh. 1001 ('501 patent) at cols. 13:35-43, 15:50-56; *see also* ¶¶ 65-67 above. A person of ordinary skill in the art would understand that even for a ribonucleotide modification, there are numerous positions within the ribonucleotide where the modifications can be incorporated such as the 2'-, 3'-, or 5'- positions of the ribose sugar, the nitrogenous base, or via an internucleoside linkage. *See* ¶ 39 and Fig. 1 above. Consistent with that understanding, the '501 patent specification states that the various modifications are “preferably located at the ribose moiety of a nucleotide” and provides a non-limiting list of positions where the modifications

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<sup>17</sup> EP 0 586 520 B1 and EP 0 618 925 B1 concern modified antisense oligonucleotides. Exh. 1062 (EP 0 586 520 B1); Exh. 1063 (EP 0 618 925 B1). To the extent there are any additional modifications enumerated in either of the two references, they would only serve to further expand the genus.

can be “attached to or replace any of the OH-groups of the ribose moiety, including but not limited to the 2’OH, 3’OH, and 5’OH position.” Exh. 1001 (’501 patent) at col. 14:21-27. *See also* ¶ 66 above.

182. Of the enumerated modifications, the ’501 patent specification provides only a few definitions. The ’501 patent specification defines the term “inverted abasics” as “nucleotides, either deoxyribonucleotides or ribonucleotides which do not have a nucleobase moiety. This kind of compound is, among others, described in Sternberger et al. (2002), *Antisense. Nucl. Ac. Drug Dev.* 12:143.” Exh. 1001 (’501 patent) at col. 14:28-32.

183. The ’501 patent specification defines the term “alkyl or any term comprising ‘alkyl’ means any carbon atom chain comprising 1 to 12, preferably 1 to 6 and more, preferably 1 to 2 C atoms.” Exh. 1001 (’501 patent) at col. 13:44-46.<sup>18</sup> A fair reading of the term “alkyl” by one of ordinary skill in the art would be that it includes, but is not limited to, C<sub>1</sub> to C<sub>12</sub>, which could be substituted or unsubstituted and/or contain primary, secondary, and tertiary alkyl groups. Thus,

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<sup>18</sup> Neither EP1 nor the ’541 provisional defines “alkyl.” Instead, they identify among the list of various modifications: “C<sub>1</sub> to C<sub>10</sub> lower alkyl, substituted lower alkyl, alkaryl or aralkyl.” *See e.g.*, Exh. 1007 (’541 provisional) at p. 12. A fair reading of this disclosure would be that the term “alkyl” includes, but is not limited to, C<sub>1</sub> to C<sub>10</sub> lower alkyl or substituted lower alkyl groups. Neither EP1 nor the ’541 provisional provides any definition, guidance, or examples of any alkyl-modified siRNA molecules, including “substituted” alkyl groups, and the various possible substitutions, and whether any such “alkyl” modified siRNA molecules would have RNAi activity.

an “alkyl” modification would encompass numerous possible species.

184. The '501 patent lists “C<sub>1</sub> to C<sub>10</sub> lower alkyl” as one of the modifications. A fair reading of this term by a person of ordinary skill in the art would be that it includes C<sub>1</sub> to C<sub>10</sub>, which could be unsubstituted or substituted and/or contain primary, secondary, and tertiary alkyl groups. The '501 patent separately calls out “substituted lower alkyl” as another possible modification but provides no description for the term. A fair reading of this term would be that it includes, but is not limited to, C<sub>1</sub> to C<sub>10</sub>, which is substituted, *i.e.*, has additional chemical groups on it.<sup>19</sup> Thus, each of the “C<sub>1</sub> to C<sub>10</sub> lower alkyl” and “substituted lower alkyl” sub-genera, which appear to narrow the breadth of “alkyl” by use of “lower,” would still encompass numerous possible species.

185. As to O-alkyl (*i.e.*, alkoxy), S-alkyl, or N-alkyl groups, the '501 patent specification does not define the term “O-, S-, or N-alkyl.” A fair reading would be that like “alkyl,” O-alkyl, S-alkyl, and N-alkyl include, but are not limited to, C<sub>1</sub> to C<sub>12</sub>, which could be substituted or unsubstituted and/or contain primary, secondary, and tertiary alkyl groups attached to an oxygen, sulphur, or nitrogen, respectively. Thus, each of the O-alkyl, S-alkyl, or N-alkyl sub-genera

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<sup>19</sup> “Substituted” has no common meaning to chemists other than to indicate that a particular portion of a molecule or a particular chemical moiety has additional chemical groups on it.

would encompass numerous possible species.<sup>20</sup>

186. As to O-alkenyl, S-alkenyl, or N-alkenyl groups, the '501 patent specification does not define the term "O-, S-, or N-alkenyl" or "alkenyl." A person of ordinary skill in the art would understand that an "alkenyl" group is formed by the removal of a hydrogen atom bonded to a doubly bonded carbon from an alkene.<sup>21</sup> A fair reading would be that the terms "O-, S-, or N-alkenyl" or "alkenyl" include, but are not limited to, C<sub>1</sub> to C<sub>12</sub>, which could be substituted or unsubstituted and/or contain primary, secondary, or tertiary alkenyl groups attached to an oxygen, sulphur, or nitrogen, respectively. Thus, each of the O-alkenyl, S-alkenyl, or N-alkenyl sub-genera would encompass numerous possible species.

187. The '501 patent specification does not define the term "heterocycloalkyl." A person of ordinary skill in the art would understand "heterocycloalkyl" refers to a radical of a saturated monocyclic hydrocarbon (*i.e.*, a cycloalkane) (with or without side chains) in which at least one ring carbon is

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<sup>20</sup> As noted above, the term "alkoxy" was not included in the list of preferred modifications in EP1 or the '541 provisional. Both applications merely list among the various modifications, O-, S- or N-alkyl modifications, with no limitation on the number of carbon atoms. *See e.g.*, Exh. 1007 ('541 provisional) at p. 12.

<sup>21</sup> The standard IUPAC meaning of an "alkene" is acyclic branched or unbranched hydrocarbons having one carbon-carbon double bond and the general formula C<sub>n</sub>H<sub>2n</sub>. Exh. 1068, Pure and Applied Chemistry, 1995, 67, 1307 (*Glossary of class names of organic compounds and reactivity intermediates based on structure (IUPAC Recommendations 1995)*) ("IUPAC 1995") at p. 1313.

replaced with an atom other than hydrogen (*i.e.*, a heteroatom).<sup>22</sup> A fair reading would be that “heterocycloalkyl” encompasses numerous cyclic hydrocarbon ring structures, including but not limited to, C<sub>3</sub> to C<sub>12</sub>, in which at least one ring carbon is replaced with a heteroatom *i.e.*, an atom other than carbon. Thus, “heterocycloalkyl” sub-genera would encompass numerous possible heterocyclic species.

188. As to the terms “alkaryl or aralkyl” and “heterocycloalkaryl,” there is no definition or guidance provided in the ’501 patent specification. The standard IUPAC meaning of “aryl” is a radical of an aromatic ring. This definition embraces polycyclic aromatic hydrocarbons as well as rings in which a carbon atom has been replaced by a heteroatom.<sup>23</sup> A fair reading of the term “alkaryl or aralkyl” would be that they encompass various alkyl-substituted aryl groups or aryl-substituted alkyl groups. A fair reading of “heterocycloalkaryl” would be that it refers to heterocycloalkyl-substituted aryls. *See also* ¶ 187 above. Thus, each of the “alkaryl or aralkyl” and “heterocycloalkaryl” sub-genera would encompass

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<sup>22</sup> The standard IUPAC meaning of “cycloalkyl” group is a radical of a saturated monocyclic hydrocarbon (*i.e.*, cycloalkane) (with or without side chains) formed by the removal of a hydrogen atom from a ring carbon atom. Exh. 1068 (IUPAC 1995) at p. 1330.

<sup>23</sup> “Aryl” groups are groups derived from arenes (monocyclic and polycyclic aromatic hydrocarbons) by removal of a hydrogen atom from a ring carbon atom. Groups similarly derived from heteroarenes are sometimes subsumed in this definition. Exh. 1068 (IUPAC 1995) at p. 1320; *see also* pp. 1318, 1340.

numerous possible species, including various heterocyclic ring structures.

189. Finally, the '501 patent specification does not provide any definition or guidance as to the terms “aminoalkylamino,” “polyalkylamino,” or “substituted silyl.” A fair reading of the terms “aminoalkylamino” and “polyalkylamino” would be that they refer to various amino derivatives, including amine-linked alkylamino derivatives. A fair reading of “substituted silyl” would be that it refers to a silyl group ( $\text{H}_3\text{Si}-$ ) having additional chemical groups on it, encompassing numerous silyl derivatives.<sup>24</sup> Thus, each of “aminoalkylamino,” “polyamino,” or “substituted silyl” sub-genera would encompass numerous possible species.

190. Claims 1-9, 12, 14, 16, 18, 20, 23, and 24 require that the siRNA molecules comprise **2'-O-alkyl** modified ribonucleotides that alternate with unmodified or differently modified ribonucleotides (including as a mixed motif for at least claims 1-9, 12, and 20) along the stretches of both strands of the siRNA molecules. Exh. 1001 ('501 patent) at claims 1-9, 12, 14, 16, 18, 20, 23, 24. As noted above, a person of ordinary skill in the art would understand the term “2'-O-alkyl” to encompass numerous possible modifications, including but not limited to,  $\text{C}_1$  to  $\text{C}_{12}$ , primary, secondary, tertiary, substituted or unsubstituted groups. Exh. 1001 ('501 patent) at col. 13:44-46; *see also* ¶¶ 178-179, 183-185 above. Thus,

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<sup>24</sup> The standard IUPAC meaning of a “silyl” group is  $\text{H}_3\text{Si}-$  or is commonly applied to hydrocarbyl derivatives of the silyl group  $\text{R}_3\text{Si}-$ . Exh. 1068 (IUPAC 1995) at p. 1366.



even for the claimed siRNA molecules having a 2'-O-alkyl modification, there would be numerous arrangements, permutations, and combinations possible along the ribonucleotide positions of the stretches of both strands for the various **2'-O-alkyl** modified ribonucleotides that alternate with unmodified or differently modified ribonucleotides, resulting in a vast collection of modified siRNA molecules.

191. Claims 1-10, 16, 17, 20, and 24 place no limits on the types of modifications for the **differently modified** nucleotides, and only require that they alternate with 2'-O-alkyl (claims 1-10, 16, 20, 24) or 2'-O-methyl (claim 17) modified ribonucleotides (including as a mixed motif for at least claims 1-10 and 20) on both stretches of both strands of the siRNA molecules. Exh. 1001 ('501 patent) at claims 1-10, 16, 17, 20, 24. Even if the claims were limited to the enumerated modifications for the differently modified ribonucleotides (which I understand they are not), the genus of the modified siRNA molecules would be vast. Exh. 1001 ('501 patent) at col. 13:35-43; *see also* ¶¶ 178-189 above. For example, there are numerous arrangements, permutations, and combinations possible along the ribonucleotide positions of the stretches of both strands for the various **differently modified** ribonucleotides that alternate with 2'-O-alkyl or 2'-O-methyl modified ribonucleotides, resulting in a vast collection of modified siRNA molecules.

192. Dependent claims 12 and 18 appear to limit the type of modifications for the differently modified ribonucleotides of the siRNA molecules to an *amino, fluoro, different alkoxy, or alkyl* modification at the *2'-position*. Exh. 1001 ('501 patent) at claims 12, 18. A person of ordinary skill in the art would understand that even for these recited modifications, the claims would encompass a vast collection of modified siRNA molecules. For example, a person of ordinary skill in the art would understand the terms “different alkoxy” or “alkyl” to encompass numerous species, including, but not limited to, C<sub>1</sub> to C<sub>12</sub>, primary, secondary, tertiary, substituted or unsubstituted groups, as long as the modification is different from the 2'-O-alkyl modification for the modified ribonucleotides. See ¶¶ 183, 185 above. Thus, there are numerous arrangements, permutations, and combinations possible along the ribonucleotide positions of the stretches of both strands for the differently modified ribonucleotides having an amino, fluoro, different alkoxy, or alkyl modification at the 2'-position that alternate with 2'-O-alkyl modified ribonucleotides, resulting in a vast collection of modified siRNA molecules.

193. Dependent claims 13, 19, 22, and 25-30 require the siRNA molecules to comprise a *2'-fluoro* modification for the differently modified ribonucleotides and require that they alternate with 2'-O-methyl modified ribonucleotides. Exh. 1001 ('501 patent) at claims 13, 19, 22, 25-30. Even for the claimed siRNA molecules having a 2'-O-methyl/2'-fluoro ribonucleotide motif, there are

numerous arrangements, permutations, and combinations possible along the ribonucleotide positions of the stretches of both strands of the siRNA molecules, resulting in a vast collection of modified siRNA molecules. Likewise, dependent claims 11, 15, and 21 require the siRNA molecules to comprise 2'-O-methyl modified ribonucleotides that alternate with *unmodified* ribonucleotides. Exh. 1001 ('501 patent) at claims 11, 15, 21. Even for the claimed siRNA molecules having a 2'-O-methyl/unmodified ribonucleotide motif, there are numerous arrangements, permutations, and combinations possible along the ribonucleotide positions of the stretches of both strands of the siRNA molecules, resulting in a vast collection of modified siRNA molecules.

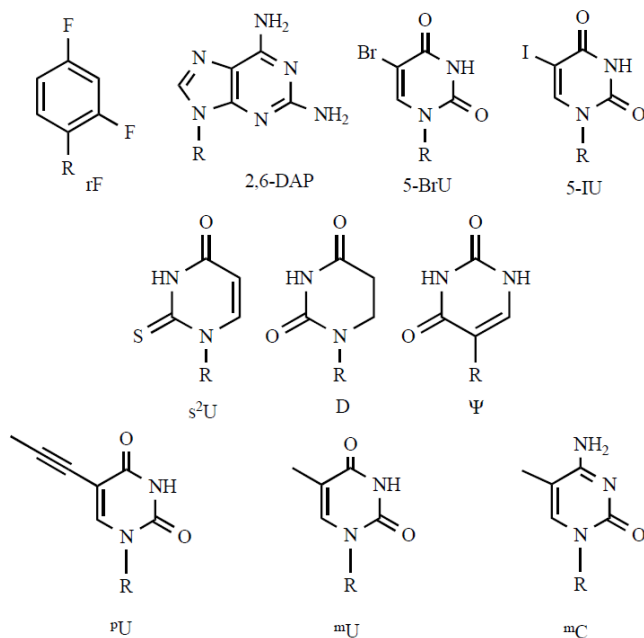
194. With the exception of claim 2, the '501 patent claims encompass, and in claims 3-8 and 26-30 require, the siRNA molecules to have an overhang on at least one strand. Exh. 1001 ('501 patent) at claims 1-30. Except for claims 4 and 26, which specify a length of one to eight nucleotides for the overhang, none of the claims specify any limit for the length of the overhangs, and encompass siRNA molecules having varied strand lengths. None of the claims specify the nucleotide composition for the overhangs either, and thus, can comprise unmodified or modified ribonucleotides, deoxyribonucleotides, or various combinations of them. Exh. 1001 ('501 patent) at claims 1, 3-30. And when one factors in the overhang requirements, the number of siRNA molecules encompassed by the '501 patent

claims is exponentially higher as there are numerous permutations, combinations, and arrangements possible along the ribonucleotide positions of the overhangs, resulting in a vast collection of structurally-diverse modified siRNA molecules.

195. Third, claims 1-30 only require that the double-stranded siRNA molecule “comprises” a first and a second strand, each of which “comprises” a first and second stretch, respectively, and each stretch “comprises” contiguous alternating 2’-O-alkyl modified ribonucleotides that alternate with unmodified or differently modified ribonucleotides. Exh. 1001 (’501 patent) at claim 1; *see also* claims 2-30. Based on the claim language and the use of the word “comprises,” a person of ordinary skill in the art would understand that the claims cover siRNA molecules having **any** chemical modification, including additional modifications and modifications that have even yet to be discovered, so long as the siRNA molecules comprise contiguous alternating 2’-O-alkyl modified ribonucleotides alternating with unmodified or differently modified ribonucleotides as required by the claims.

196. For example, apart from the modifications discussed above, the claimed siRNA molecules could include any type of nucleotide base modifications, including, but not limited to, 2,4-difluorotoluyil ribonucleoside; 2,6-diaminopurine; 5-bromouridine; 5-iodouridine; dihydrouridine; pseudouridine; 5-propynyluridine; 5-methyluridine; and 5-methylcitydine. Exh. 1032 (Gaglione M. and Messere A.,

*Recent Progress in Chemically Modified siRNAs*, Mini-Reviews in Medicinal Chemistry, 10(7):578-95 (2010)) (“Gaglione 2010”) at p. 581; *see also* Exh. 1035 (Deleavey 2009) at Fig. 16.3.5 (depicting base-modified nucleotides using in siRNA).

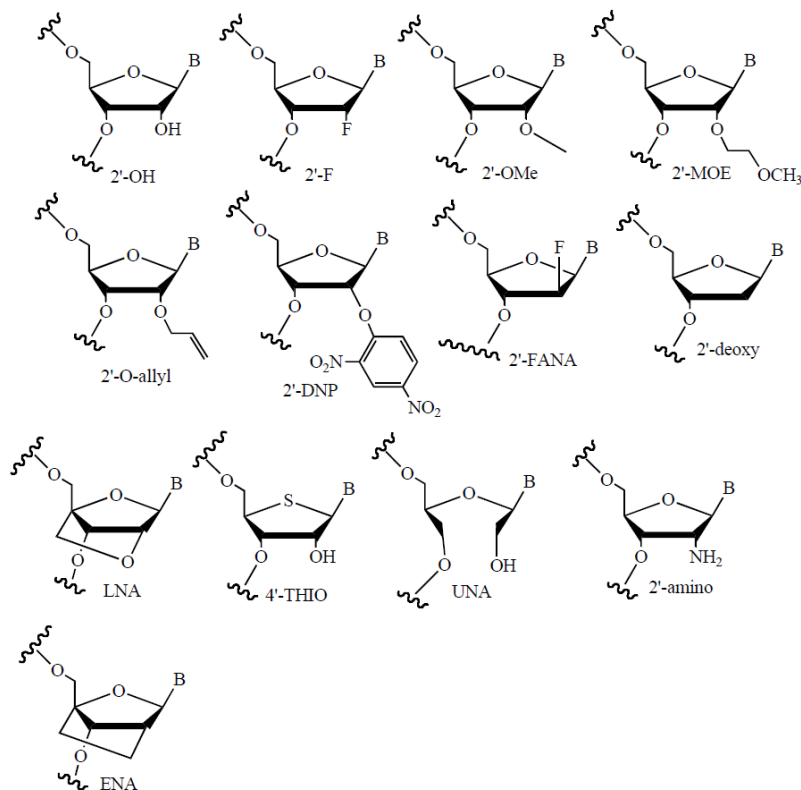


**Fig. (1).** Nucleobase modifications in siRNA. rF: 2,4-difluorotoluidyl ribonucleoside; 2,6-DAP: 2,6-diaminopurine; 5-BrU: 5-bromouridine; 5-IU: 5-Iodouridine; s<sup>2</sup>U: 2-thiouridine; D: dihydrouridine; ψ: pseudouridine; P<sup>1</sup>U: 5-propynyluridine; m<sup>1</sup>U: 5-methyluridine; m<sup>1</sup>C: 5-methylcytidine

Exh. 1032 (Gaglione 2010) at p. 581, Fig. 1.

197. The claimed siRNA molecules could include any type of nucleotide sugar modifications, including, but not limited to, 2'-O-(2-methoxyethyl) (2'-MOE), 2'-O-allyl, 2'-deoxy-2'-fluoro-β-D-arabino nucleic acids (2'-FANA), locked nucleic acid (LNA), and unlocked nucleic acid (UNA). The claimed siRNA molecules could also include sugar modifications at positions other than the 2'-position such as 4'-thio. Exh. 1032 (Gaglione 2010) at pp. 582-83; *see also*

Exh. 1035 (Deleavey 2009) at Fig. 16.3.3 (depicting sugar units that have been successfully used to modify siRNA duplexes).



**Fig. (2).** Sugar modifications in siRNA. 2'-F: 2'-fluoroRNA; 2'-OMe: 2'-O-Methyl RNA; 2'-MOE: 2'-O-(2-methoxyethyl)RNA; 2'-O-allyl: 2'-O-allyl RNA; 2'-DNP: 2'-O-(2,4-dinitrophenyl) RNA; 2'-FANA: 2'-deoxy-2'-fluoro- $\beta$ -D-arabino nucleic acids; 2'-deoxy: 2'-deoxyribonucleotide; LNA: locked nucleic acid; 4'-Thio: 4'-thioribonucleoside; UNA: unlocked nucleic acid; 2'-amino: 2'-amino RNA; ENA: ethylene-bridge nucleic acid.

Exh. 1032 (Gaglione 2010) at p. 583, Fig. 2 (showing a non-exhaustive list of sugar modifications).

198. The claimed siRNA molecules could also include any type of siRNA backbone modifications, including, but not limited to, phosphodiester, phosphorothioate, boranophosphate, amide-linked, morpholino, and peptide nucleic acid (PNA). Exh. 1032 (Gaglione 2010) at pp. 583-84; *see also* Exh. 1035 (Deleavey 2009) at Fig. 16.3.4 (depicting internucleotide linkages used in siRNA).

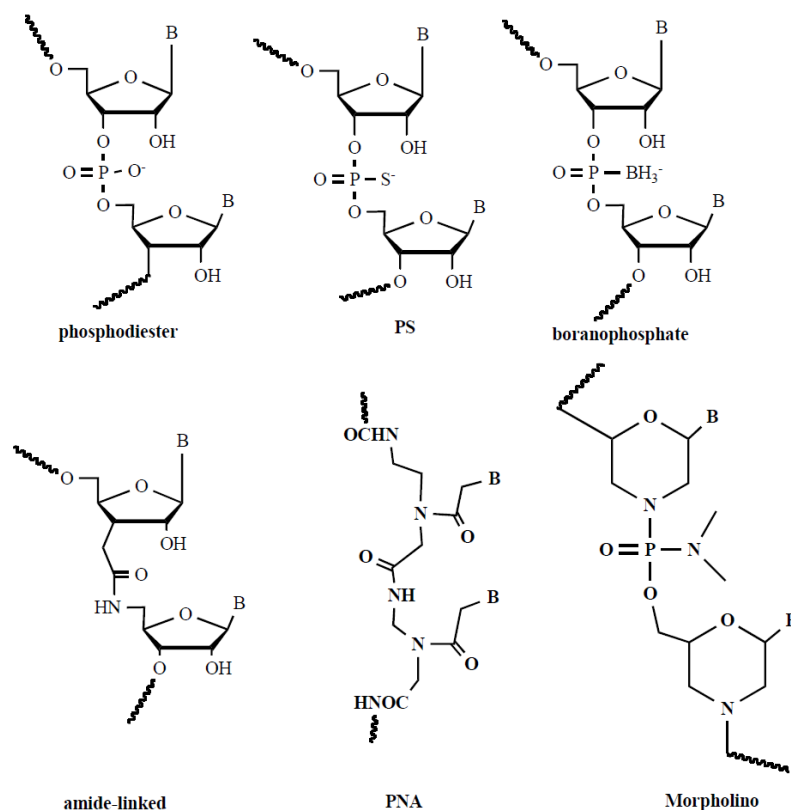


Fig. (3). Backbone modifications in siRNA. PS: phosphorothioate linkage; PNA: peptide nucleic acid.

Exh. 1032 (Gaglione 2010) at p. 584, Fig. 3 (showing exemplary siRNA backbone modifications).

199. Many of these modifications include dramatically redesigned sugar structures or other novel chemistries nowhere disclosed in the '501 patent and that were discovered after August 2002 (the filing of the earliest applications to which the '501 patent claims priority) as being useful in duplex siRNA. Exh. 1035 (Deleavey 2009) at Figs. 16.3.3-16.3.5 and pp. 16.3.5-10. Moreover, for each of the various chemical modifications, there are numerous permutations, combinations, and arrangements possible along the ribonucleotide positions of the stretches of both strands. Thus, the '501 patent claims cover a vast genus of

chemically-modified siRNA molecules.

**c. The duplex architecture of the siRNA molecules**

200. Claims 1 and 9-24 do not specify any duplex architecture for the double-stranded siRNA molecules and only require them to have a double-stranded structure; claims 2-8 and 25-30 specify that the double-stranded siRNA molecules be blunt ended on one or both ends, or have overhangs at the 3'- or 5'-ends of the first or second strands. Exh. 1001 ('501 patent) at claims 1-30; *see also* ¶ 194 above. The claims also require each *stretch* of the double-stranded siRNA molecule consist of at least 17 and fewer than 30 ribonucleotides. Exh. 1001 ('501 patent) at claims 1-30. Thus, the claims encompass siRNA molecules having varied duplex stretch lengths ranging from 17 to 30 ribonucleotides. Except for claim 9, which requires the first and second *strands* to consist of at least 18 nucleotides, the claims do not place any limits on the overall length of the two *strands* of the double-stranded siRNA molecule and would encompass siRNA molecules having additional nucleotides on one or both ends of one or both strands, *i.e.*, the overhangs. Claims 4 and 26 specify that the overhangs consist of one to eight nucleotides. Exh. 1001 ('501 patent) at claims 4, 26. Thus, the claims would also encompass siRNA molecules having various structural (or duplex) architectures, including, but not limited to, blunt ends on one or both ends, overhangs on the 3'- or 5'-end or both of one or both strands, fork siRNA, or other



asymmetric duplex architectures. Exh. 1090 (Chang C. *et al.*, *Structural Diversity Repertoire of Gene Silencing Small Interfering RNAs*, *Nucleic Acid Therapeutics*, 21(3):125-31 (2011)) (“Chang 2011”) at p. 126; Exh. 1032 (Gaglione 2010) at pp. 584-85; Exh. 1035 (Deleavey 2009) at Fig. 16.3.2 (depicting functional siRNA architectures); Exh. 1033 (Nawrot B. and Sipa K., *Chemical and Structural Diversity of siRNA Molecules*, *Current Topics in Medicinal Chemistry* 6(9):913-25 (2006)) (“Nawrot 2006”) at p. 916; *see also* ¶ 55 above.

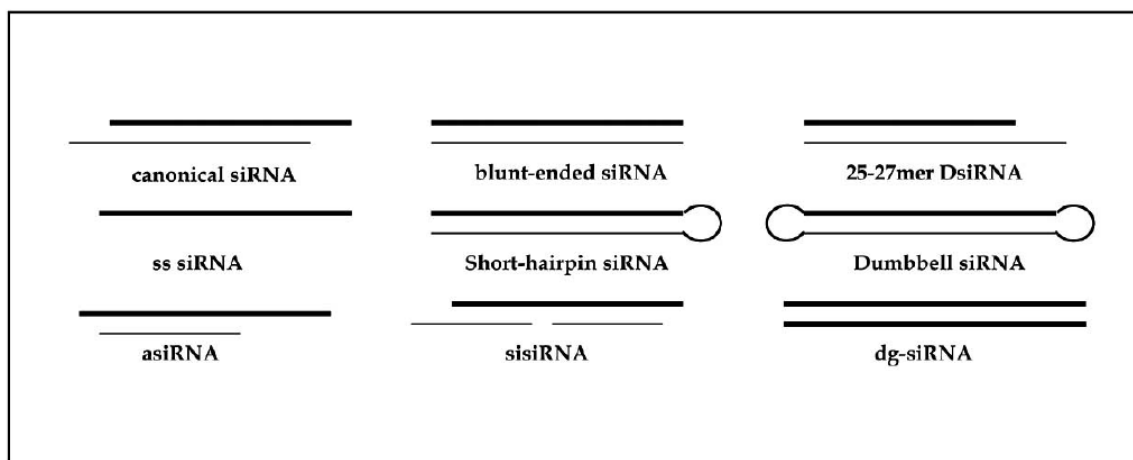


Fig. (4). Duplex architecture modifications. (Bold line: antisense strand, thin line: sense strand).

Exh. 1032 (Gaglione 2010) at p. 585, Fig. 4 (showing the structural diversity of siRNA duplexes). But the specification only includes examples of 2'-O-methyl modified siRNA molecules (none of which meet the requirement for two different modifications) that have a duplex length of 21 or 19 nucleotides and are blunt ended on both ends. Exh. 1001 ('501 patent) at Examples 7-11; *see also* ¶¶ 79-81 above.

201. Additionally, the claims could encompass siRNA molecules with any

type of end modifications and siRNA conjugates at either or both the 5'- and 3'-ends of one or both strands, including, but not limited to, conjugates comprising cholesterol, lipids, inverted abasics, biotin, polymers, peptides, antibodies, aptamers, and inorganic nanostructured materials with or without forming nanoconstructs. Exh. 1032 (Gaglione 2010) at pp. 586-87.

siRNA conjugate	site of conjugation on siRNA	target siRNA
Cholesterol-siRNA	3'-end of sense strand <sup>a</sup> 5'-end of sense strand	Apo-B-1 siRNA p38 MAP kinase siRNA
$\alpha$ -tocopherol-siRNA	5'-end of antisense strand <sup>a</sup>	Apo-B-1 siRNA
Lipid-siRNA	3'-end of sense strand <sup>a</sup>	Apo-B-1 siRNA
TAT-siRNA	3'-end amine of antisense strand <sup>b</sup> 5'-end thiol of sense strand	GFP siRNA/CDK9 siRNA p38 MAP kinase siRNA
Penetratin-siRNA	5'-end thiol of sense strand 5'-end thiol of sense strand	Luciferase siRNA p38 MAP kinase siRNA
Transportan-siRNA	5'-end thiol of sense strand <sup>b</sup>	Luciferase siRNA
PEG-siRNA	3'-end amine of sense strand <sup>b</sup> 5'-end thiol of sense strand	Luciferase siRNA/RecQL1 siRNA
Peptide analogue of insulin-like growth factor 1-siRNA	5'-end amine of sense strand	Insulin receptor substrate 1 siRNA
Antibody-siRNA	3'-end biotin of sense strand	Luciferase siRNA
Aptamer-siRNA	5'-end of sense strand 5'-end of sense strand	Lamini A/C siRNA Bcl-2 siRNA/PLK1 siRNA
Quantum dot-siRNA	5'-end thiol of sense strand	GFP siRNA
Iron oxide nanoparticle-siRNA	5'-end thiol of antisense strand	GFP siRNA/survivin siRNA
Carbon nanotube-siRNA	5'-end thiol of sense strand	Luciferase siRNA

<sup>a</sup> Chemical modification of backbone nucleotide: partial 2'-O-methyl sugar modification, phosphorothioate linkage.

<sup>b</sup> Reaction with duplex siRNA.

Exh. 1032 (Gaglione 2010) at p. 587, Table 1 (listing examples of siRNA conjugates).

202. In sum, although the '501 patent claims tend to impose some structural requirements (*i.e.*, the requirement for contiguous alternating 2'-O-alkyl modified ribonucleotides on both strands that alternate with unmodified or

differently modified ribonucleotides and are phase-shifted, the length of the stretches of the double-stranded siRNA molecule, the requirement for a blunt end or an overhang), the genus of chemically-modified double-stranded siRNA molecules that could be encompassed by the claims would nonetheless be structurally diverse, enormous, and potentially limitless. In my opinion, considering the various possibilities at each level—target gene, target nucleic acid sequences, siRNA duplex architecture, and at the nucleotide level, where there are numerous possible substitutions, arrangements, permutations, and combinations of modifications at each nucleotide position—the number of siRNA molecules encompassed by the claimed genus would be vast or potentially unlimited.

203. I understand from counsel that during prosecution of the '987 patent, the Applicant submitted a declaration from one of the inventors, Dr. Kaufmann, to explain “how to calculate the number of possible ways of arranging 2'-modified and unmodified nucleotides on sense and antisense strands of a double-stranded RNA molecule where each strand is 23 nucleotides long.” Exh. 1061 (Feb. 8, 2008 Declaration of Dr. Kaufmann) at ¶ 2. Dr. Kaufmann calculated that there were “ $2^{23}$  possible ways of arranging modified and unmodified nucleotides on each strand [of a 23-nucleotide dsRNA], and the arrangements on each strand are independent. Accordingly, in such a double stranded molecule there are  $2^{23} \times 2^{23} = 2^{46} = 7.0 \times 10^{13}$  possible arrangements.” Exh. 1061 (Feb. 8, 2008 Declaration of Dr.

Kaufmann) at ¶ 5. I also understand from counsel that Applicant represented to the U.S. patent office that Dr. Kaufmann's calculation assumed the dsRNA contained no modifications other than 2'-O-methyl modified ribonucleotides. Dr. Kaufmann's calculation of 70 trillion is consistent with my opinion that the genus of the siRNA molecules containing one modification is incredibly large. Here, the genus includes an unlimited list of modifications for the differently modified ribonucleotides and various 2'-O-alkyl modifications for the modified ribonucleotides, which can be used in many different combinations. Therefore, the claimed genus is enormous and potentially limitless.

204. The '501 patent attempts to claim a platform technology, *i.e.*, a modification strategy that can be broadly applied across most or all sequences and duplex lengths, and consistently provides active, stable siRNAs. However, the examples of internally modified siRNA molecules disclosed in the patent specification are of the same type: those having internal 2'-O-methyl modified ribonucleotides, with the remaining ribonucleotides being unmodified across both strands, and having the same duplex architecture (blunt ended on both ends). There are no examples in the patent specification of any siRNA molecules having two different ribonucleotide modifications within a strand as required by the claims. Nor are there examples of siRNA molecules having modification patterns other than 2'-O-methyl modifications or having varied duplex lengths and

architectures as encompassed by this structurally diverse genus, and no demonstration that such diverse siRNA molecules exhibit any RNA interference activity. There is also no data provided in the '501 patent specification to provide any confidence that the activity observed for the few, and structurally similar, 2'-O-methyl modified siRNA molecules that target one of three genes (none of which have two differently modified ribonucleotides, any modification other than a 2'-O-methyl modification, a duplex length of 17 nucleotides or greater than 21 nucleotides, or an overhang), can be extrapolated to the vast genus of modified siRNA molecules now claimed.

**ii. The specification fails to show that the inventors were in possession of the genus of siRNA molecules claimed**

**a. The 2'-O-alkyl modified and differently modified ribonucleotides**

205. The '501 patent claims a vast genus of modified siRNA molecules having *any 2'-O-alkyl* modification for the modified ribonucleotides and *any modification* for the differently modified ribonucleotides that have a phase-shifted arrangement across both strands and targeting any gene. Exh. 1001 ('501 patent) at claims 1-30; *see also* ¶¶ 178-199, 202-204 above. For example, claims 1-9, 12, 14, 16, 18, 20, 23, and 24 do not place any limits on the type of 2'-O-alkyl modification for the modified ribonucleotides; claims 1-10, 16, 17, 20, and 24 do not place any limits on the types of modifications for the differently modified

ribonucleotides; dependent claims 12 and 18 specify that the siRNA molecules comprise an amino, fluoro, different alkoxy, or alkyl modification at the 2'-position for the differently modified ribonucleotides; dependent claims 13, 19, 22, and 25-30 specify that the siRNA molecules comprise a 2'-O-methyl/2'-fluoro ribonucleotide motif; and claims 11, 15, and 21 specify that the siRNA molecules have a 2'-O-methyl/unmodified ribonucleotide motif. *See* ¶¶ 190-194 above. And, none of the claims 1-30 exclude the possibility that they could encompass siRNA molecules with any number of additional chemical modifications as long as they meet the minimum claim requirements. *See* ¶¶ 178-199 above.

206. However, the '501 patent specification does not adequately reflect the structural diversity of the claimed genus of modified siRNA. As described in paragraphs 71-83 above, the disclosures in the '501 patent are narrow. While the patent specification generally refers to "2'-O-alkyl" modifications and identifies 2'-O-methyl and 2'-O-ethyl groups as the preferred groups (Exh. 1001 ('501 patent) at col. 27:53-54), the examples are limited to siRNA molecules comprising **2'-O-methyl** modified and **unmodified** nucleotides (Exh. 1001 ('501 patent) at Examples 7-11) and those having terminal amino or inverted abasic end cap modifications with all of the nucleotides being unmodified (Exh. 1001 ('501 patent) at Example 6). And they all target one of three genes, namely PTEN, Akt, and p110β. Exh. 1001 ('501 patent) at Examples 1-13, Figs. 1-16. The examples

and specification state that *alternating 2'-O-methyl and unmodified* siRNAs are preferred, and more specifically, siRNA molecules containing alternating single 2'-O-methyl modified and unmodified nucleotides *across the entire strands*. Exh. 1001 ('501 patent) at cols. 17:53-18:11; *see also* col. 31:6-26 (discussing stability and RNAi activity of contiguous alternating single 2'-O-methyl modifications across both strands of molecules V13, V14, V15, and V12 and noting an unexpected preference for molecules V15 and V12 in terms of RNAi activity, where the 2'-O-methyl modifications occurred at every odd nucleotide position of the antisense strand beginning at the 5'-end through the 3'-end).

207. Of the approximately 120 siRNA molecules disclosed in the '501 patent specification, less than half (about 49) of them have internal 2'-O-methyl modified ribonucleotides on one or both strands, with the remaining ribonucleotides being unmodified.<sup>25</sup> Exh. 1001 ('501 patent) at Examples 7-11, Figs. 10-16; *see also* ¶¶ 79-81 above. And of those, about 25 2'-O-methyl modified siRNA molecules are non-duplicative; the majority of the 2'-O-methyl modified molecules of Figures 10-15 are duplicative of one another. Exh. 1001

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<sup>25</sup> The patent specification includes siRNA molecules that are unmodified and have overhangs, mismatches, or end modifications, or are fully modified across one or both strands or encompass loop structures. Exh. 1001 ('501 patent) at Examples 1-13, Figs. 1-18. However, these siRNA molecules would not be encompassed by the '501 patent claims at least because they do not contain stretches of modified ribonucleotides alternating with unmodified or differently modified ribonucleotides on both strands as required by the '501 patent claims.

(’501 patent) at Examples 7-11; Figs. 10-16; *see also* **Appendix B**. And of the 2’-O-methyl modified siRNA molecules, less than 10 are reported in the patent specification as having RNAi activity (*e.g.*, 86AB (PTENAB V10) (Fig. 11), 86AB (Fig. 12B), 94A2B1 (PTENAB V15), 94A2B2 (PTENAB V12), Akt1AB V5, and p110 $\beta$ AB V5). Exh. 1001 (’501 patent) cols. 28:42-44, 29:33-37, 30:4-13, 30:34-61, 31:6-17, 31:60-63, 32:10-15; *see also* Examples 7-11, Figs. 10-16. And the internally modified siRNA molecules have the same nucleotide sequence for PTEN, Akt, or p110 $\beta$ , respectively, and the same duplex architecture, *i.e.*, they are blunt ended on both ends. Exh. 1001 (’501 patent) at Figs. 10-16. A person of ordinary skill in the art would not consider the examples of siRNA molecules having one duplex architecture (*i.e.*, blunt ended) comprising one type of modification pattern (2’-O-methyl alternating with unmodified) targeting the same nucleotide sequences of one of three genes (PTEN, Akt, or p110 $\beta$ ) to be representative of the claimed genus of modified siRNA molecules having any duplex architecture comprising any type of modifications that target any gene.

208. A person of ordinary skill in the art would also not consider the examples of blunt ended siRNA molecules having **2’-O-methyl** ribonucleotides alternating with **unmodified** ribonucleotides to be representative of the claimed genus of modified siRNA molecules, which includes siRNA molecules having various modification patterns or motifs within each stretch of each strand of the



double-stranded siRNA molecule, including modified ( $M_m$ ) ribonucleotides having **any** 2'-O-alkyl modification alternating with (i) unmodified (U) ribonucleotides (*e.g.*,  $M_m/U/M_m$  motif); (ii) **differently** modified ( $D_n$ ) ribonucleotides (*e.g.*,  $M_m/D_n/M_m$  motif); and (iii) unmodified (U) **or** differently modified ( $D_n$ ) ribonucleotides, *i.e.*, a mixed motif (*e.g.*,  $M_m/U/M_m/D_n$  motif). *See also* ¶¶ 178-180 above. As noted above, the siRNA molecules having internal modifications in the figures, the examples, and the sequence listing, are of the **same type**: those having the **same** modification (2'-O-methyl) and pattern, with the remaining nucleotides being **unmodified** and the **same** duplex architecture (blunt ended on both ends). Exh. 1001 ('501 patent) at Examples 7-11; *see also* ¶¶ 79-81, 206-207 above.

Nowhere does the specification disclose any siRNA molecules having a modification pattern comprising any modification other than a **2'-O-methyl** modification for the **2'-O-alkyl** modified ribonucleotides as encompassed by at least claims 1-9, 12, 14, 16, 18, 20, and 23-24. The specification also fails to disclose any siRNA molecules having **two different** modifications as encompassed by at least claims 1-10, 12, 13, 16-20, 22, and 24-30, including a **2'-fluoro** modification as required by claims 13, 19, 22, and 25-30. The specification also fails to disclose any siRNA molecules having a **mixed** modification pattern comprising modified ribonucleotides that alternate with unmodified or differently modified ribonucleotides as encompassed by at least claims 1-10, 12, and 20.

There is also no data that any such siRNA molecules would be stable and active, which is the problem the '501 patent claims to solve. Exh. 1001 ('501 patent) at Examples 1-13, Figs. 1-19.

209. Moreover, while the '501 patent specification (and the priority applications) includes generic disclosures which when combined may encompass additional modified siRNA molecules of the '501 patent claims, those generic disclosures fail to demonstrate possession of the claimed genus of siRNA molecules.

210. As described in paragraphs 65-70 and Section X above, the '501 patent specification (and the priority applications, including their original claims) generally asserts that the ribonucleic acid molecules of the invention may be unmodified, may include end-modifications, may have patterns of modifications, including modified ribonucleotides alternating with unmodified or differently modified ribonucleotides, or may be blunt ended or have overhangs at the 5'-end or the 3'-end of one or both strands. The inventors include this broad generic text to summarize their invention, elements of which when combined may encompass siRNA molecules that fall within the scope of the '501 patent claims. However, in my opinion, each of these disclosures provides little more than an attempt to stake out a vast collection of siRNA molecules containing those features that the inventors hoped to possess. But as explained throughout my declaration, simply

defining the contours for these types of siRNA molecules in the nascent and complex field of RNAi is not enough to show possession. I believe this is the case for three reasons:

211. First, the inventors distinguish themselves from the prior art, for example in the use of overhangs, throughout the details of their examples and embodiments – yet the vast genera described in the summary of their invention encompass overhang-containing molecules which were included in the prior art, and for which they did not possess any embodiments in chemically-modified form. *See* ¶¶ 221-227 below. The inventors also state that siRNA molecules must have a minimum duplex length of 18-19 nucleotides for RNAi – yet the vast genera described in the summary of their invention encompass molecules having a 17-nucleotide duplex length, for which they also did not possess any embodiments in chemically modified form, and which had RNAi activity. *See* ¶¶ 215-219 below. The inventors cannot explicitly distinguish their invention from much of the chemical space included in these broad generic statements at the same time as claiming that their invention actually encompasses all of this chemical space.

212. Second, the broad, generic text used to summarize their invention is so broad that it fails to provide any meaningful distinction as to the limits of their invention. The genera described in their summary of the invention section encompass even more of chemical space than is encompassed by the already vast

claims. *See* ¶¶ 173-204 above. A person of ordinary skill in the art would recognize these broad descriptions as reflecting a desire to possess a broad genus of double-stranded RNA molecules rather than actual possession of a specific class of useful siRNAs.

213. Third, the field of siRNA technology was in its infancy in 2002. *See* ¶¶ 228-235 below. Over the intervening 16 years, it has required extensive empirical work and multiple inventive steps to bring even a small subset of the molecules encompassed by these broad disclosures to the point of practical utility. The field of RNAi is complex; the underlying biological mechanisms and protein machinery involved in RNAi were not well understood and largely undiscovered in 2002; and the impact of chemical modifications on siRNA functionality and their interplay with target sequence and the duplex architecture could not be predicted and would need to be experimentally tested. *See* ¶¶ 228-235, 251-271 below. The field required extensive, creative, empirical work to reduce to practice a variety of the siRNAs encompassed by the generic text recited here, nowhere disclosed in the '501 patent specification.

214. Therefore, in my opinion, the inclusion of broad, generic statements, which taken together would encompass an unlimited collection of siRNAs, does not reflect possession of the invention now claimed in the '501 patent.

**b. The length of the double-stranded siRNA molecules and duplex architectures**

215. Claims 1-30 also require that the first and the second *stretches* of the claimed double-stranded siRNA molecules each consist of *at least 17 and fewer than 30* ribonucleotides. Exh. 1001 ('501 patent) at claims 1-30. Claim 8 requires that the each of the first and second *strands* consists of at least 18 nucleotides. Exh. 1001 ('501 patent) at claim 8. A person of ordinary skill in the art would understand that claims 1-30 include siRNA molecules having duplex lengths of *17 nucleotides*.

216. However, the '501 patent specification repeatedly states that the RNAi molecules must have a minimum duplex length of 18 or 19 nucleotides for RNAi activity. Exh. 1001 ('501 patent) at cols. 7:65-66 ("FIGS. 5A-5B show that the duplex length of the RNAi molecules has to be at least 18-19 nucleotides."), 12:22-26 ("the complementarity between the first stretch of nucleotides and the target RNA has to be 18-19 nucleotides; *stretches of as little as 17 nucleotides* even with two sequence specific overhangs *are not functional* in mediating RNAi") (emphasis added); 25:44-48 ("Having established the *minimal* duplex length of 18 or more than 18 nucleotides for functional siRNA molecules....") (emphasis added), *see also* cols. 12:46-60, 26:14-18; ¶¶ 74-75 above.

217. The examples of the '501 patent demonstrate that "the double strand duplex of the siRNA molecules has to be longer than 17 base pairs to show activity

whereas [siRNA] molecules with 17 base pair long duplexes or shorter ***are not functional*** even if sequence specific overhangs are added.” Exh. 1001 (’501 patent) at col. 24:49-54 (emphasis added). Based on the results, the inventors concluded that “***active siRNA duplexes should be at least 18 nt or longer***” and “the ***minimum*** requirement for optimum RNAi mediated interference is thus a ***duplex length of 18 or 19 nucleotides.***” Exh. 1001 (’501 patent) at cols. 24:65-25:2, 25:22-27 (emphasis added).

218. Additionally, in EP1, the ’541 provisional, and EP2 (each of which are incorporated by reference in the ’501 patent specification at col. 1:24-27), the inventors distinguished their invention from the prior art by stating:

“This length requirement [of 18 or 19 nucleotides] is clearly different from the technical teaching of the prior art such as, for example, the international patent application number WO 01/75164. It is within the present invention that any further design, both according to the present invention and as described in the prior art, can be realised in connection with an interfering ribonucleic acid having said length characteristics, i.e. a length of 18 or 19 nucleotides.”

Exh. 1006 (EP1) at p. 11; Exh. 1007 (’541 provisional) at p. 11; Exh. 1008 (EP2) at p. 12. Like the ’501 patent, EP1, the ’541 provisional, and EP2 also teach that “the doublestrand duplex of the siRNA molecules ***has to be longer than 17 base pairs to show activity*** whereas molecules with 17 base pair long duplexes or shorter are ***not functional*** even if sequence-specific overhangs are added.” Exh. 1006 (EP1) at p. 31; Exh. 1007 (’541 provisional) at p. 31; Exh. 1008 (EP2) at p.

35 (emphasis added). And, the inventors “established the *minimal duplex length of 18 or more than 18 nucleotides for functional siRNA molecules.*” Exh. 1006 (EP1) at p. 32; Exh. 1007 ('541 provisional) at p. 32; Exh. 1008 (EP2) at p. 36 (emphasis added).

219. Based on the '501 patent specification and the examples, a person of ordinary skill in the art would understand that the inventors were not in possession of any siRNA molecules of the claimed genus of siRNA molecules having a duplex length of 17 nucleotides included in claims 1-30 of the '501 patent and which the patent specification emphasizes as being outside the minimum structural requirement for the RNAi molecules of the invention.

220. Moreover, as discussed in paragraph 200 above, the claims encompass siRNA molecules having varied duplex stretch lengths ranging from 17 to 30 ribonucleotides. Exh. 1001 ('501 patent) at claims 1-30. Except for claims 4, 9, and 26, which require the first and second *strands* to consist of at least 18 nucleotides (claim 9) and have an overhang of one to eight nucleotides (claims 4 and 26), the claims do not place any limits on the overall length of the two *strands* of the double-stranded siRNA molecule and would encompass siRNA molecules having additional nucleotides on one or both ends of one or both strands, *i.e.*, the overhangs. Exh. 1001 ('501 patent) at claims 1-30. A person of ordinary skill in the art would understand that the claims include siRNA molecules having diverse

duplex architectures, such as those having varying stretch and strand lengths, including those having duplex lengths of up to 30 ribonucleotides. And as noted in paragraphs 79-81 above, the only 2'-O-methyl modified siRNA molecules disclosed in the '501 patent specification (and in EP2) are those having a duplex length of 21 nucleotides or 19 nucleotides that are blunt ended on both ends. *See also* ¶ 148 above. Likewise, the only 2'-O-methyl modified siRNA molecules disclosed in either EP1 or the '541 provisional are those having a duplex length of 21 nucleotides that are blunt ended on both ends. *See* ¶ 139 above. None of the applications disclose any contiguous alternating modified ribonucleotides alternating with unmodified or differently modified ribonucleotides having a duplex length of less than 19 nucleotides or greater than 21 nucleotides as encompassed by claims 1-30, or those having duplex architectures other than blunt ends on both ends as encompassed by claims 1 and 3-30 of the '501 patent. *See also* ¶¶ 200-204 above.

**c. The requirement for overhangs**

221. Claims 1 and 3-30 encompass 2'-O-alkyl modified siRNA molecules having *overhangs* on one or both ends of one or both strands. Exh. 1001 ('501 patent) at claims 1, 3-30. Claims 3-8 and 26-30 require the 2'-O-alkyl modified siRNA molecules to have *an overhang on at least one end* of one strand. Exh. 1001 ('501 patent) at claims 3-8, 26-30. Claims 5, 7, 27, and 29 specify that the



**3'-end** of the antisense (first) or sense (second) strands of the claimed siRNA molecules have an overhang; claims 6, 8, 28, and 30 specify that the **5'-end** of the antisense (first) or sense (second) strands of the claimed siRNA molecules have an overhang. Exh. 1001 ('501 patent) at claims 5-8, 27-30. With the exception of claims 4 and 26, none of the claims place any limits on the number of nucleotides that can be included within the overhangs. Exh. 1001 ('501 patent) at claims 1, 3-30. And none of the claims place any limits on the composition of the overhangs, whether they contain deoxyribonucleotides, unmodified or modified ribonucleotides, including complementary ribonucleotides, or various combinations of them. A person of ordinary skill in the art would understand that the claims encompass siRNA molecules having varied nucleotide lengths for the overhangs as well as varied nucleotides within the overhang, as long as they meet the minimum claim requirements.

222. However, the '501 patent specification fails to disclose any 2'-O-alkyl modified siRNA molecule that has an overhang as encompassed by claims 1 and 3-30 and as required by claims 3-8 and 26-30. All of the examples disclosed in the '501 patent specification (and in the priority applications) are missing one or more claim elements. For example, all the examples of siRNA molecules that have an overhang on at least one strand do **not** have any 2'-O-alkyl modified **and** unmodified or differently modified ribonucleotides on both strands as required by

the '501 patent claims; they are either unmodified, have terminal amino or inverted abasic end cap modifications with all of the nucleotides being unmodified, or are fully 2'-O-methyl modified (molecule 79A79B of Example 5, Fig. 8B). Exh. 1001 ('501 patent) at Examples 1-6, 11, Figs. 3-9, 16 (molecule V1); *see also* ¶¶ 72-78 above. A person of ordinary skill in the art would not consider the examples of siRNA molecules having overhangs to be representative of the claimed genus of 2'-O-alkyl modified siRNA because they all lack 2'-O-alkyl modified and unmodified or differently modified ribonucleotides.

223. Likewise, all the examples of siRNA molecules that have 2'-O-methyl modified and unmodified ribonucleotides on both strands are ***blunt ended on both ends***, *i.e.*, do ***not*** have any overhang as required by the '501 patent claims. Exh. 1001 ('501 patent) at Examples 7-11, Figs. 10-16; *see also* ¶¶ 79-81 above. A person of ordinary skill in the art would not consider the examples of ***blunt ended*** siRNA molecules having 2'-O-methyl ribonucleotides alternating with unmodified ribonucleotides described in the '501 patent specification (or the priority applications) to be representative of the claimed genus of 2'-O-alkyl modified siRNA molecules that require an ***overhang*** on at least one strand.

224. Moreover, as discussed above, the inventors repeatedly distinguished their “blunt-ended” duplex design from the prior art duplex design that included ***3'-overhangs*** based on their observation that “3'-overhangs are not required for

RNAi activity.” Exh. 1001 (’501 patent) at cols. 24:23-29, 11:23-27 (referring to the prior art duplex design described by Tuschl *et al.* as “provid[ing] for a length of 21 to 23 nucleotides and ***a modification at the 3’ end***”), cols. 14:66-15:1 (“This [blunt-ended] design of RNAi clearly differs from, e.g., that described by Tuschl *et al.* in WO 01/75164, ***which contains a 3’-overhang***.”), *see also* cols. 14:55-15:36, 15:65-16:2 (stating that the RNAi molecules of the present invention, including those having combinations of two or more design elements may be blunt ended or have a 5’-overhang); col. 25:22-27; ¶¶ 61, 69-70 above.

225. Moreover, based on the examples in the patent specification, the inventors stated that “a blunt duplex was ***more stable*** than the duplex with overhangs.” Exh. 1001 (’501 patent) at 26:34-36 (Example 5) (emphasis added), *see also* Fig. 8A (demonstrating higher serum stability over time of blunt ended unmodified siRNA molecule 28A28B compared to its counterpart siRNA molecules 18A18B and 30A30B having deoxy overhangs at the 3’- and 5’-ends, respectively); ¶¶ 76-77 above; *see also* ¶ 73 above.

226. And, in EP1, the ’541 provisional, and EP2 (each of which are incorporated by reference in the ’501 patent specification at col. 1:24-27), the inventors also distinguished their blunt-ended invention from the prior art by stating that “[t]his [blunt-ended] design of RNAi is thus ***clearly different*** from, e.g., the one of Tuschl *et al.* as specified in international patent application WO

01/75164 *which discloses a 3'-overhang*,” based on their experimental results. Exh. 1006 (EP1) at p. 14 (emphasis added); Exh. 1007 ('541 provisional) at p. 14; Exh. 1008 (EP2) at pp. 15-17; *see also* ¶¶ 140, 150 above. Like the '501 patent, in EP1, the '541 provisional, and EP2, the inventors concluded based on their experimental results that “3'-overhangs are not essential for RNAi activity” and further stated that “a *blunt* duplex is *more stable* than the duplex with overhangs.” Exh. 1006 (EP1) at pp. 31, 33 (emphasis added); Exh. 1007 ('541 provisional) at pp. 31, 33; Exh. 1008 (EP2) at pp. 34, 37-38; *see also* ¶¶ 141, 150 above.

227. Based on the '501 patent specification and the examples, a person of ordinary skill in the art would understand that the inventors were not in possession of any 2'-O-alkyl modified siRNA molecules, including those having contiguous alternating 2'-O-alkyl modified ribonucleotides alternating with unmodified or differently modified ribonucleotides, having an overhang at any end (5'-, 3'-end, or both) on any strand (sense, antisense, or both) as encompassed by claims 1 and 3-30 of the '501 patent, and as required by claims 3-8 and 26-30. A person of ordinary skill in the art would understand that the properties of blunt ended 2'-O-methyl modified siRNA disclosed in the patent specification and those additionally having an overhang as required by the '501 patent claims are not comparable. As shown by Kraynack and Baker, multiple 2'-O-methyl modified siRNAs that were active in a blunt ended architecture were found to be inactive in an architecture

containing 3'-overhangs. Exh. 1060 (Kraynack B.A. and Baker B.F., *Small Interfering RNAs Containing Full 2'-O-Methylribonucleotide-Modified Sense Strands Display Argonaute2/eIF2C2-Dependent Activity*, RNA 12:163-76 (2006)) ("Kraynack 2006") at p. 172; *see also* ¶ 229 below.

**iii. The lack of predictability of the effect of modifications on RNAi activity and stability**

228. The lack of possession is compounded by the fact that different modifications interact in different ways. Further, the fact that a particular pattern of modifications is observed to be useful for one or even several sequences does not mean that it will be universally applicable (or even that it is the best starting point for optimization of the pattern of other sequences). For example, a 2000 article by Parrish *et al.*, demonstrated that the interaction of a 2'-aminocytidine or a 2'-aminouridine modification with normal RNA was different as compared to the interaction of a 2'-fluorouridine modification with normal RNA of dsRNA molecules. Exh. 1034 (Parrish S. *et al.*, *Functional Anatomy of a dsRNA Trigger: Differential Requirement for the Two Trigger Strands in RNA Interference*, Molecular Cell, Vol. 6:1077-87 (2000)) ("Parrish 2000") at p. 1081, Fig. 5 (demonstrating the effects of sugar modifications at the 2'-position of dsRNA and that "[m]odification of uracil with 2'-fluorouracil was compatible with RNAi activity, while modification with 2'-aminouracil or 2'-aminocytidine produced a decrease in activity comparable to that seen with the deoxynucleotide

modification.”)

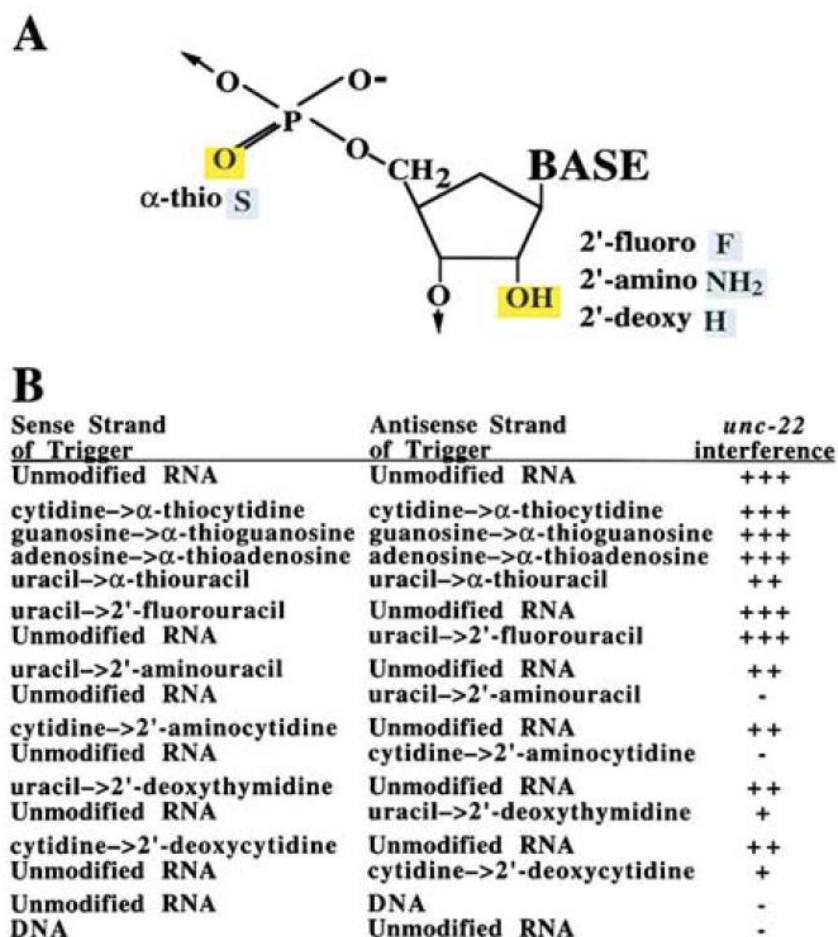


Figure 5 of Exh. 1034 (Parrish 2000) at p. 1081.

229. In a separate study, as discussed above, Kraynack and Baker investigated the effects of construct design (*i.e.*, duplex architecture), target site, and sugar-phosphate backbone composition on RNAi-mediated silencing. Exh. 1060 (Kraynack 2006). They demonstrated that the activity of modified siRNAs with full 2'-O-methyl sense strands was dependent on construct design (*i.e.*, duplex architecture) and that the activity of the 2'-O-methyl modified siRNAs having a blunt end 20-base pair RNA duplex was different compared to those having a

canonical 19-base pair RNA duplex with 3'-dTdT overhangs. Exh. 1060

(Kraynack 2006) at pp. 166-67, Fig. 2. Based on these results, the authors

concluded:

“Although the underlying basis of the effect of construct design and sequence on the activity of modified siRNAs has yet to be fully understood, one may conclude that usage of a *single* design and/or sequence is *not* sufficient when defining the scope and limitations of a given modification for usage in the RNAi pathway.”

Exh. 1060 (Kraynack 2006) at p. 173 (emphasis added). This study highlighted the interplay between duplex architecture, sequence, and chemical architecture, and their impact on functionality of chemically-modified siRNAs. *See also* Exh. 1072 (Choung 2006) at p. 921, Fig. 3 (demonstrating impact of single or groups of 2'-O-methyl modified ribonucleotides on duplex activity and that siRNAs having groups of 2'-O-methyl modified ribonucleotides in a phase-shifted pattern had reduced stability and activity), Fig. 5 (demonstrating the impact of incorporating 2'-O-methyl or 2'-fluoro pyrimidines and phosphorothioate backbone modifications on RNAi activity and stability).

230. As to strand length extensions and overhangs, subsequent work has demonstrated that the location and composition of the overhangs can impact RNAi activity, including the duration of silencing. For example, Chang *et al.* demonstrated that single and double strand extensions (comprising target-unrelated sequences) at the 3'- or 5'-ends of the antisense and sense strands of conventional

19-mer siRNA affected gene silencing activity differently, depending on the location (sense or antisense strand) and position (3'- or 5'-end) to which the extensions were attached. Exh. 1088 (Chang C.I. *et al.*, *A Structure-Activity Relationship Study of siRNAs With Structural Variants*, Biochem. Biophys. Res. Commun. 359(4):997-1003 (2007)) ("Chang 2007"). The authors observed that a 3' sense extension was the only single strand extension modification in their study that did not negatively impact siRNA gene silencing efficiency. Exh. 1088 (Chang 2007) at p. 1003, Fig. 1C. Single or double strand extensions at the 3'-end of the antisense strand, the 5'-end of either the sense or the antisense strand, or both, resulted in siRNA molecules with diminished gene silencing efficiency compared to control. Exh. 1088 (Chang 2007) at pp. 998-1001, Figs. 1C, 2C, 3C.

231. Likewise Strapps *et al.* demonstrated that the character of the two-nucleotide 3'-overhang of the antisense (guide) strand has a profound effect on the duration of silencing activity of the siRNA molecules. Exh. 1084 (Strapps W.R. *et al.*, *The siRNA Sequence and Guide Strand Overhangs are Determinants of In Vivo Duration of Silencing*, Nucleic Acids Research, 38(14):4788-97 (2010)) ("Strapps 2010"). The authors examined the impact on gene silencing *in vitro* and *in vivo* of antisense 3'-overhangs having (i) ribonucleotides complementary to positions -1 and -2 in the target RNA, (ii) 2'-O-methyl modified versions of those complementary nucleotides, (iii) deoxythymidine overhangs, and (iv) 2'-O-



methyluridine overhangs. Exh. 1084 (Strapps 2010) at pp. 4790-94, Figs. 1-4. The authors observed that the ribonucleotide overhangs extended the duration of silencing as compared to deoxythymidine overhangs *in vitro* and *in vivo*. Exh. 1084 (Strapps 2010) at p. 4796. The authors also demonstrated that “siRNAs that have very similar capacities for reduction of mRNA levels in tissue culture cells and *in vivo* have distinct durations of silencing that appear dependent upon the composition of the siRNA.” Exh. 1084 (Strapps 2010) at p. 4789. The authors also noted that “duration of activity appears to be affected by both the underlying sequence of the siRNAs and the chemical nature of the guide strand overhangs.” Exh. 1084 (Strapps 2010) at p. 4796; *see also* Exh. 1036 (Bramsen J. and Kjems J., *Development of Therapeutic-Grade Small Interfering RNAs by Chemical Engineering*, *Frontiers in Genetics* 3(154):1-21 (2012)) (“Bramsen 2012”) at p. 9 (noting that “siRNA overhang composition does influence their affinity for the Ago2 PAZ domain and siRNA potencies.”).

232. Similarly, subsequent work has demonstrated that synthetic RNA duplexes 25-30 nucleotides in length (which the claims allow for) could trigger efficient RNAi, but require processing by different cellular machinery. Exh. 1090 (Chang 2011) at p. 127. For example, Kim *et al.* demonstrated that 27-bp-long duplexes were processed efficiently by Dicer with varying potencies depending on the target site and that the introduction of end modifications compromised RNAi

activity. Exh. 1085 (Kim D.H. *et al.*, *Synthetic dsRNA Dicer Substrates Enhance RNAi Potency and Efficacy*, *Nature Biotechnology*, 23(2):222-26 (2005)) at pp. 222-23, 225, Figs. 1, 3; *see also* Exh. 1086 (Rose S.D. *et al.*, *Functional Polarity is Introduced by Dicer Processing of Short Substrate RNAs*, *Nucleic Acids Research*, 33(13):4140-56 (2005)). These 27 bp-long siRNA structures are known as “Dicer-substrate siRNA” as they are processed by Dicer into 21-mer siRNAs (thus, requiring engagement with other cellular machinery not involved in processing 19-21 nt siRNAs), before entering RISC, and triggering RNAi. Exh. 1090 (Chang 2011) at p. 127. This interaction with different biological processes requires different and non-interchangeable design considerations when applying chemical modifications to these longer duplexes. Thus, Collingwood *et al.*, examined the effect of chemical modification patterns, including those incorporating 2’-O-methyl and 2’-fluoro modifications, of 27-mer Dicer-substrate siRNA (DsiRNA) duplexes on their relative potency, ability to be cleaved by Dicer, and potential for immune activation. Exh. 1038 (Collingwood M.A. *et al.*, *Chemical Modification Patterns Compatible with High Potency Dicer-Substrate Small Interfering RNAs*, *Oligonucleotides* 18:187-200 (2008)) (“Collingwood 2008”) at pp. 190-92, Table 1, Fig. 2. The authors observed that some modifications “profoundly reduced function although other patterns maintained high potency,” and that “[e]ffects of sequence context were observed, where the relative potency of modification

patterns varied between sites,” “making it important to examine the behavior of modification patterns at different sites in different target genes.” Exh. 1038 (Collingwood 2008) at pp. 187, 190, Fig. 2. These studies demonstrate that duplex length, duplex architecture, target sequence, and chemical modifications have a complex interplay in terms of their effect on RNAi activity, and that it would be impossible to extend design principles derived for conventional 19-21 nt siRNAs to their longer congeners.

233. The examples of the '501 patent specification also demonstrate that the same modifications can have dramatically different effects on siRNA serum stability or RNAi activity or both, depending on the type of modification, position, and the extent of the modifications. Examples 1 and 6 demonstrate that introducing amino cap modifications at the 3'- and 5'-ends of the siRNA molecules can impact functionality. Exh. 1001 ('501 patent) at cols. 23:46-54; 27:14-37; Figs. 3, 9; *see also* ¶¶ 72, 78 above. Additionally, Examples 7-11 demonstrate that for siRNA molecules having internal 2'-O-methyl modified and unmodified nucleotides (none of which have two different modifications or an overhang), the 2'-O-methyl modifications can impact not only the activity of the RNAi molecules but also their stability. *See e.g.*, Exh. 1001 ('501 patent) at col. 32:4-30, Example 11, Fig. 16 (comparing stability and RNAi activity of molecules V3, V4, V5, V6), cols. 31:60-32:15, Fig. 15B-C (comparing stability and RNAi

activity of molecules V13, V14, V15, and V12), cols. 30:62-31:20; *see also* ¶¶ 79-83 above; Exh. 1028 (Czauderna 2003) at pp. 2710-13 (acknowledging a lack of understanding of the effect of chemical modifications on siRNA activity or stability).

234. The lack of possession of the claimed invention is further compounded by the fact that the claimed genus of siRNA molecules is enormous and would encompass structurally diverse modified siRNA molecules—those having different chemical and duplex architectures, as well as targeting an unlimited number of genes. *See* Section XI.A.i above. The specification fails to provide any correlation between structure and function for the claimed siRNA molecules. There is no data provided in the '501 patent specification to provide any confidence that the activity observed for the few, and structurally similar, 2'-O-methyl modified siRNA molecules, (none of which have a modification other than a 2'-O-methyl modification, two differently modified ribonucleotides, duplex lengths greater than 21 nucleotides or less than 19 nucleotides, or an overhang as required by the claims) can be extrapolated to the vast genus of contiguous alternating 2'-O-alkyl modified siRNA molecules claimed.

235. From outside the RNAi field, it may look deceptively simple to find active sequences and designs for oligonucleotide therapeutics as sequences are selected as complementary strands to a chosen target mRNA. However, this

generates thousands of options for a target site (tiling along the target mRNA), and at each site there is a complex interplay of sequence, chemical modifications, various possible oligonucleotide lengths, duplex or single-stranded architecture, targeting ligand or delivery vehicle, etc. *See generally* Exh. 1035 (Deleavey 2009); Exh. 1036 (Bramsen 2012); Exh. 1037 (Haringsma H.J. *et al.*, *mRNA Knockdown by Single Strand RNA is Improved by Chemical Modifications*, *Nucleic Acids Research* 40: 4125-36 (2012)) and Exh. 1038 (Collingwood 2008) at pp. 187-200 (both discussing the interplay of different kinds of modifications with different siRNA designs); Exh. 1039 (Behlke M., *Chemical Modification of siRNAs for In Vivo Use*, *Oligonucleotides* 18:305-20 (2008)) (“Behlke 2008”) and Exh. 1027 (Watts 2008) (both discussing the interplay of modifications with different sequences); *see also* ¶¶ 53-56, 176-177 above. If sequences are not screened in the context of appropriate chemical modifications, they will not be stable enough to be effective. Conversely, if a sequence is incompatible with a chosen modification pattern, the sequence may be eliminated from a screen even if it might have been an excellent therapeutic lead sequence under a different modification pattern. Exh. 1039 (Behlke 2008) at p. 306; Exh. 1040 (Pei Y. and Tuschl T., *On the Art of Identifying Effective and Specific siRNAs*, *Nature Methods* 3:670-76 (2006)) (“Pei and Tuschl 2006”) at pp. 670, 674. Clearly, there are numerous obstacles that need to be addressed when developing an RNAi molecule,

particularly as a therapeutic, and a precise balance of these multiple variables (*e.g.*, sequence selection, siRNA architecture, modification pattern, delivery vehicle, etc.) is critical. *See also* ¶¶ 53-56, 252-264 below.

**iv. The specification fails to describe the claimed genus of siRNA molecules that have RNAi activity**

236. As noted above, “siRNA” by definition requires the molecules to have RNA interference activity. *See* ¶¶ 122-127 above. Thus, it is my opinion that the ’501 patent specification fails to demonstrate possession of the claimed genus of siRNA molecules that have RNAi activity. A person of ordinary skill in the art would understand that not all of the siRNA molecules encompassed by the claimed genus of modified siRNA molecules would have RNAi activity. For example, some of the modifications or configurations may render the siRNA molecules inactive or may negatively impact stability. In other words, a person of ordinary skill in the art would understand that the claims combine the structural features that are satisfied by an enormous genus of structurally diverse modified siRNA molecules targeting any gene with the function of having RNAi activity that are satisfied by an unknown and possibly a subset of undisclosed siRNA molecules that are part of the structurally diverse genus of siRNA molecules. Nowhere does the specification include *any* example of an siRNA molecule that has any modification other than a 2’-O-methyl modification, has two different modifications, a duplex length greater than 21 nucleotides or less than 19

nucleotides, or has an overhang as required by the '501 patent claims, and has RNAi activity, and no guidance is provided that would allow a person of ordinary skill in the art to predict which of the siRNA molecules of the claimed genus would have RNAi activity. A person of ordinary skill in the art would not know which of those siRNA molecules would have RNAi activity without making and testing them. This is an additional reason for why the '501 patent specification fails to describe the genus of 2'-O-alkyl modified siRNA molecules claimed.

237. In sum, the '501 patent claims encompass an enormous number of structurally diverse 2'-O-alkyl modified siRNA molecules that have a particular pattern of contiguous alternating 2'-O-alkyl modified ribonucleotides alternating with unmodified or differently modified ribonucleotides that may be blunt ended or have an overhang on one or both ends of one or both strands. However, all of the examples of siRNA molecules having internal modifications in the figures, the examples, and the sequence listing, are of the *same type*: those having the *same* modification (2'-O-methyl) and pattern, with the remaining nucleotides being *unmodified*, and the *same* duplex architecture (blunt ended on both ends). Exh. 1001 ('501 patent) at Examples 7-11; *see also* ¶¶ 79-81, 206-227 above. The '501 patent specification (and the priority applications) provides no disclosure of (i) any modified siRNA molecules having a modification pattern comprising a 2'-*O-alkyl* modification other than 2'-O-methyl for the modified ribonucleotides as

encompassed by at least claims 1-9, 12, 14, 16, 18, 20, 23, and 24,

(ii) any modified siRNA molecules having ***two different*** modifications, including a ***2'-fluoro*** modification, as encompassed by at least claims 1-10, 12, 13, 16-20, 22, and 24-30, (iii) any modified siRNA molecules having a ***mixed*** modification pattern, *i.e.*, modified ribonucleotides alternating with both unmodified and differently modified ribonucleotides as encompassed by at least claims 1-10, 12 and 20, (iv) any modified siRNA molecules having ***overhangs*** as encompassed by claims 1 and 3-30, (v) any modified siRNA molecules having duplex stretch lengths ***greater than 21 nucleotides*** as encompassed by claims 1-30, (vi) any modified siRNA molecules having a duplex stretch length of ***17 nucleotides*** as encompassed by claims 1-30 that have RNAi activity, and which the specification states is outside the minimum structural requirement of 18-19 nucleotides for RNAi activity, and (vii) any ***structure-activity relationships*** to guide in the design of those claimed siRNA molecules. Combined with the lack of predictability of the effect of chemical modifications on RNAi activity (and stability) of siRNA molecules, a person of ordinary skill in the art would understand that the inventors were ***not*** in possession of the vast genus of modified siRNA molecules encompassed by the '501 patent claims.



## **XII. The '501 Claims Lack Enablement Support**

### **A. The '501 Patent Specification Fails to Teach How to Make and Use the siRNA Molecules Without Undue Experimentation**

238. It is my understanding that to provide enablement support for generic claims like the challenged claims, *i.e.*, claims to a genus of double-stranded siRNA molecules comprising a first and a second strand comprising complementary first and second stretches having the same length, respectively, wherein (i) the first and second *stretches* each comprise contiguous alternating 2'-O-alkyl modified ribonucleotides alternating with unmodified or differently modified ribonucleotides (ii) the 2'-O-alkyl modified ribonucleotides of the first *strand* are shifted by one ribonucleotide relative to the 2'-O-alkyl modified ribonucleotides on the second *strand*, and (iii) each of the first and second *stretches* consists of at least 17 and fewer than 30 ribonucleotides, and that are useful for RNAi, the specification must teach one of ordinary skill in the art how to make and use the claimed invention without undue experimentation. *See also* ¶¶ 33-36 above. The '501 patent specification does not disclose or provide any guidance to practice the vast genus of siRNA molecules that meet the claims. This is for several reasons.

239. First, claims 1-30 encompass an enormous (and potentially limitless) genus of structurally diverse siRNA molecules having contiguous alternating 2'-O-alkyl modified ribonucleotides targeting any gene. Second, the claims require that the modified siRNA molecules be useful for RNAi, which is what the specification

teaches was the purpose of the invention. Further, the '501 patent specification generally states that one or both strands can have various patterns of modifications, including the claimed pattern of modifications; siRNA molecules can be blunt ended or have overhangs; and that the stretches/strands can have varying nucleotide lengths, but without any illustrating sequence, any working example, and any guidance that would allow one of ordinary skill in the art to practice what is claimed. The specification fails to provide any modified siRNA molecules that are useful for RNAi and have (i) a modification *other than a 2'-O-methyl* modification for the 2'-O-alkyl modified ribonucleotides, (ii) *two different* modifications, including a *2'-fluoro* modification, (iii) a *mixed* modification pattern, *i.e.*, 2'-O-alkyl modified ribonucleotides alternating with both unmodified and differently modified ribonucleotides, (iv) *overhangs* on one or both ends of one or both strands, (v) a duplex length *greater than 21 nucleotides*, or (vi) a duplex length of *17 nucleotides*, which the specification states is not useful for RNAi. The patent specification does not provide any *structure-activity relationships* to guide in the design of those claimed siRNA molecules or predict which of the claimed siRNA molecules would be useful for RNAi. To the extent persons of ordinary skill might be able to practice the claimed invention based on the teachings in the '501 patent specification, my opinion is that such practice would require undue experimentation for the reasons discussed below.

**i. The nature of the invention and the breadth of the claims**

240. The claims are directed to double-stranded siRNA molecules comprising contiguous alternating 2'-O-alkyl modified ribonucleotides alternating with unmodified or differently modified ribonucleotides on both strands where the 2'-O-alkyl modified ribonucleotides are in a phase-shifted pattern, and the siRNA molecules consist of stretches of 17-30 ribonucleotides. Exh. 1001 ('501 patent) at claims 1-30; *see also* ¶¶ 173, 178, 205 above.

241. A person of ordinary skill in the art would understand that the purpose of the invention was to provide interfering RNA molecules, including the claimed double-stranded siRNAs, that are both stable and active, *i.e.*, exhibited RNAi activity in a biological system, including in a living cell. Exh. 1001 ('501 patent) at col. 2:29-31 ("It is apparent, therefore, that synthetic interfering RNA molecules that are both stable and active in a biochemical environment such as a living cell are greatly to be desired."). A person of ordinary skill in the art would also understand that the inventors contemplated that the claimed siRNA molecules would be useful for mediating RNAi. Exh. 1001 ('501 patent) at col. 12:41-45 ("[A]ll of the ribonucleic acids of the present invention are suitable to cause or being involved in methods of RNA mediated interference"); *see also* ¶¶ 59-64, 122-127 above.

**a. The claimed genus of double-stranded siRNA molecules is vast**

242. As discussed in Section XI.A.i above, the claimed genus of siRNA molecules is enormous, and would include a potentially unlimited number of structurally diverse modified siRNA molecules:

- The claimed genus of siRNA molecules is not limited by sequence, target gene, or organism, and can encompass siRNA molecules targeting any gene, including those that have yet to be identified (§§ 174-177). And even for a single target gene, this would result in thousands of potential sequences or target sites (§§ 176-177);
- The claimed genus includes siRNA molecules having various patterns or motifs within each strand, including: (i) 2'-O-alkyl modified ( $M_m$ ) ribonucleotides alternating with unmodified (U) ribonucleotides (*e.g.*,  $M_m/U/M_m$  motif); (ii) 2'-O-alkyl modified ( $M_m$ ) ribonucleotides alternating with differently modified ( $D_n$ ) ribonucleotides (*e.g.*,  $M_m/D_n/M_m$  motif); and (iii) 2'-O-alkyl modified ( $M_m$ ) ribonucleotides alternating with unmodified (U) or differently modified ( $D_n$ ) ribonucleotides, *i.e.*, a mixed motif (*e.g.*,  $M_m/U/M_m/D_n$  motif) (§§ 178-180);
- The claimed genus encompasses siRNA molecules having diverse chemical architectures, including various modifications such as sugar modifications, base modifications, and internucleoside linkages, and those enumerated in the '501 patent specification, so long as the siRNA molecules comprise contiguous alternating 2'-O-alkyl modified ribonucleotides alternating with unmodified or differently modified ribonucleotides as required by the claims (§§ 181-199);
- The claimed genus encompasses siRNA molecules having diverse duplex architectures, including blunt ends on one or both ends, overhangs on one or both ends of one or both strands, forks, and conjugates (§§ 200-201); and
- With the exception of a few dependent claims (claims 2, 4, 9, and 26), the claims do not place any limits on the strand lengths of the siRNA molecules, and encompass siRNA molecules of varying strand lengths,

so long as the first and second stretches consist of 17-30 ribonucleotides (¶ 200).

243. As noted in paragraphs 65-67 and 178-194 above, the '501 patent specification makes reference to numerous modifications that could be incorporated into the siRNA molecules, and provides a non-limiting list of possible modifications, including various sub-genera. Exh. 1001 ('501 patent) at cols. 13:31-46, 14:22-32, 16:3-14. The '501 patent specification and the claims contemplate numerous variables and configurations for the chemical and duplex architectures that could be incorporated in the siRNA molecules. See ¶¶ 195-204, 243 above. A person of ordinary skill in the art would also understand that not all of the siRNA molecules of this genus would be useful for RNAi. For example, some of the modifications or configurations may render the siRNA molecules inactive or unstable (*i.e.*, may negatively impact stability), making them useless for RNAi. Thus, the claims encompass an enormous genus of structurally diverse modified siRNA molecules targeting any gene, of which an unknown and possibly a subset of undisclosed siRNA molecules would be useful for RNAi. And, a person of ordinary skill in the art would need to make and test the various siRNA molecules to determine whether or not they would be useful for RNAi. In my opinion, the size and structural diversity of the claimed genus would increase the burden placed on a person of ordinary skill in the art to be able to practice the claimed invention.

**ii. The presence or absence of working examples and the amount of guidance in the specification**

**a. The '501 patent specification does not provide enough guidance to allow a person of ordinary skill in the art to practice the claimed invention**

244. As discussed above, claims 1-30 encompass a vast genus of structurally diverse modified siRNA molecules targeting any gene. However, the '501 patent specification only provides examples of siRNA molecules (none of which meet the requirement for two different modifications) that target one of three genes, namely PTEN, Akt, and p110 $\beta$ . Exh. 1001 ('501 patent) at Examples 1-13. Additionally, the '501 patent specification discloses in the figures, the examples, and the sequence listing, about 25 siRNA molecules having internal ribonucleotide modifications on one or both strands, which are of the *same* type: siRNA molecules having the *same* modification (2'-O-methyl) and pattern, with the remaining nucleotides being *unmodified* and the same duplex architecture (blunt ended on both ends). Exh. 1001 ('501 patent) at Examples 7-11; *see also* ¶¶ 71, 79-81, 206-207, 220, 223 above. And even within this narrow subset of siRNA molecules (about 25), none of which have overhangs, the '501 patent specification teaches that not all of them were stable and active, which is the problem the '501 patent claims to solve (Exh. 1001 ('501 patent) at cols. 2:29-31, 2:35-37, 11:19-34), and the nature, position, or extent of nucleotide modification, affects RNAi activity (and also stability). *See* ¶ 207 above; *see also* ¶¶ 79-83,

240-241 above.

245. As explained in paragraphs 206-227 above, a person of ordinary skill in the art would not consider the siRNA molecules disclosed in the '501 patent specification to be representative of the vast genus of siRNA molecules claimed. There is no data provided in the '501 patent specification to provide any confidence that the activity observed for the few, and structurally similar, 2'-O-methyl modified siRNA molecules can be extrapolated to the enormous genus of structurally diverse modified siRNA molecules claimed. *See* ¶¶ 173-204 above.

246. Likewise, as noted above, the claims include modified siRNA molecules having various modification patterns or motifs within each strand of the siRNA molecules, including alternating 2'-O-alkyl modified/unmodified (*e.g.*,  $M_m/U/M_m$  motif); alternating 2'-O-alkyl modified/differently modified (*e.g.*,  $M_mD_n/M_m$  motif); and alternating 2'-O-alkyl modified/unmodified or differently modified (*e.g.*,  $M_m/U/M_m/D_n$  mixed motif), and those having diverse duplex architectures. Exh. 1001 ('501 patent) at claims 1-30; *see also* ¶¶ 178-180, 200 above. The '501 patent specification generally lists various modifications that could be incorporated in an siRNA molecule (Exh. 1001 ('501 patent) at col. 13:35-43) and generally describes assays for screening the activity and stability of siRNA molecules (Exh. 1001 ('501 patent) at Examples 1-13). However, it provides no guidance to a person of ordinary skill in the art on how to select the

various modifications for the 2'-O-alkyl modified or the differently modified ribonucleotides, including the claimed combinations of modifications, to aid in the design of siRNA molecules that would be useful for RNAi. The patent specification also provides no correlation between structure and function for the claimed siRNA molecules to allow a person of ordinary skill in the art to predict which of the claimed siRNA molecules would be useful for RNAi. This is particularly important as the location and position of modified, unmodified, or differently modified ribonucleotides on both strands can impact RNAi activity (and stability). *See* ¶¶ 252-264 below; *see also* ¶¶ 228-235 above (discussing the lack of predictability of the effect of chemical modifications on RNAi activity).

247. Additionally, whereas claims 1-30 include siRNA molecules having duplex lengths of **17 nucleotides** (Exh. 1001 ('501 patent) at claims 1-30; *see also* ¶¶ 200, 215 above), there is no teaching in the '501 patent specification of any siRNA molecules having a duplex length of 17 nucleotides that **have** RNAi activity. Exh. 1001 ('501 patent) at Examples 1-13. In fact, the '501 patent specification (and the priority applications) repeatedly states that the **minimum** duplex length of the siRNA molecule must be at least 18-19 nucleotides **for RNAi activity**, and that stretches of as little as 17 nucleotides, even with sequence specific overhangs, are **not functional for RNAi**. Exh. 1001 ('501 patent) at col. 7:65-66 and Fig. 5, col. 12:22-26; *see also* cols. 12:46-60, 24:49-54, 24:65-25:2,



25:22-27, 25:44-48, 26:14-18, Fig. 7A, and ¶¶ 216-219 above. A person of ordinary skill in the art would understand from these disclosures that siRNA molecules having a duplex length of 17 nucleotides would **not** be useful for RNAi. In my opinion, the '501 patent specification does not provide any guidance for a person of ordinary skill in the art to make and use modified siRNA molecules having duplex lengths of 17 nucleotides for RNAi.

248. Further, as discussed above, with the exception of claim 2, the claims encompass, and in claims 3-8 and 26-30 require, the siRNA molecules to have overhangs at the 3'- or 5-ends of the antisense (first) or sense (second) strands. Exh. 1001 ('501 patent) at claims 1-30; *see also* ¶¶ 200, 220 above. However, the only 2'-O-methyl modified siRNA molecules disclosed in the '501 patent specification (and the priority applications) are those that are blunt ended on both ends. Exh. 1001 ('501 patent) at Examples 7-11; *see also* ¶¶ 79-81 above. The patent specification provides no guidance on the duplex architecture, including the relative location (sense or antisense strand or both) and position (3'- or 5'-end or both) of the overhang, the overall length of the overhang, the number and types (modified or unmodified) nucleotides to include in the overhang, including various ribonucleotides or deoxyribonucleotides. In fact, as explained in paragraphs 221-227 above, based on the '501 patent specification, a person of ordinary skill in the art would understand that the inventors distinguish their “blunt-ended” approach

from the use of 3'-overhangs, in particular, that "a blunt [ended] duplex is *more stable* than the duplex molecule with overhangs." Exh. 1001 ('501 patent) at *e.g.*, col. 26:34-36 (emphasis added). In my opinion, the '501 patent specification does not provide any guidance for a person of ordinary skill in the art to make and use modified siRNA molecules having overhangs. As shown by Kraynack and Baker, chemical modification patterns suitable for blunt ended duplexes may not be functional in the context of overhang-containing duplexes. Exh. 1060 (Kraynack 2006) at p. 172; *see also* ¶¶ 227, 229 above. Thus, the examples of blunt ended duplexes provided in the patent specification would not enable a person of ordinary skill in the art to make or use analogous overhang-containing siRNAs.

249. Likewise, while claims 1-30 include siRNA molecules having duplex lengths less than 19 or greater than 21 nucleotides (Exh. 1001 ('501 patent) at claims 1-30; *see also* ¶¶ 200, 220 above), the only 2'-O-methyl modified siRNA molecules in the '501 patent specification are those having a duplex length of 21 or 19 ribonucleotides (Figs. 12-16); EP1 and the '541 provisional only disclose 2'-O-methyl modified siRNA molecules having a duplex length of 21 ribonucleotides (Figs. 11-13).

250. The '501 patent specification generally states in the various embodiments that one or both strands can have various patterns of modifications; siRNA molecules can be blunt ended or have overhangs; and that the

stretches/strands can have varying nucleotide lengths. Exh. 1001 ('501 patent) at *e.g.*, cols. 2:35-6:3, 16:25-18:49; *see also* Fig. 2. But, the '501 patent specification does not disclose any modified siRNA molecules that are useful for RNAi and have (i) a modification ***other than a 2'-O-methyl*** modification for the ***2'-O-alkyl*** modified ribonucleotides as encompassed by at least claims 1-9, 12, 14, 16, 18, 20, 23, and 24; (ii) ***two different*** modifications, including a ***2'-fluoro*** modification, as encompassed by at least claims 1-10, 12, 13, 16-20, 22, and 24-30; (iii) a ***mixed*** modification pattern, *i.e.*, 2'-O-alkyl modified ribonucleotides alternating with both unmodified and differently modified ribonucleotides as encompassed by at least claims 1-10, 12 and 20; (iv) an ***overhang*** as encompassed by claims 1 and 3-30; (v) a duplex length ***greater than 21 nucleotides*** as encompassed by claims 1-30; or (vi) a duplex length of ***17 nucleotides*** as encompassed by claims 1-30 that have RNAi activity, which the specification states is not useful for RNAi. Nor does the '501 patent specification provide any guidance that would allow a person of ordinary skill in the art to predict which of the siRNA molecules of the claimed genus would be useful for RNAi. *See also* ¶ 236 above. The specification does not provide sufficient information for one of ordinary skill in the art to account for such variables as compatibility of various modifications with each other and the duplex architecture (*i.e.*, effect on stability) and the impact of chemical modifications and duplex architecture on proper recognition and function of the

siRNA by cellular RNAi protein machinery (*i.e.*, effect on RNAi activity). *See* ¶¶ 50-52 above; *see also* Exh. 1035 (Deleavey 2009) at pp. 16.3.2-3 (describing the interactions between duplex siRNAs with the cellular machinery, including nucleases and the RISC complex); ¶¶ 53-56, 228-235 above. In my opinion, this lack of guidance would increase the burden placed on a person of ordinary skill in the art to be able to practice the claimed invention.

**iii. The level of ordinary skill, the state of the prior art, and the nature and predictability of the field**

251. The '501 patent claims are directed to modified double-stranded siRNA. Exh. 1001 ('501 patent) at claims 1-30. At all relevant time-frames (2002-2017), the field of the '501 patent (interfering RNA molecules) would have been complex and as a result, a person of ordinary skill in the art would have had a degree of education or experience as discussed in paragraph 38 above.

**a. The impact of modifications on siRNA function and stability could not be predicted and would need to be tested experimentally**

252. While nucleotide chemistry was generally well known as of 2002-2003 and the use of chemically-modified nucleotides in other technologies such as antisense oligonucleotides or ribozymes (which operate to alter expression of target genes by a different mechanism than siRNAs) was known, the use of modified nucleotides in *siRNAs* and their impact on activity and stability of siRNAs was not well understood. Exh. 1041 (Kurreck J., *Antisense Technologies:*

*Improvement through Novel Chemical Modifications*, Eur. J. Biochem. 270:1628-44 (2003)) at pp. 1630, 1638-40. It was not until May 2001 when Elbashir *et al.*, first demonstrated that siRNAs could be used to trigger an RNAi response in mammalian cells, a transformational result that opened up a new approach to the study of the role of proteins in mammalian cells and the possibility that siRNAs could eventually be used as gene-specific therapeutics. Exh. 1042 (Elbashir 2001) at p. 497.

253. In the early 2000s, the mechanism of RNAi and the impact of various nucleotide modifications on RNAi activity and stability were just beginning to be understood. As noted in paragraphs 228-235 above, Parrish *et al.*, demonstrated the differences in interactions of various modifications, including 2'-amino or 2'-fluoro modifications with normal RNA of double-stranded RNA molecules. Exh. 1034 (Parrish 2000) at p. 1081, Fig. 5. Amarzguioui *et al.*, noted in 2003 that "[t]he mechanism of RNAi is not well understood" and examined the effect of mutations and chemical modifications on efficacy and duration of action of siRNAs. Exh. 1043 (Amarzguioui M. *et al.*, *Tolerance for Mutations and Chemical Modifications in a siRNA*, Nucleic Acids Research 31(2):589-95 (2003)) ("Amarzguioui 2003") at p. 589. Likewise, in 2003, Chiu and Rana, in an effort to address the issue of siRNA stability and further dissect the mechanism of RNAi in human cells, demonstrated for the first time that 2'-OH was not required for

siRNA to enter the RNAi pathway and that incorporation of various chemical modifications, including 2'-O-methyl modified nucleotides or 2'-fluoro pyrimidines (C and U), in one or both strands of the siRNA molecules affected activity as well as stability. Exh. 1044 (Chiu Y.L. and Rana T.M. *et al.*, *siRNA Function in RNAi: A Chemical Modification Analysis*, RNA 9:1034-48 (2003)) ("Chiu and Rana 2003") at pp. 1035, 1037 (Table 1). These early studies (and those of others) demonstrated that the effect of modifications on RNAi activity and stability depended on the location (sense or antisense strand), position (5'- or 3'- ends or any of the internal nucleotides) and number of modified nucleotides within the base-paired region of the siRNA duplex. Exh. 1045 (Patkaniowska A. and Tuschl T. *et al.*, Chapter 4, *Gene Silencing by Synthetic siRNA Duplexes in Mammalian Cell Culture*, RNA Interference (RNAi) Nuts & Bolts of RNAi Technology 71-88 (2003 ed.)) ("Patkaniowska 2003") at p. 81. These studies also demonstrated that the antisense strand was more sensitive to modifications than the sense strand, and that "[b]y careful selection of modifications and adjustments to the specific targeting sequence it should be possible to generate fully nuclease resistant yet active siRNAs, which may prove useful for *in vivo* applications. Conditions for optimal pharmacokinetic and pharmacodynamic distribution of siRNAs remain to be established." Exh. 1045 (Patkaniowska 2003) at p. 81.

254. The lack of understanding of the effect of chemical modifications on

siRNA activity or stability is also evidenced by the inventors' own work from 2003 (after the filing of EP1 and the '541 provisional), where they acknowledged that they did not know whether the increase in stability conferred by alternating 2'-O-methyl modifications along the siRNA molecule "will also improve the pharmacodynamic properties of these molecules, and it is very likely that further modifications and improvements in delivery are necessary to enable the use of synthetic siRNAs *in vivo*." Exh. 1028 (Czauderna 2003) at pp. 2710-13; *see also* p. 2705. The inventors acknowledged that it was "puzzling that not all siRNAs with alternating 2'-O-methyl modifications on both strands were functional," and that "the specific nucleotide positions of the internal 2'-O-alkyl modifications in the siRNA duplex are crucial to balance the beneficial stabilising effects with activity reducing effects." Exh. 1028 (Czauderna 2003) at p. 2713. The inventors also acknowledged that "[a]ccording to our data it is essential to balance the effects of 2'-O-alkyl modifications on stability against the negative effects on activity, which makes it important to be aware of various design principles." Exh. 1028 (Czauderna 2003) at p. 2715. Based on their observations, they offered the following four principles:

- Modifications at the 5'-hydroxyl group of the antisense strand should be avoided since end modifications (-NH<sub>2</sub> or inverted abasic) at this position abolish RNAi inducing activity;

- Alternating 2'-O-alkyl modification but not blocks of 2'-O-alkyl modification lead to significant resistance against serum-derived nucleases without significant loss of RNAi activity;
- 2'-O-methyl-modified siRNAs are more active when the incorporated modifications are on every second nucleotide, facing a non-modified nucleotide; and
- siRNAs in which the alternating modifications at the 2'-hydroxyl group start with the most terminal nucleotides of the antisense strand are more efficient than siRNAs which begin this modification at the second nucleotide of the antisense strand.

Exh. 1028 (Czauderna 2003) at p. 2715.

255. I note that nowhere in their 2003 publication do the inventors even suggest that siRNA molecules comprising *two different* modifications would have RNAi activity, let alone one that has contiguous alternating modified ribonucleotides having any modification other than a 2'-O-methyl modification alternating with unmodified or differently modified ribonucleotides or an overhang as required by the '501 patent claims.

256. As noted in paragraph 233 above, the '501 patent specification includes several examples demonstrating that the impact of chemical modifications on siRNA activity or stability could not be predicted. Examples 1 and 6 demonstrate that introducing amino cap modifications at the 3'- and 5'-ends of the siRNA molecules can negatively impact functionality. Exh. 1001 ('501 patent) at Examples 1, 6, Figs. 3, 9. Examples 7-11 demonstrate that siRNA molecules having internal 2'-O-methyl modified and unmodified nucleotides, whether as



single or group modifications on one or both strands, can impact not only the activity of the RNAi molecules but also their stability. Exh. 1001 ('501 patent) at Examples 7-11, Figs. 10-16.

257. Moreover, in 2003, the precise mechanisms by which siRNAs mediated gene silencing, including the structure and function of various proteins in the RNAi machinery and how they interacted with the siRNA molecules, were not known. In my opinion, while a person of ordinary skill in the art would have been guided by a few general principles for designing synthetic siRNAs, such as having an 18 to 21 nucleotide length or including nucleotide overhangs (Exh. 1045 (Patkaniowska 2003) at p. 74; *see also* Exh. 1040 (Pei and Tuschl 2006)), a person of ordinary skill in the art would not have been able to predict which modification strategies would be effective at mediating RNAi or improving stability without knowing how the individual siRNA molecules and sequences interacted with the underlying RNAi protein machinery. Without this knowledge, a person of ordinary skill in the art could not have reliably developed structure-activity relationships (SARs) or made correlations between structure and function that would allow the exploration of rational design strategies for modified siRNAs. As Alnylam's own scientists acknowledged, as of 2004, "[t]he effects of chemical modifications are being evaluated and certain rules are emerging, although some mixed observations exist. This may be due to differences among the gene targets

employed or to the different sequences employed.” Exh. 1046 (Manoharan M., *RNA Interference and Chemically Modified Small Interfering RNAs*, Current Opinion in Chemical Biology 8:570-79 (2004)) at p. 577.

258. Additional insights into the possible mechanism of RNAi were gained in mid-2004, when the crystal structure of Argonaute protein, a protein implicated in the RNAi pathway, was first solved, but many questions regarding the details of the mechanism for siRNA-guided cleavage still remained. Exh. 1047 (Song J.J. *et al.*, *Crystal Structure of Argonaute and Its Implication for RISC Slicer Activity*, Science 305:1434-37 (2004)) at 1436, Fig. 4. The same group also was one of the first to suggest that the Argonaute proteins were the catalytic components of RISC, the RNA-induced silencing complex. Exh. 1048 (Liu J. *et al.*, *Argonaute2 is the Catalytic Engine of Mammalian RNAi*, Science 305:1437-41 (2004)) at p. 1441. And, although insights into the structure and function of various proteins involved in the RNAi machinery, including Argonaute, have been reported over the years, even as recently as 2013, Meister could correctly state that “there are still major gaps in our understanding of Argonaute function on the molecular level.” Exh. 1049 (Meister G., *Argonaute Proteins: Functional Insights and Emerging Roles*, Nature Reviews Genetics 14:447-56 (2013)) at p. 456.

259. As noted in paragraphs 252-254 above, the early studies on modified siRNAs through 2003 focused on examining the effects on RNAi of *single*

modification strategies (on one or both strands), or at select nucleotide positions along one or both strands, with the remaining nucleotides being *unmodified*.

McSwiggen *et al.* were the first to demonstrate in 2003 that siRNA molecules having *two different* 2'-modifications, including 2'-O-methyl or 2'-fluoro pyrimidines, at internal nucleotide positions had RNAi activity. Exh. 1064 (International Publication No. WO 03/070918 by McSwiggen *et al.*) ("McSwiggen PCT") at pp. 98-103 (Example 5), 142-46 (Table I), Figs. 4-16. And, as discussed below, in 2005, Allerson *et al.*, published one of the first reports of fully modified siRNA molecules having a combination of *two different* 2'-modifications that not only improved stability but improved potency compared to unmodified siRNA. Exh. 1016 (Allerson 2005) at p. 901. This study demonstrated that a fully-modified siRNA molecule having a combination of *two different* 2'-modifications—2'-O-methyl and 2'-fluoro at alternating positions across both strands—not only was more stable but was over 500-fold more potent than the unmodified siRNA. Exh. 1016 (Allerson 2005) at pp. 902-03, Figs. 2-3, Table I. This increase in potency was considered surprising and highlighted that the effect of specific combinations of chemical modifications on siRNA activity could not have been predicted based on structure alone.

260. Also in 2005, Morrissey *et al.* published one of the first reports of *in vivo* activity of a fully modified siRNA molecule completely lacking 2'-OH

residues. Exh. 1065 (Morrissey D.V. *et al.*, *Activity of Stabilized Short Interfering RNA in Mouse Model of Hepatitis B Virus Replication*, *Hepatology* 41:1349-56 (2005)) (“Morrissey 2005”) at pp. 1354-55. This study demonstrated that a specific combination of chemical modifications—2’-fluoro substitutions on all the pyrimidines (*i.e.*, cytidine and uridine), 2’-deoxyribose or 2’-O-methyl substitutions on all the purines (*i.e.*, adenosine and guanosine) of the sense or antisense strand, respectively, and 5’- and 3’-inverted abasic end caps on the sense strand—not only improved stability, but resulted in a more effective level of silencing *in vivo*, which could not have been predicted based on structure alone. Exh. 1065 (Morrissey 2005) at pp. 1351, 1354-55. The authors acknowledged that chemical modifications were likely sequence dependent and may preclude the development of simple rules that would be broadly applicable:

“Given that RNAi requires recognition of siRNAs by the RNA-induced silencing complex protein complex, it is not surprising that siRNA function is sensitive to chemical modification. ***The interplay between internucleotide linkage changes, base and sugar modifications, and effects on helical character are likely to be complex and may preclude simple rules about which modifications are permissible. In addition, modifications are likely to have dissimilar impact on different primary sequences.***”

Exh. 1065 (Morrissey 2005) at p. 1354 (emphasis added). Alnylam’s own work shows that even for a particular target (VEGF), there was no single best modification strategy that was universally applicable and provided effective

siRNAs across all the siRNA duplexes tested. *See e.g.*, Exh. 1083 (WO 2005/089224 by de Fougerolles *et al.*) at Table 6 (testing eight different modification strategies in 17 different parent siRNA duplexes targeting VEGF, including alternating 2'-O-methyl modifications across both strands and selective incorporation of 2'-O-methyl or 2'-fluoro modifications at pyrimidines on one or both strands, and showing that not all of the modified siRNA retained RNAi activity). Alnylam's data shows even for a particular siRNA duplex, the location and position of the 2'-O-methyl modifications matters and differently impacts RNAi activity. Exh. 1083 (WO 2005/089224) at *e.g.*, Table 6, p. 117.

261. Over the years, as the requirements of RNAi are being better understood, so has the complexity increased. As I noted in 2008:

“siRNA duplexes have been chemically modified in a wide variety of ways. *Some of the results in the literature, however, seem to contradict one another, or to work on one system but not another. This field is still very young, and it will take time for the more robust and universal modifications to be recognized as such.* In the meantime, it is useful to have many options so that at least one of the chemistries can be used to modify an siRNA without compromising its potency.”

Exh. 1027 (Watts 2008) at p. 843 (emphasis added). Thus, even as of 2008, as I acknowledged, the chemical modification of siRNA was considered a very young field.

262. And, as noted in paragraphs 228-235 above, the lack of predictability

is also compounded by the fact that different modifications interact in different ways. Further, the fact that a particular pattern of modifications is observed to be useful for one or even several sequences does not mean that it will be universally applicable (or even that it is the best starting point for optimization of the pattern of other sequences). It became increasingly understood as of 2008 that “[t]he precise choice of chemical modifications to employ can vary with the design [duplex architecture] of the siRNA used, specific sequence, intended application, and method of delivery” and that “the precise design of the siRNA employed can influence the choice of pattern of chemical modifications suitable for use.” Exh. 1039 (Behlke 2008) at p. 306. It has also become increasingly clear since 2008 that “[t]here is no single ‘best’ modification pattern to recommend; rather, choice of the precise modification pattern employed can vary with the design of the siRNA, application, route of delivery, and optional use of delivery aids, such as liposomes or nanoparticles.” Exh. 1039 (Behlke 2008) at p. 314.

263. And despite the fact that the state of the art has evolved by leaps and bounds since the initial discovery of RNAi in mammalian cells in 2001, the chemical modification of siRNA is still considered a rapidly developing field of study. Exh. 1035 (Deleavey 2009) at p. 16.3.16. As I acknowledged in 2009:

***“While rapid progress has been made in this field of study, several frontiers remain to be explored with chemically modified siRNAs. Most of the currently used strategies have significant sequence dependence; thus, a remaining***

*challenge is to find universal chemical modification strategies, or to elucidate rules to allow reliable prediction of which modifications and architectures will be effective for a given sequence.* Significant advances in siRNA delivery technologies have also been achieved, and in combination with chemical modification strategies, the prospects for siRNA therapeutics are looking up.”

Exh. 1035 (Deleavey 2009) at p. 16.3.16 (emphasis added). Even as of 2009, finding a universally applicable chemical modification strategy that is independent of sequence was still considered to be a major challenge. Likewise, rules for reliably predicting which modifications and architecture would be effective for a given siRNA sequence remained to be elucidated.

264. Thus, while the state of the art has evolved considerably over the last decade, even in light of more recent insights, a person of ordinary skill in the art would not find any guidance, examples, or direction in the ’501 specification that would have allowed them to practice the invention now claimed. Moreover, the impact of modifications on siRNAs on function and stability could not be predicted and would need to be tested experimentally. In my opinion, this lack of predictability would increase the burden placed on a person of ordinary skill in the art to be able to practice the claimed invention. *See also* ¶¶ 228-235 above.

#### **iv. Quantity of experimentation necessary**

265. As noted above, the genus of chemically-modified siRNA molecules encompassed by the ’501 patent claims is very large and possibly unlimited, and

includes structurally diverse modified siRNAs. Moreover the '501 patent specification gives no guidance or examples as to a strategic direction in which to embark in exploring this vast chemical space. Identifying the siRNA molecules of this vast genus that would be useful for RNAi would require a person of ordinary skill in the art to engage in a systematic design, synthesis, and screening process. And, as I discussed in paragraphs 257-263 above, because the effect of modifications on RNAi activity and/or stability is considered to be sequence specific (Exh. 1035 (Deleavey 2009) at p. 16.3.11), for each target sequence, a person of ordinary skill in the art would need to make and test the numerous possible permutations, combinations, and arrangements of the various types of 2'-O-alkyl modified or differently modified ribonucleotides relative to each other or the unmodified ribonucleotides within the stretches of the strands of the siRNA molecules, and any additional combinations of design strategies, including strand lengths, overhang requirements, duplex architectures, or other modifications, (which the claims allow for) and test them for activity and stability.

266. As discussed in paragraph 262 above, the precise choice of chemical modifications to employ can vary with the design of the siRNA used, specific sequence, intended application, and method of delivery. Exh. 1039 (Behlke 2008) at p. 306. Thus, for each siRNA molecule, a person of ordinary skill in the art would need to make the following determinations, depending on the intended



application and method of delivery: (1) select target nucleic acid or sequences, (2) select a duplex architecture for the siRNA molecules, (3) select a chemical modification strategy, (4) synthesize the siRNA molecule, and (5) test the siRNA molecule for activity and stability. At the very least, items (1), (2), and (3) require original thought and insight and non-intuitive leaps, nowhere disclosed in the '501 patent specification. And each of the steps would involve considerable work:

- For example, selecting a target nucleic acid itself would involve at minimum a consideration of the genetic basis of the disease, whether siRNA would be a viable approach for treating the disease, whether siRNA molecules can be delivered to the relevant tissue or target site. Even if the target gene is known, selection of a target site within the corresponding RNA is a process that requires choices to be made based on non-trivial biological insights.
- Selecting an siRNA architecture would involve a consideration of factors such as the tissues of therapeutic relevance and the different bioavailability and inherent potency of different architectures. There may also be interplay between architecture and sequence (from the previous point) on factors like potency and stability.
- Selecting a modification strategy is based on extensive structure-activity relationship analyses for each architecture, then additional modification studies for each siRNA sequence to determine the optimal chemical modification strategies for a particular siRNA. When novel chemical modifications are tested, pre-existing insights need to be reconfirmed, as the interactions between different types of modifications can vary.
- Synthesizing each of the chemically-modified siRNAs requires understanding the coupling and deprotection conditions of any modifications involved, setting up the syntheses, deprotecting the oligomers, purifying the oligomers, characterizing their identity and purity, then measuring their concentration and annealing complementary strands.

- Designing appropriate, robust, and reproducible *in vitro* and or *in vivo* assays is required before testing can be carried out. This often involves the use of purified enzymes or biomatrices to test stability, the use of silencing assays in multiple cell types to examine the RNAi activity and stability of the siRNA molecules, including measurement of mRNA or protein knockdown, followed by establishing dose-response curves, stability assays, identification of degradation products of the siRNA molecules, immunostimulatory activity assays, specificity assays often based on high-throughput sequencing or microarrays, and then animal studies if a therapeutic use is envisioned. The results of these assays are integrated to identify promising leads for further testing, or to redesign, reassess, or retool the design strategy.

Exh. 1035 (Deleavey 2009) at pp. 16.3.3-16; Exh. 1050 (de Fougerolles A.R. and Maraganore J.M., Chapter 16, *Discovery and Development of RNAi Therapeutics*, Antisense Drug Technology: Principles, Strategies and Applications (2d ed. 2008)) (“de Fougerolles 2008”) at pp. 466-81; *see also* Exh. 1036 (Bramsen 2012); ¶¶ 176-177, 235 above.

267. Moreover, while various modifications can confer advantageous properties to siRNAs, “it is also clear that the effectiveness of modifications has strong sequence dependence. Many modifications are beneficial in some siRNA regions but detrimental in others, and enhance activity in some sequence contexts, but reduce potency in others.” Exh. 1035 (Deleavey 2009) at p. 16.3.11. Thus, determining whether the siRNA molecules having the claimed structural features would be functional could not be predicted from structure alone and would require screening, which would take substantial time and effort.

268. Numerous publications over the years confirm that the design of

modified siRNA molecules that are active and stable has been an empirical process. For example, Behlke noted that site selection is critical to the performance of siRNA compounds and that work on unmodified siRNA molecules has led to the development of excellent site selection and design criteria as well as computer assisted algorithms to facilitate this process. Exh. 1039 (Behlke 2008) at p. 306. However, Behlke went on to note that despite the progress made, empiric testing would be necessary and that design rules and site-selection criteria for modified siRNAs were lacking in the field:

“It is important to note that the use of chemical modifications can alter the potency of a siRNA and frequently a chemically modified siRNA will show lower potency than the unmodified RNA version of that same sequence, especially when extensively modified. Specifically, certain modification patterns have been demonstrated to impair the ability of an RNA sequence to trigger an RNAi effect whereas other patterns have less of an impact on potency. These effects vary with sequence, such that a given modification pattern can be effective at one site yet reduce potency of a siRNA at a second site. ***Thus empiric testing is usually necessary to ensure that a modified siRNA is effective when a known potent unmodified siRNA is converted to a modified form.*** Alternatively, initial site screening can be done using only modified duplexes, bypassing use of unmodified RNA entirely. ***Design rules and site selection criteria specific for modified siRNAs have not been reported.***”

Exh. 1039 (Behlke 2008) at p. 306 (emphasis added); *see also* Exh. 1040 (Pei and Tuschl 2006) at p. 670 (noting that computer-based approaches or tools “are not perfect” and that “[n]ot every selected siRNA meets the desired thresholds of

potency and specificity, so that experimental proof of downregulation of targeted mRNA or protein ***remains important***, not even considering the evaluation of off-target effects.”) (emphasis added). And, although siRNA candidate selection guidelines and computer-based approaches or tools were becoming available in 2006 that could “increase the likelihood of identifying effective and specific siRNAs at the expense of eliminating many potentially functional and specific siRNAs,” they did ***not*** eliminate “that need [for siRNAs] to be experimentally validated to identify potent and specific siRNAs for a given target gene.” Exh. 1040 (Pei and Tuschl 2006) at pp. 670, 674. And, I note that these computer-based selection tools had not been formulated in 2002-2003, and none had been reported even as of 2008 for modified siRNAs of the type claimed in the ’501 patent (any 2’-O-alkyl modified ribonucleotides alternating with any differently modified or unmodified ribonucleotides). Exh. 1039 (Behlke 2008) at p. 306. As Alnylam’s own scientists noted:

“Prediction of the nucleotide sequence and chemical modifications required to yield an ideal siRNA duplex remains a work in progress .... At present, the most prudent and robust strategy is to synthesize and screen a substantial library of siRNA duplexes for each target of interest (perhaps even ‘tiling’ the entire messenger RNA) to identify the most promising candidates.”

Exh. 1076 (Bumcrot D. *et al.*, *RNAi Therapeutics: A Potential New Class of Pharmaceutical Drugs*, *Nature Chemical Biology*, 2(12):711-19 (2006)) at p. 714.

269. As with *in vitro* selection of lead candidates, a person of ordinary skill in the art would consider potency, specificity, and stability as the three most important attributes to take into account when designing and selecting siRNA. Exh. 1050 (de Fougérolles 2008) at p. 466. And, using a combination of bioinformatics, chemical modifications, and empirical testing, while it would have been possible to “very rapidly, in a span of several months, identify potent, specific and stable *in vitro* active lead siRNA candidates to any target of interest” (Exh. 1050 (de Fougérolles 2008) at p. 467), that certainly would not have been the case in 2002-2003 (when the earliest applications to which the ’501 patent claims priority were filed) and would not eliminate the need for empirical experimental research, screening, testing, or validation all together. And while it may take several months today to identify *in vitro* active lead siRNA candidates for a target gene of interest, the claims are not limited to any target gene. In my opinion, the amount of experimentation required to design, synthesize, screen, test, and identify siRNA molecules in the potentially unlimited genus of siRNA molecules that are functional—have RNAi activity—would be significant and undue. As de Fougérolles noted in 2008:

“Predicting the nucleotide sequence and chemical modifications required to yield an ideal RNAi therapeutic ***still remains a work in progress***. While much progress has been made in understanding what attributes are required to identify an *in vitro* active and stable siRNA, ***much less is known about how well those attributes translate into***

***identifying in vivo active siRNAs... At present, in order to identify robust in vitro active lead candidate siRNAs suitable for subsequent in vivo study, the practical and prudent approach is to synthesize and screen a library of siRNA duplexes for potency, specificity, nuclease stability, and immunostimulatory activity.”***

Exh. 1050 (de Fougérolles 2008) at p. 469 (emphasis added). This highlights the fact that even though the field had advanced as of 2008, the biopharmaceutical industry continued to rely on an “empiric approach.” Exh. 1050 (de Fougérolles 2008) at p. 469. And while a person of ordinary skill in the art may have a better understanding of the design principles that could get one closer to identifying a stable, functional siRNA in less time today, that certainly was not the case in 2002-2003, and would not eliminate the need for empirical testing all together.

270. The lack of sufficient working examples or guidance in the specification, and the lack of a correlation between structure and function in the specification only contribute to the problem. A person of ordinary skill in the art would understand from the specification that the effect of each and every modification on activity and stability of an siRNA molecule would need to be carefully considered and tested. *See also* ¶¶ 71-83, 254-264 above. In my opinion, significant work would be necessary to select, design, synthesize, and screen siRNA molecules encompassed by the claimed genus of siRNA molecules containing contiguous alternating 2'-O-alkyl modified ribonucleotides alternating with unmodified or differently modified ribonucleotides and having varied duplex

architectures and to determine which of those would be useful for RNAi as required by the claims. At best, the patent provides an unsubstantiated hypothesis that siRNAs containing multiple different modifications might have RNAi activity. What would be needed to test this hypothesis would be nothing short of a full-blown research project, requiring a person of ordinary skill in the art to engage in an iterative, trial and error process; one that is complicated by the lack of any meaningful guidance in the '501 patent specification that could serve as preliminary data.

271. Finally, I note that although the significant legacy of nucleic acid chemistry developed for antisense oligonucleotide therapeutics sped up the evolution of RNAi technology, the molecular requirements for effective recruitment of the RNAi enzymatic machinery and the double-stranded nature of RNAi imposed a unique set of limitations on the chemical modification of siRNAs, which took years of investigation, creativity, and experimentation to overcome. Exh. 1030 (Khvorova A. and Watts J.K., *The Chemical Evolution of Oligonucleotide Therapies of Clinical Utility*, *Nature Biotechnology* 35(3):238-48 (2017)) at p. 242; *see also* Exh. 1029 (Kaczmarek J.C. *et al.*, *Advances in the Delivery of RNA Therapeutics: From Concept to Clinical Reality*, *Genome Medicine* 9(60):1-6 (2017)) at p. 12. The fact that the first siRNA-based therapeutic is only now poised to enter the market in 2018, almost 20 years since

the discovery of RNAi in mammalian cells, is a testament to the numerous obstacles and limitations that had to be overcome and highlights the need for years of multi-disciplinary research and collaboration between academia and the biopharmaceutical industry. In my opinion, this is further evidence of the unpredictability in the field of the '501 patent and demonstrates that the amount of experimentation needed was not only undue but also required innovative steps, nowhere disclosed in the '501 patent specification.

### **XIII. The Original Claims of the '968 Application Fail to Provide Written Description and Enablement Support**

272. As noted in paragraph 57 above, the '501 patent was filed on May 8, 2017 as the '968 application. As filed, the '968 application included 30 claims. Exh. 1004 ('968 prosecution history, Original Claims) at claims 1-30. Original claims 1-30 of the '968 application are identical to the issued claims 1-30 of the '501 patent. Exh. 1001 ('501 patent) at claims 1-30. Thus, for the reasons discussed above, original claims 1-30 fail to demonstrate possession of the vast genus of modified siRNA molecules and fail to teach how to practice the claimed invention without undue experimentation.

273. It is therefore my opinion that the specification as a whole, including the original claims, does not demonstrate possession or provide any guidance for the vast genus of modified siRNA molecules of the '501 patent claims. *See also* Sections XI-XII above.



#### **XIV. Unpatentability Over the Prior Art (Claims 1-5, 7, 9-27 and 29)**

274. I understand that a patent claim is invalid for anticipation if a single piece of prior art contains each and every element recited in the claim. *See* ¶ 29 above. In my opinion, Allerson 2005 meets all of the limitations of claims 1, 2, 9, 10, 12, 13, 16-20, 22, 24, and 25; Choung 2006 meets all of the limitations of claims 1, 3-5, 7, 9-11, 14, 15, 20, 21, and 23; and Podbevsek 2010 meets at least all of the structural limitations of claims 1, 16, 19, 26, 27, and 29 of the '501 patent for the reasons discussed below.

##### **A. Allerson 2005 Meets All of the Limitations of Claims 1, 2, 9, 10, 12, 13, 16-20, 22, 24, and 25 of the '501 Patent**

275. Allerson 2005, by authors Allerson *et al.*, is entitled “Fully 2’-Modified Oligonucleotide Duplexes with Improved in Vitro Potency and Stability Compared to Unmodified Small Interfering RNA,” and published in the Journal of Medicinal Chemistry in 2005 and online on January 20, 2005. Exh. 1016 (Allerson 2005) at p. 901. I understand that Allerson 2005 was not in front of the Examiner during prosecution of the '501 patent and is not cited anywhere in the '501 patent.

276. As discussed in paragraphs 26-28 above, I understand that Allerson 2005 qualifies as prior art because it was published on January 20, 2005.

##### **i. Overview of Allerson 2005**

277. Allerson 2005 discloses “a small interfering RNA (siRNA) motif,

consisting entirely of 2'-O-methyl and 2'-fluoro nucleotides, that displays enhanced plasma stability and increased in vitro potency.” Exh. 1016 (Allerson 2005) at p. 901. Allerson 2005 is “the first report of such a potent fully modified motif, which may represent a useful design for therapeutic oligonucleotides.” Exh. 1016 (Allerson 2005) at p. 901. Allerson 2005 notes that its motif, which includes 2'-O-methyl and 2'-fluoro modified nucleotides, was novel even when compared with a previously described pattern—an “alternating” motif “using 2'-OMe and unmodified (2'-OH) nucleotides.” Exh. 1016 (Allerson 2005) at p. 901 (citing to Reference (21), a paper co-authored by the '501 patent's inventors, Exh. 1028 (Czauderna 2003)).

278. The duplexes used in Allerson 2005's structure-activity-relationship (SAR) study were designed to target one of two sites within the coding region of the human PTEN mRNA, which had previously been reported as valid target sites for siRNA. Exh. 1016 (Allerson 2005) at p. 901. Modified and unmodified duplexes were introduced into HeLa cells using a cationic lipid transfection reagent (lipofectin). *In vitro* activity was measured by performing RT-PCR on the PTEN mRNA and comparing to mRNA levels of untreated cells. All PTEN signals were normalized to total RNA, as measured with RiboGreen.

279. Allerson 2005 provides a detailed description of the sequences, modification patterns, and data showing that its 2'-O-methyl and 2'-fluoro motifs

are capable of RNAi. For example, Figure 2 depicts duplexes of alternating 2'-O-methyl (red) and 2'-fluoro (green) modified nucleotide motifs, including duplex 8. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Figure 2 also shows that each chemically-modified duplex can inhibit endogenous PTEN mRNA expressed in HeLa cells. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2 (duplexes 8, 11).

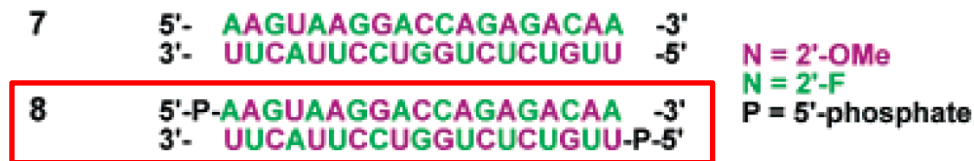
280. Allerson 2005 concludes that “[t]he alternating 2'-F/2'-OMe motif appears to be an attractive design for creating functionally active and stable RNA duplexes.” Exh. 1016 (Allerson 2005) at pp. 903.

281. In my opinion, Allerson 2005 discloses at least one double-stranded siRNA molecule having contiguous alternating 2'-O-methyl and 2'-fluoro modifications on both strands and discloses that it has RNAi activity.

**a. Allerson 2005 discloses siRNA duplex 8**

282. Allerson 2005 discloses several double-stranded siRNA targeting PTEN mRNA that have contiguous alternating 2'-O-methyl and 2'-fluoro modified nucleotides in the sense and antisense strands, including duplexes 7, 8, 10, and 11. Exh. 1016 (Allerson 2005) at pp. 901-02, Fig. 2.

283. One such siRNA, “duplex 8,” contains alternating 2'-O-methyl and 2'-fluoro modified nucleotides on both strands. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2A. Duplex 8 of Figure 2 is reproduced below.



Exh. 1016 (Allerson 2005) at p. 902, Fig. 2A.

284. Below is an annotated version of the duplex 8 construct:

Sense Strand	5'	P-a <u>a</u> g <u>u</u> a <u>a</u> g <u>g</u> a <u>c</u> c <u>a</u> g <u>a</u> g <u>a</u> c <u>a</u> a	3'
Antisense Strand	3'	u <u>u</u> c <u>a</u> u <u>u</u> c <u>c</u> u <u>g</u> g <u>u</u> c <u>u</u> c <u>u</u> g <u>u</u> -P	5'

Lower case = 2'-O-methyl ribonucleotide                      P = 5'-phosphate  
Lower case = 2'-fluoro ribonucleotide

Exh. 1016 (Allerson 2005) at p. 902, Fig. 2A.

285. Duplex 8 is blunt ended on both ends and both the sense and antisense strands have 19 nucleotides that are complementary to one another (*i.e.*, each C, G, U, and A on one strand is base-paired with a G, C, A, and U on the other strand, respectively). Exh. 1016 (Allerson 2005) at p. 902, Fig. 2A (PTEN mRNA reduction by duplexes targeted to site A). Allerson 2005 states that “the bottom strand of each duplex is complementary to the target mRNA [PTEN mRNA].”

Exh. 1016 (Allerson 2005) at p. 902; *see also* ¶ 278 above.

286. Duplex 8 is a fully-modified siRNA molecule having 2'-O-methyl modified nucleotides alternating with 2'-fluoro modified nucleotides on both the sense and antisense strands. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. As shown above, in duplex 8, each 2'-O-methyl modified ribonucleotide of the antisense strand is base-paired with a 2'-fluoro modified ribonucleotide on the

sense strand; each 2'-fluoro modified ribonucleotide on the antisense strand is base-paired with a 2'-O-methyl modified ribonucleotide on the sense strand, *i.e.*, there is a phase shift. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2; *see also* Exh. 1001 ('501 patent) at cols. 17:28-36, 17:56-18:2, 18:12-18, Fig. 2B. Additionally, in duplex 8, the 2'-O-methyl modified ribonucleotides are located at odd nucleotide positions (1, 3, 5, 7, etc.) on the antisense strand (from the 5' - to 3'-end) and they are at even nucleotide positions (2, 4, 6, 8, etc.) on the sense strand (from the 5' - to the 3'-end).

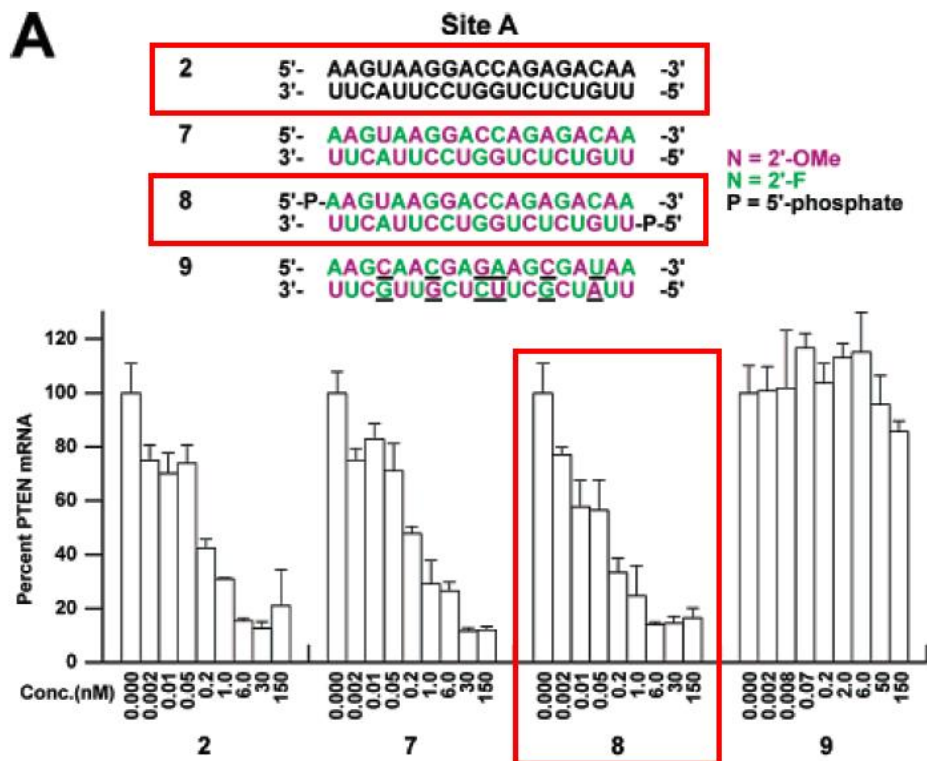
**b. Allerson 2005 discloses that the siRNA duplex 8 has RNAi activity**

287. Allerson 2005 includes data on RNAi activity for several siRNA, and in particular, includes a figure and a table showing duplex 8's activity in inhibiting PTEN mRNA. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2, Table 1.

288. Allerson 2005 describes the RNAi activity experiment as testing the design of the duplexes to "target one of two sites within the coding region of the human PTEN mRNA." Exh. 1016 (Allerson 2005) at p. 901. Modified and unmodified duplexes were introduced into HeLa cells using a cationic lipid transfection reagent. The subsequent "[i]n vitro activity was measured by performing RT-PCR on the PTEN mRNA and comparing to mRNA levels of untreated cells." Exh. 1016 (Allerson 2005) at p. 901.

289. The results of the experiment showing the RNAi activity of duplex 8

as compared to its unmodified analogue, duplex 2, were depicted in Figure 2A, excerpts of which I have annotated below:



Exh. 1016 (Allerson 2005) at p. 902, Fig. 2A.

290. Figure 2A demonstrates that administration of duplex 8 causes a marked decrease in the levels of PTEN mRNA. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2A. And as shown in Figure 2A, this activity was dose dependent with higher concentrations of duplex 8 resulting in lower percentages of PTEN mRNA. This *in vitro* data shows that duplex 8 has RNAi activity.

- ii. **Allerson 2005 meets all of the limitations of claims 1, 2, 9, 10, 12, 13, 16-20, 22, 24, and 25 of the '501 patent**

291. Based on the disclosures of Allerson 2005 described above, and in

particular, the siRNA duplex 8, Allerson 2005 meets all of the limitations of claims 1, 2, 9, 10, 12, 13, 16-20, 22, 24, and 25 of the '501 patent.<sup>26</sup>

**a. Allerson 2005 meets all of the limitations of claim 1**

292. Claim 1 of the '501 patent recites:

“A double-stranded siRNA molecule against a target nucleic acid,

wherein the double-stranded siRNA molecule comprises a first strand and a second strand,

wherein the first strand comprises a first stretch that is complementary to the target nucleic acid,

wherein the second strand comprises a second stretch that is the same length as the first stretch and is complementary to the first stretch,

wherein the first strand and the second strand form a double-stranded structure comprising the first stretch and the second stretch,

wherein the first stretch and the second stretch each comprises contiguous alternating 2'-O-alkyl ribonucleotides,

wherein the 2'-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides,

wherein the 2'-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2'-O-alkyl ribonucleotides of the second strand, and

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<sup>26</sup> Allerson 2005 discloses duplexes 7, 8, 10, and 11, each one of which would meet all the limitations of 1, 2, 9, 10, 12, 13, 16-20, 22, 24, and 25 of the '501 patent for the same reasons that duplex 8 meets all the claim limitations and the duplexes would be considered interchangeable for purposes of the analysis.

wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides.”

Exh. 1001 (’501 patent) at claim 1.

**i. “A double-stranded siRNA molecule against a target nucleic acid”**

293. Claim 1 of the ’501 patent recites “a double-stranded siRNA molecule against a target nucleic acid.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 278 and 283-285 above, duplex 8 is a double-stranded siRNA molecule that targets the nucleic acid PTEN mRNA. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Thus, duplex 8 meets this limitation in claim 1. *See also* ¶¶ 287-290 above.

**ii. “wherein the double-stranded siRNA molecule comprises a first strand and a second strand”**

294. Claim 1 of the ’501 patent recites “wherein the double-stranded siRNA molecule comprises a first strand and a second strand.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 283-285 above, duplex 8 meets this limitation because it is a double-stranded molecule having an antisense (first) strand and a sense (second) strand. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2.

**iii. “wherein the first strand comprises a first stretch that is complementary to the target nucleic acid”**

295. Claim 1 of the ’501 patent recites “wherein the first strand comprises a first stretch that is complementary to the target nucleic acid.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 278 and 283-285 above, the



antisense (first) strand of duplex 8 is complementary to the target nucleic acid, PTEN mRNA. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Thus, the antisense strand comprises a first stretch of 19 nucleotides that is complementary to the target nucleic acid, PTEN mRNA, and duplex 8 meets this limitation.

**iv. “wherein the second strand comprises a second stretch that is the same length as the first stretch and is complementary to the first stretch”**

296. Claim 1 of the '501 patent recites “wherein the second strand comprises a second stretch that is the same length as the first stretch and is complementary to the first stretch.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 283-285 above, the sense (second) strand of duplex 8 is the same length as the antisense (first) strand; both are 19 nucleotides in length. As also shown above, the sense (second) strand of duplex 8 is complementary to the antisense (first) strand. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Thus, the sense and the antisense strand comprise second and first stretches of equal length (19 nucleotides), respectively, that are complementary to one another and duplex 8 meets this limitation.

**v. “wherein the first strand and the second strand form a double-stranded structure comprising the first stretch and the second stretch”**

297. Claim 1 of the '501 patent recites “wherein the first strand and the second strand form a double-stranded structure comprising the first stretch and the

second stretch.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 283-285 above, duplex 8 meets this limitation because the antisense (first) and the sense (second) strands, which comprise the first and second stretches, hybridize to form a double-stranded structure. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2.

**vi. “wherein the first stretch and the second stretch each comprises contiguous alternating 2’-O-alkyl ribonucleotides”**

298. Claim 1 of the ’501 patent recites “wherein the first stretch and the second stretch each comprises contiguous alternating 2’-O-alkyl ribonucleotides.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 283-286 above, in duplex 8, the antisense (first) and the sense (second) strands, which comprise the first and second stretches, respectively, each have contiguous alternating 2’-O-methyl modified ribonucleotides, which are a species of 2’-O-alkyl modified ribonucleotides, across both strands. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Thus, duplex 8 meets this limitation.

**vii. “wherein the 2’-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides”**

299. Claim 1 of the ’501 patent recites “wherein the 2’-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 283-286 above, duplex 8 meets this limitation because the alternating 2’-O-methyl modified

ribonucleotides alternate with 2'-fluoro modified ribonucleotides (*i.e.*, differently modified ribonucleotides) across both strands. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2.

**viii. “wherein the 2'-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2'-O-alkyl ribonucleotides of the second strand, and”**

300. Claim 1 of the '501 patent recites “wherein the 2'-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2'-O-alkyl ribonucleotides of the second strand.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 283-286 above, duplex 8 has contiguous alternating 2'-O-methyl ribonucleotides that alternate with 2'-fluoro modified ribonucleotides. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. As also noted in paragraph 286 above, in duplex 8, the alternating 2'-O-methyl modified ribonucleotides on the antisense (first) strand are shifted by one relative to the 2'-O-methyl modified ribonucleotides on the sense (second) strand, *i.e.*, are in a phase-shift. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Thus, duplex 8 meets this limitation.

**ix. “wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides”**

301. Claim 1 of the '501 patent recites “wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 283-285 above, the

antisense (first) and the sense (second) strands of duplex 8 are 19 ribonucleotides in length. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Thus, both strands comprise first and second stretches consisting of at least 17 and fewer than 30 ribonucleotides and duplex 8 meets this limitation.

302. In sum, duplex 8 meets all of the limitations of claim 1.

**b. Allerson 2005 meets all of the limitations of claim 2**

303. Claim 2 is dependent on claim 1. Claim 2 additionally requires that “the double-stranded siRNA molecule is blunt ended on both ends.” Exh. 1001 (’501 patent) at claim 2.

304. As discussed in paragraphs 283-285 above, duplex 8 is blunt ended on both ends. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Because duplex 8 meets all the limitations of claim 1, as demonstrated in paragraphs 292-302 above, and because duplex 8 is blunt ended on both ends, duplex 8 meets all of the limitations of claim 2.

**c. Allerson 2005 meets all of the limitations of claim 9**

305. Claim 9 is dependent on claim 1. Claim 9 additionally requires that “each of the first strand and the second strand consists of at least 18 nucleotides.” Exh. 1001 (’501 patent) at claim 9.

306. As discussed in paragraphs 283-285 above, both strands of duplex 8 contain 19 ribonucleotides. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Because

duplex 8 also meets all the limitations of claim 1, as demonstrated in paragraphs 292-302 above, and because duplex 8 has two strands that are each 19 nucleotides long, duplex 8 meets all of the limitations of claim 9.

**d. Allerson 2005 meets all of the limitations of claims 10, 12, 13, 20, and 22**

307. Claims 10 and 12 are dependent on claim 1. Claim 10 additionally requires that “the 2’-O-alkyl ribonucleotides are 2’-O-methyl ribonucleotides.” Claim 12 additionally requires that “the differently modified ribonucleotides have an amino, fluoro, different alkoxy, or alkyl modification at the 2’-position.” Exh. 1001 (’501 patent) at claims 10, 12.

308. As discussed in paragraphs 283-286 above, duplex 8 has 2’-O-methyl modified ribonucleotides. As also discussed above, duplex 8 has 2’-fluoro modified ribonucleotides, *i.e.*, the fluoro modification is at the 2’-position. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Because duplex 8 also meets all the limitations of claim 1, as demonstrated in paragraphs 292-302 above, and because duplex 8 has 2’-O-methyl modified ribonucleotides and 2’-fluoro modified ribonucleotides, duplex 8 meets all of the limitations of claims 10 and 12.

309. Claim 13 is dependent on claim 10 and additionally requires “the 2’-O-methyl ribonucleotides alternate with 2’-fluoro ribonucleotides.” As discussed in paragraphs 283-286 above, duplex 8 has contiguous alternating 2’-O-methyl modified ribonucleotides that alternate with 2’-fluoro modified ribonucleotide

across both strands. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Because duplex 8 also meets all the limitations of claim 10 as demonstrated in paragraphs 307-308 above, and because duplex 8 has 2'-O-methyl modified ribonucleotides that alternate with 2'-fluoro modified ribonucleotides on both strands, duplex 8 meets all of the limitations of claim 13.

310. Claim 20 is dependent on claim 1 and additionally requires "the 5' ribonucleotide of the first stretch is a 2'-O-alkyl modified ribonucleotide." Claim 22 is dependent on claim 13 and additionally requires "the 5' ribonucleotide of the first stretch is a 2'-O-methyl ribonucleotide." Exh. 1001 ('501 patent) at claims 20, 22.

311. As discussed in paragraphs 283-286 above, in the blunt ended duplex 8, the antisense (first) strand, and therefore the first stretch, begins with a 2'-O-methyl modified ribonucleotide, which is a species of 2'-O-alkyl modified ribonucleotides, at the first nucleotide position from the 5'-end. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Because duplex 8 meets all the limitations of claims 1 and 13 as demonstrated in paragraphs 292-302 and 309 above, and because the 5' ribonucleotide of the first stretch of duplex 8 is a 2'-O-methyl ribonucleotide (*i.e.*, a 2'-O-alkyl ribonucleotide), duplex 8 meets all of the limitations of claims 20 and 22.

**e. Allerson 2005 meets all of the limitations of claims 16-19 and 24**

312. Claim 16 is dependent on claim 1. Claim 16 additionally requires that “the first stretch and the second stretch each comprises contiguous alternating single 2’-O-alkyl ribonucleotides, and wherein the single 2’-O-alkyl ribonucleotides alternate with the differently modified ribonucleotides.” Exh. 1001 (’501 patent) at claim 16.

313. As discussed in paragraphs 283-286 above, both the antisense (first) and sense (second) strands of duplex 8 contain contiguous alternating single 2’-O-methyl modified ribonucleotides, which are a species of 2’-O-alkyl modified ribonucleotides, alternating with 2’-fluoro modified ribonucleotides. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Thus, each of the first and second stretches comprise contiguous alternating single 2’-O-methyl modified ribonucleotides that alternate with 2’-fluoro modified ribonucleotides. Because duplex 8 also meets all the limitations of claim 1, as demonstrated in paragraphs 292-302 above, and because each of the first and second stretches of duplex 8 comprise single 2’-O-methyl modified ribonucleotides (*i.e.*, 2’-O-alkyl modified ribonucleotides) that alternate with 2’-fluoro modified ribonucleotides (*i.e.*, differently modified ribonucleotides), duplex 8 meets all of the limitations of claim 16.

314. Claims 17-19 are dependent on claim 16. Claim 17 additionally requires that “the single 2’-O-alkyl ribonucleotides are 2’-O-methyl

ribonucleotides.” Claim 18 additionally requires that “the differently modified ribonucleotides have an amino, fluoro, different alkoxy, or alkyl modification at the 2’-position.” Claim 19 additionally requires that “the single 2’-O-alkyl ribonucleotides are 2’-O-methyl ribonucleotides, and the differently modified ribonucleotides are 2’-fluoro ribonucleotides.” Exh. 1001 (’501 patent) at claims 17-19.

315. As discussed in paragraphs 283-286 above, duplex 8 has contiguous alternating single 2’-O-methyl ribonucleotides (*i.e.*, a 2’-O-alkyl modified ribonucleotide) that alternate with 2’-fluoro modified ribonucleotides. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Because duplex 8 also meets all the limitations of claim 16, as demonstrated in paragraphs 312-313 above, and because each of the first and second stretches comprise contiguous alternating single 2’-O-methyl modified ribonucleotides that alternate with 2’-fluoro modified ribonucleotides, duplex 8 meets the limitations of claims 17-19.

316. Claim 24 is dependent on claim 16 and additionally requires that “the 5’ ribonucleotide of the first stretch is a 2’-O-alkyl modified ribonucleotide that alternates with a differently modified ribonucleotide.” As discussed in paragraphs 283-286 above, in the blunt ended duplex 8, the antisense (first) strand, and therefore the first stretch, begins with a 2’-O-methyl modified ribonucleotide (*i.e.*, a 2’-O-alkyl modified ribonucleotide) at the first nucleotide position from the 5’-



end. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Additionally, as discussed above, the 2'-O-methyl modified ribonucleotides alternate with 2'-fluoro modified ribonucleotides on both strands. Because duplex 8 meets all the limitations of claim 16 as demonstrated in paragraphs 312-313 above, and because the 5' ribonucleotide of the first stretch is a 2'-O-methyl ribonucleotide (*i.e.*, a 2'-O-alkyl modified ribonucleotide) that alternates with a 2'-fluoro modified ribonucleotide, duplex 8 meets all of the limitations of claim 24.

**f. Allerson 2005 meets all of the limitations of claim 25**

317. Claim 25 is dependent on claim 19. Claim 25 additionally requires that “the double-stranded siRNA molecule is blunt ended on at least one end.” Exh. 1001 ('501 patent) at claim 25.

318. As discussed in paragraphs 283-285 above, duplex 8 is blunt ended on both ends. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2. Because duplex 8 meets all the limitations of claim 19, as demonstrated in paragraphs 314-315 above, and because duplex 8 is blunt ended on both ends, and thus, is blunt ended on at least one end, duplex 8 meets all of the limitations of claim 25.

319. In sum, Allerson 2005 meets all of the structural limitations of claims 1, 2, 9, 10, 12, 13, 16-20, 22, 24, and 25 of the '501 patent. Additionally, siRNA by definition is required to have RNAi activity (*see* Section IX.C.i above). As discussed in paragraphs 287-290 above, duplex 8 has RNAi activity as

demonstrated by the inhibition of PTEN mRNA levels in Fig. 2. Thus, Allerson 2005 meets this additional requirement of claims 1, 2, 9, 10, 12, 13, 16-20, 22, 24, and 25. *See also* ¶ 293 above.

**B. Choung 2006 Meets All of the Limitations of Claims 1, 3-5, 7, 9-11, 14, 15, 20, 21, and 23 of the '501 Patent**

320. Choung 2006, by authors Choung S. *et al.*, is entitled “Chemical Modification of siRNAs to Improve Serum Stability Without Loss of Efficacy,” and was published in Biochemical and Biophysical Research Communications in 2006 and online on February 20, 2006. Exh. 1072 (Choung 2006) at p. 919. Choung 2006 is listed as a cited reference for the '501 patent under “Other Publications.” Exh. 1001 ('501 patent) at p. 3. I understand that Choung 2006 was not discussed by the Examiner during prosecution of the '501 patent.

321. As discussed in paragraphs 26-28 above, I understand that Choung 2006 qualifies as prior art because it was published on February 20, 2006.

**i. Overview of Choung 2006**

322. Choung 2006 examined the effect of various chemical modification strategies on serum stability and RNAi activity of Sur10058, an siRNA specifically targeting survivin, a protein implicated in the apoptosis pathway. Exh. 1072 (Choung 2006) at p. 919. In the study, the authors “introduced various combinations of chemical modifications into Sur10058 in order to identify the modified structures which can greatly enhance its serum stability without reducing

its knockdown efficiency.” Exh. 1072 (Choung 2006) at p. 920.

323. The authors screened 82 siRNAs specifically targeting survivin, identified “highly effective siRNA inhibitors including Sur10058,” introduced various chemical modifications into Sur10058, and tested their effects on serum stability and activity. Exh. 1072 (Choung 2006) at pp. 919-20. Various modified and unmodified duplexes, including 2’-O-methyl modified siRNA, were introduced into HeLa cells using a cationic lipid transfection reagent (Lipofectamine 2000). *In vitro* activity was measured by performing RT-PCR on the survivin mRNA, comparing to mRNA levels of cells treated with Non-silencing siRNA (NC). All survivin signals were normalized to GAPDH. Northern blot analysis was also performed, with  $\beta$ -actin as an internal control. Exh. 1072 (Choung 2006) at p. 920.

324. Choung 2006 provides a detailed description of the sequences, modification patterns, and data showing that its 2’-O-methyl modified siRNA molecules have RNAi activity. For example, Figures 2, 3, and 5 demonstrate the effects of 2’-O-methyl modifications incorporated as 5’- and 3’-end modifications, alternating modifications, and as combinations of pyrimidine modifications on Sur10058 activity and stability. Exh. 1072 (Choung 2006) at Figs. 2, 3, 5.

325. In my opinion, Choung 2006 discloses a double-stranded siRNA molecule having contiguous alternating 2’-O-methyl modified and unmodified

ribonucleotides on both strands and discloses that it has RNAi activity.

**a. Choung 2006 discloses siRNA duplex Sur10058-Me-AL3**

326. Choung 2006 discloses several double-stranded 2'-O-methyl modified siRNA specifically targeting survivin, a protein implicated in both anti-apoptosis and the regulation of cytokinesis, including siRNA construct Sur10058-Me-AL3. Exh. 1072 (Choung 2006) at pp. 921-23, Fig. 3. The authors state: "All siRNA duplexes have the sense strand sequence of 5'-AAG GAG AUC AAC AUU UUC A dTdT-3'. The 19 nucleotides represent the target sequence in survivin (NM\_001168) and dTdT is a 2-nt 3' overhang. The antisense strand was composed of nucleotides that are complementary to the target sequence and the dTdT 3' overhang sequence." Exh. 1072 (Choung 2006) at p. 920.

327. Sur10058-Me-AL3, which contains alternating 2'-O-methyl and unmodified ribonucleotides and has 3'-overhangs is reproduced below. The top strand is the sense strand and the bottom strand is the antisense strand.

**6) Sur10058-Me-AL3**

5'            aaggagaucaacauuuuca dTdT 3'  
3' dTdT uuccucuaguuguaaaagu 5'

Lower case = unmodified

**Lower case** = 2'-O-methyl modified

dTdT = dideoxythymidine overhang

Exh. 1072 (Choung 2006) at p. 923, Fig. 3A.

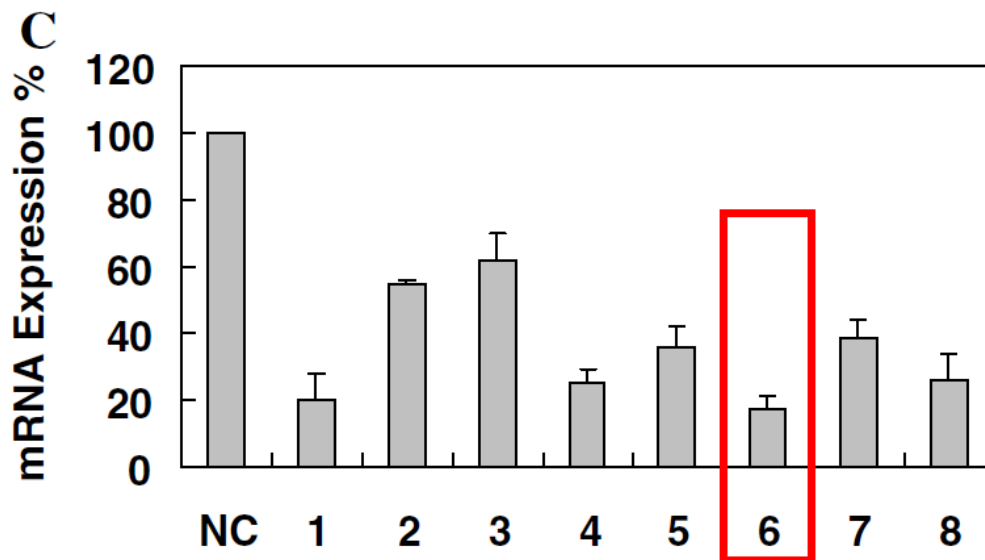
328. In Sur10058-Me-AL3, each of the sense (top) and the antisense (bottom) strands have a strand length of 21 nucleotides with two deoxythymidine overhangs at the 3'-ends. The sense and antisense strands have a stretch of 19 nucleotides that are complementary to another (*i.e.*, each C, G, U, and A on one strand is base-paired with a G, C, A, and U on the other strand, respectively). Exh. 1072 (Choung 2006) at p. 923, Fig. 3A. Choung 2006 states that “the antisense strand was composed of nucleotides that are complementary to the target sequence [in survivin].” Exh. 1072 (Choung 2006) at p. 920; *see also* ¶ 326 above.

329. As to the modifications, in Sur10058-Me-AL3, both the sense and the antisense strands comprise contiguous alternating single 2'-O-methyl modified and unmodified ribonucleotides across the 19-nucleotide stretch of each strand. Exh. 1072 (Choung 2006) at p. 923, Fig. 3A. As shown above, in Sur10058-Me-AL3, each 2'-O-methyl modified ribonucleotide on the antisense strand is base-paired with an unmodified ribonucleotide on the sense strand; each unmodified ribonucleotide on the antisense strand is base-paired with a 2'-O-methyl modified ribonucleotide on the sense strand, *i.e.*, there is a phase shift. Exh. 1072 (Choung 2006) at p. 923, Fig. 3A; *see also* Exh. 1001 ('501 patent) at cols. 17:28-36, 17:56-18:2, 18:12-18, Fig. 2B. Additionally, in Sur10058-Me-AL3, the 2'-O-methyl modified ribonucleotides are located at odd nucleotide positions (1, 3, 5, 7, etc.) on the antisense strand (from the 5'- to 3'-end) and they are at even nucleotide

positions (2, 4, 6, 8, etc.) on the sense strand (from the 5'- to the 3'-end).

**b. Choung 2006 discloses that the siRNA duplex Sur10058-Me-AL3 has RNAi activity**

330. Choung 2006 includes data on RNAi activity and survivin mRNA expression following transfection of Sur10058-Me-AL3 in Fig. 3C:



Exh. 1072 (Choung 2006) at p. 923, Fig. 3C.

331. As shown in Fig. 3C, transfection of Sur10058-Me-AL3 (2nM) in HeLa cells causes a marked decrease in the levels of survivin mRNA. Exh. 1072 (Choung 2006) at p. 923, Fig. 3C. This *in vitro* data shows that Sur10058-Me-AL3 has RNAi activity.

**ii. Choung 2006 meets all of the limitations of claims 1, 3-5, 7, 9-11, 14, 15, 20, 21, and 23 of the '501 patent**

332. Based on the disclosures of Choung 2006 described above, and in particular, the siRNA duplex Sur10058-Me-AL3, Choung 2006 meets all of the

limitations of claims 1, 3-5, 7, 9-11, 14, 15, 20, 21, and 23 of the '501 patent.

**a. Choung 2006 meets all of the limitations of claim 1**

333. Claim 1 of the '501 patent recites:

“A double-stranded siRNA molecule against a target nucleic acid,

wherein the double-stranded siRNA molecule comprises a first strand and a second strand,

wherein the first strand comprises a first stretch that is complementary to the target nucleic acid,

wherein the second strand comprises a second stretch that is the same length as the first stretch and is complementary to the first stretch,

wherein the first strand and the second strand form a double-stranded structure comprising the first stretch and the second stretch,

wherein the first stretch and the second stretch each comprises contiguous alternating 2'-O-alkyl ribonucleotides,

wherein the 2'-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides,

wherein the 2'-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2'-O-alkyl ribonucleotides of the second strand, and

wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides.”

Exh. 1001 ('501 patent) at claim 1.

**i. “A double-stranded siRNA molecule against a target nucleic acid”**

334. Claim 1 of the '501 patent recites “a double-stranded siRNA molecule

against a target nucleic acid.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 326-328 above, Sur10058-Me-AL3 is a double-stranded siRNA molecule that targets the nucleic acid survivin mRNA. Exh. 1072 (Choung 2006) at pp. 920, 923, Fig. 3. Thus, Sur10058-Me-AL3 meets this limitation in claim 1. *See also* ¶¶ 330-331 above.

**ii. “wherein the double-stranded siRNA molecule comprises a first strand and a second strand”**

335. Claim 1 of the ’501 patent recites “wherein the double-stranded siRNA molecule comprises a first strand and a second strand.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 326-328 above, Sur10058-Me-AL3 meets this limitation because it is a double-stranded molecule having an antisense (first) strand and a sense (second) strand. Exh. 1072 (Choung 2006) at p. 923, Fig. 3.

**iii. “wherein the first strand comprises a first stretch that is complementary to the target nucleic acid”**

336. Claim 1 of the ’501 patent recites “wherein the first strand comprises a first stretch that is complementary to the target nucleic acid.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 326-328 above, the antisense (first) strand of Sur10058-Me-AL3 comprises a 19-nucleotide stretch that is complementary to the target nucleic acid, survivin mRNA. Exh. 1072 (Choung 2006) at pp. 920, 923, Fig. 3. Thus, the antisense strand comprises a first stretch of



19 nucleotides that is complementary to the target nucleic acid, survivin mRNA, and Sur10058-Me-AL3 meets this limitation.

**iv. “wherein the second strand comprises a second stretch that is the same length as the first stretch and is complementary to the first stretch”**

337. Claim 1 of the '501 patent recites “wherein the second strand comprises a second stretch that is the same length as the first stretch and is complementary to the first stretch.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 326-328 above, the sense (second) strand of Sur10058-Me-AL3 comprises a 19-nucleotide stretch that is complementary to the 19-nucleotide stretch of the antisense (first) strand. Exh. 1072 (Choung 2006) at pp. 920, 923, Fig. 3. Thus, the sense and the antisense strand comprise second and first stretches of equal length (19 nucleotides), respectively, that are complementary to one another and Sur10058-Me-AL3 meets this limitation.

**v. “wherein the first strand and the second strand form a double-stranded structure comprising the first stretch and the second stretch”**

338. Claim 1 of the '501 patent recites “wherein the first strand and the second strand form a double-stranded structure comprising the first stretch and the second stretch.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 326-328 above, Sur10058-Me-AL3 meets this limitation because the antisense (first) and the sense (second) strands, which comprise the first and second

stretches, hybridize to form a double-stranded structure. Exh. 1072 (Choung 2006) at pp. 920, 923, Fig. 3.

**vi. “wherein the first stretch and the second stretch each comprises contiguous alternating 2’-O-alkyl ribonucleotides”**

339. Claim 1 of the ’501 patent recites “wherein the first stretch and the second stretch each comprises contiguous alternating 2’-O-alkyl ribonucleotides.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 326-329 above, in Sur10058-Me-AL3, the antisense (first) and the sense (second) strands, which comprise the first and second 19-nucleotide stretches, respectively, each have contiguous alternating 2’-O-methyl modified ribonucleotides, which are a species of 2’-O-alkyl modified ribonucleotides, across the 19-nucleotide stretches of both strands. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Thus, Sur10058-Me-AL3 meets this limitation.

**vii. “wherein the 2’-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides”**

340. Claim 1 of the ’501 patent recites “wherein the 2’-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 326-329 above, Sur10058-Me-AL3 meets this limitation because the alternating 2’-O-methyl modified ribonucleotides alternate with unmodified ribonucleotides across the 19-

nucleotide stretches of both strands. Exh. 1072 (Choung 2006) at p. 923, Fig. 3.

**viii. “wherein the 2’-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2’-O-alkyl ribonucleotides of the second strand, and”**

341. Claim 1 of the ’501 patent recites “wherein the 2’-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2’-O-alkyl ribonucleotides of the second strand.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 326-329 above, Sur10058-Me-AL3 has contiguous alternating 2’-O-methyl ribonucleotides that alternate with unmodified ribonucleotides. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. As also noted in paragraph 329 above, in Sur10058-Me-AL3, the alternating 2’-O-methyl modified ribonucleotides on the antisense (first) strand are shifted by one relative to the 2’-O-methyl modified ribonucleotides on the sense (second) strand, *i.e.*, are in a phase-shift. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Thus, Sur10058-Me-AL3 meets this limitation.

**ix. “wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides”**

342. Claim 1 of the ’501 patent recites “wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides.” Exh. 1001 (’501 patent) at claim 1. As discussed in paragraphs 326-328 above, the antisense (first) and the sense (second) strands of Sur10058-Me-AL3 have

complementary first and second stretches, respectively, having a length of 19 ribonucleotides. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Thus, both strands comprise first and second stretches consisting of at least 17 and fewer than 30 ribonucleotides and Sur10058-Me-AL3 meets this limitation.

343. In sum, Sur10058-Me-AL3 meets all of the limitations of claim 1.

**b. Choung 2006 meets all of the limitations of claims 3-5 and 7**

344. Claim 3 is dependent on claim 1. Claim 3 additionally requires that “at least one end of the double-stranded siRNA molecule has an overhang.” Exh. 1001 (’501 patent) at claim 3.

345. As discussed in paragraphs 326-328 above, in Sur10058-Me-AL3, both the sense and the antisense strands have an overhang at the 3’-ends. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Thus, Sur10058-Me-AL3 has an overhang on both ends of the siRNA molecule, and therefore on at least one end. Because Sur10058-Me-AL3 meets all the limitations of claim 1, as demonstrated in paragraphs 333-343 above, and because Sur10058-Me-AL3 has an overhang on at least one end, Sur10058-Me-AL3 meets all of the limitations of claim 3.

346. Claims 4, 5, and 7 are dependent on claim 3. Claim 4 additionally requires that “the overhang has one to eight nucleotides.” Claim 5 additionally requires that “the 3’ end of the first strand has an overhang.” Claim 7 additionally requires that the “the 3’ end of the second strand has an overhang.” Exh. 1001

(’501 patent) at claims 4, 5, 7.

347. As discussed in paragraphs 326-328 above, Sur10058-Me-AL3 has an overhang of two nucleotides (deoxythymidines, dTdT) on the 3’-end of both the antisense (first) and the sense (second) strands. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Because Sur10058-Me-AL3 meets all the limitations of claim 3, as demonstrated in paragraphs 344-345 above, and because Sur10058-Me-AL3 has an overhang of two nucleotides on the 3’-ends of the first and second strands, Sur10058-Me-AL3 meets all the limitations of claims 4, 5, and 7.

**c. Choung 2006 meets all of the limitations of claim 9**

348. Claim 9 is dependent on claim 1. Claim 9 additionally requires that “each of the first strand and the second strand consists of at least 18 nucleotides.” Exh. 1001 (’501 patent) at claim 9.

349. As discussed in paragraphs 326-328 above, both strands of Sur10058-Me-AL3 contain 21 ribonucleotides. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Because Sur10058-Me-AL3 also meets all the limitations of claim 1, as demonstrated in paragraphs 333-343 above, and because Sur10058-Me-AL3 has two strands that are each 21 nucleotides long, Sur10058-Me-AL3 meets all of the limitations of claim 9.

**d. Choung 2006 meets all of the limitations of claims 10, 11, 20, and 21**

350. Claim 10 is dependent on claim 1. Claim 10 additionally requires that

“the 2’-O-alkyl ribonucleotides are 2’-O-methyl ribonucleotides.” Exh. 1001 (’501 patent) at claim 10. As discussed in paragraphs 326-329 above, Sur10058-Me-AL3 has 2’-O-methyl modified ribonucleotides. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Because Sur10058-Me-AL3 also meets all the limitations of claim 1, as demonstrated in paragraphs 333-343 above, and because Sur10058-Me-AL3 has 2’-O-methyl modified ribonucleotides, Sur10058-Me-AL3 meets all of the limitations of claim 10.

351. Claim 11 is dependent on claim 10 and additionally requires that “the 2’-O-methyl ribonucleotides alternate with the unmodified ribonucleotides.” Exh. 1001 (’501 patent) at claim 11. As discussed in paragraphs 326-329 above, in Sur10058-Me-AL3, the 2’-O-methyl modified ribonucleotides alternate with unmodified ribonucleotides on both stretches of both strands. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Because Sur10058-Me-AL3 meets all the limitations of claim 10, as demonstrated in paragraph 350 above, and because the 2’-O-methyl ribonucleotides alternate with unmodified ribonucleotides, Sur10058-Me-AL3 meets all of the limitations of claim 11.

352. Claim 20 is dependent on claim 1 and additionally requires “the 5’ ribonucleotide of the first stretch is a 2’-O-alkyl modified ribonucleotide.” Exh. 1001 (’501 patent) at claim 20. As discussed in paragraphs 326-329 above, in Sur10058-Me-AL3, the antisense (first) strand, and therefore the first stretch of 19

nucleotides, begins with a 2'-O-methyl modified ribonucleotide, which is a species of 2'-O-alkyl modified ribonucleotides, at the first nucleotide position from the 5'-end. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Because Sur10058-Me-AL3 meets all the limitations of claim 1, as demonstrated in paragraphs 333-343 above, and because the 5' ribonucleotide of the first stretch of Sur10058-Me-AL3 is a 2'-O-methyl ribonucleotide (*i.e.*, a 2'-O-alkyl modified ribonucleotide), Sur10058-Me-AL3 meets all of the limitations of claim 20.

353. Claim 21 is dependent on claim 11 and additionally requires that “the 5' ribonucleotide of the first stretch is a 2'-O-methyl ribonucleotide.” Exh. 1001 ('501 patent) at claim 21. As discussed in paragraphs 326-329 above, in Sur10058-Me-AL3, the antisense (first) strand, and therefore the first stretch of 19 nucleotides, begins with a 2'-O-methyl modified ribonucleotide, at the first nucleotide position from the 5'-end. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Because Sur10058-Me-AL3 meets all the limitations of claim 11, as demonstrated in paragraph 351 above, and because the 5' ribonucleotide of the first stretch of Sur10058-Me-AL3 is a 2'-O-methyl ribonucleotide, Sur10058-Me-AL3 meets all of the limitations of claim 21.

**e. Choung 2006 meets all of the limitations of claims 14, 15, and 23**

354. Claim 14 is dependent on claim 1. Claim 14 additionally requires that “the first stretch and the second stretch each comprises contiguous alternating

single 2'-O-alkyl ribonucleotides, and wherein the single 2'-O-alkyl ribonucleotides alternate with the unmodified ribonucleotides.” Exh. 1001 ('501 patent) at claim 14.

355. As discussed in paragraphs 326-329 above, in Sur10058-Me-AL3, the 19-nucleotide stretches of the antisense (first) and the sense (second) strands contain contiguous alternating single 2'-O-methyl modified ribonucleotides, which are a species of 2'-O-alkyl modified ribonucleotides, alternating with unmodified ribonucleotides. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Thus, each of the first and second stretches comprise contiguous alternating single 2'-O-methyl modified ribonucleotides that alternate with unmodified ribonucleotides. Because Sur10058-Me-AL3 also meets all the limitations of claim 1, as demonstrated in paragraphs 333-343 above, and because each of the first and second stretches of Sur10058-Me-AL3 has contiguous alternating single 2'-O-methyl modified ribonucleotides (*i.e.*, 2'-O-alkyl modified ribonucleotides) that alternate with unmodified ribonucleotides, Sur10058-Me-AL3 meets all of the limitations of claim 14.

356. Claims 15 is dependent on claim 14 and additionally requires that “the single 2'-O-alkyl ribonucleotides are 2'-O-methyl ribonucleotides.” Exh. 1001 ('501 patent) at claim 15. As discussed in paragraphs 326-329 above, Sur10058-Me-AL3 has contiguous alternating single 2'-O-methyl ribonucleotides that



alternate with unmodified ribonucleotides. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Because Sur10058-Me-AL3 also meets all the limitations of claim 14, as demonstrated in paragraphs 354-355 above, and because the single 2'-O-alkyl modified ribonucleotides are 2'-O-methyl ribonucleotides, Sur10058-Me-AL3 meets all of the limitations of claim 15.

357. Claim 23 is dependent on claim 14 and additionally requires that “the 5' ribonucleotide of the first stretch is a 2'-O-alkyl modified ribonucleotide that alternates with an unmodified ribonucleotide.” Exh. 1001 ('501 patent) at claim 23. As discussed in paragraphs 326-329 above, in Sur10058-Me-AL3, the antisense (first) strand, and therefore the first stretch of 19 nucleotides, begins with a 2'-O-methyl modified ribonucleotide, which is a species of 2'-O-alkyl modified ribonucleotides, at the first nucleotide position from the 5'-end. Exh. 1072 (Choung 2006) at p. 923, Fig. 3. Because Sur10058-Me-AL3 meets all the limitations of claim 14, as demonstrated in paragraphs 354-355 above, and because the 5' ribonucleotide of the first stretch of Sur10058-Me-AL3 is a 2'-O-methyl ribonucleotide (*i.e.*, a 2'-O-alkyl modified ribonucleotide), Sur10058-Me-AL3 meets all of the limitations of claim 23.

358. In sum, Choung 2006 meets all of the structural limitations of claims 1, 3-5, 7, 9-11, 14, 15, 20, 21, and 23 of the '501 patent. Additionally, siRNA by definition is required to have RNAi activity (*see* Section IX.C.i above). As

discussed in paragraphs 330-331 above, Sur10058-Me-AL3 has RNAi activity as demonstrated by the inhibition of survivin mRNA levels in Fig. 3. Thus, Choung 2006 meets this additional requirement of claims 1, 3-5, 7, 9-11, 14, 15, 20, 21, and 23. *See also* ¶ 334 above.

**C. Podbevsek 2010 Meets All of the Structural Limitations of Claims 1, 16, 19, 26, 27, and 29 of the '501 Patent**

359. Podbevsek 2010, by authors Podbevsek P. *et al.*, is entitled “Solution-state Structure of a Fully Alternately 2’-F/2’-OMe Modified 42-nt Dimeric siRNA Construct,” and published in Nucleic Acids Research in 2010 and online on July 12, 2010. Exh. 1082 (Podbevsek 2010) at p. 7298. I understand that Podbevsek 2010 was not in front of the Examiner during prosecution of the '501 patent and is not cited anywhere in the '501 patent.

360. As discussed in paragraphs 26-28 above, I understand that Podbevsek 2010 qualifies as prior art because it was published on July 12, 2010.

**i. Overview of Podbevsek 2010**

361. Podbevsek 2010 discloses “a high-resolution solution structure of a stable 42-nt RNA dimeric construct.” Exh. 1082 (Podbevsek 2010) at p. 7298. Podbevsek 2010 describes that “[t]wo 21-nt siRNA that efficiently hybridize consist of ribose units that were alternately substituted by 2’-fluoro and 2’-methoxy [*i.e.*, 2’-O-methyl)] groups.” Exh. 1082 (Podbevsek 2010) at p. 7298. The authors state that they undertook the present study “to analyze conformational

preorganization of the two 21-nt RNAs separately and after hybridization into the dimeric construct by solution state NMR.” Exh. 1082 (Podbevsek 2010) at p. 7299. Podbevsek 2010 states that “[s]tructural characterization of the dimeric construct provides molecular details which correlate with the high potency of the modified siRNA.” Exh. 1082 (Podbevsek 2010) at p. 7299.

362. Podbevsek 2010 notes that “[a] completely 2’-F/2’-OMe modified dimeric RNA construct was previously shown to be a highly potent RNAi trigger in HeLa cells.” Exh. 1082 (Podbevsek 2010) at p. 7304 (citing to Reference (18), by Allerson *et al.*, Exh. 1016 (Allerson 2005)). The authors report that in the current study, they “present a 3D structure of a 42-nt dimeric alternately 2’-F/2’-OMe modified construct consisting of a Watson–Crick base paired duplex with additional 3’ UU overhangs and a 5’ phosphate group on the antisense strand, which mimics naturally occurring siRNAs produced in cells by the Dicer enzyme.” Exh. 1082 (Podbevsek 2010) at p. 7304. They observed that the “overall thermal stability of the duplex, reflected by a  $T_m$  of 81.7°C, is not appreciably different from the stability of an identical duplex lacking the 3’ UU overhangs, with a previously reported  $T_m$  of 82.0°C.” Exh. 1082 (Podbevsek 2010) at p. 7305 (citing to Reference (18) by Allerson *et al.*, Exh. 1016 (Allerson 2005)). I note that the authors refer to duplex 11 of Allerson *et al.*, which was reported by Allerson *et al.* to have a  $T_m$  of 82.0°C. Exh. 1016 (Allerson 2005) at Table 1.

363. Podbevsek 2010 additionally observed that “[a]ll nucleotides in the construct exhibit a predominantly N-type sugar pucker, which is usually found in RNA molecules,” and that “[t]he RNA like conformation greatly improves the binding affinity for the target RNA, which is desired for synthetic siRNA oligonucleotides.” Exh. 1082 (Podbevsek 2010) at p. 7305. The authors noted that “[i]t is not surprising that the introduction of 2’-fluoro and 2’-OMe modifications does not have any significant impact on the structure of the construct. Deviations from the native A-type RNA structure could reduce the efficiency of the RNAi mechanism.” Exh. 1082 (Podbevsek 2010) at p. 7305.

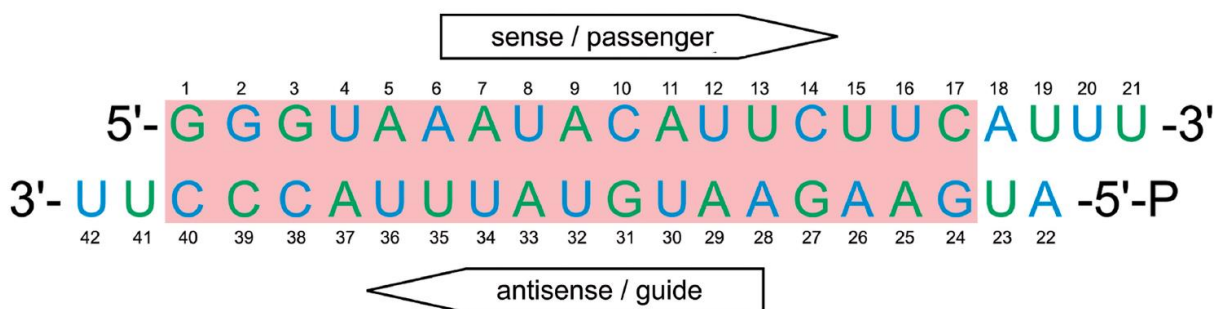
364. Podbevsek 2010 concludes that “the alternating 2’-F/2’-OMe motif appears to be a promising design for synthetic siRNA oligonucleotides with high serum stability.” Exh. 1082 (Podbevsek 2010) at p. 7305.

365. In my opinion, Podbevsek 2010 discloses a double-stranded siRNA molecule having contiguous alternating 2’-O-methyl modified and 2’-fluoro modified ribonucleotides and 3’-end overhangs that meets at least the structural elements of the ’501 patent claims.

**a. Podbevsek 2010 discloses a fully 2’-F/2’-OMe modified siRNA duplex**

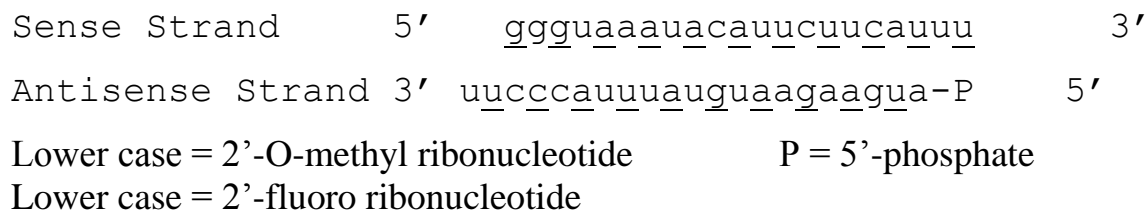
366. Podbevsek 2010 discloses in Figure 2 a “fully modified 2’-F/2’-OMe modified siRNA duplex,” which the authors describe as “a 42-nt dimeric RNA construct which consists of 19 Watson-Crick base pairs, 3’ UU overhangs in both

strands, which are found in naturally occurring siRNAs, and a 5' phosphate on the antisense strand, which is required for correct positioning within the RISC.” Exh. 1082 (Podbevsek 2010) at p. 7299. The 2'-F/2'-OMe siRNA construct of Figure 2 is reproduced below:



Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2.

367. Below is an annotated version of the 2'-F/2'-OMe siRNA duplex:



Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2.

368. In the 2'-F/2'-OMe siRNA duplex, each of the sense (top) and the antisense (bottom) strands have a length of 21 nucleotides with two modified uridine (2'-F/2'-OMe uridine) overhangs at the 3'-ends. The sense and antisense strands have a stretch of 19 nucleotides that are complementary to another (*i.e.*, each C, G, U, and A on one strand is base-paired with a G, C, A, and U on the other strand, respectively). Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2. The

authors state that “[t]he two 21-nt oligos efficiently hybridize thus forming an A-type double helix with 3’ UU overhangs on both strands. The helical segment is completely complementary and exhibits 19 Watson–Crick base pairs.” Exh. 1082 (Podbevsek 2010) at pp. 7304-05, *see also* p. 7299. The antisense strand of the 2’-F/2’-OMe siRNA duplex of Podbevsek 2010 comprises a 19-nucleotide stretch that is complementary to its target nucleic acid, PTEN mRNA. *See* Exh. 1087 (NCBI Nucleotide Blast Results for the 2’-F/2’-OMe siRNA duplex) at p.8 (showing the 19-nucleotide stretch of the sense strand shares 100% sequence identity with a nucleotide sequence of PTEN mRNA).

369. I note the 19-nucleotide base-paired region of the 2’-F/2’-OMe siRNA duplex is identical in sequence and modification pattern to duplex 10 and duplex 11 of Allerson 2005 targeting the site B of PTEN mRNA, that were fully 2’-F/2’-OMe modified 19-mer siRNAs and blunt ended on both ends. Exh. 1016 (Allerson 2005) at p. 902, Fig. 2B. Podbevsek 2010 (referring to the duplexes of Allerson 2005) states:

“a highly potent siRNA duplex, which targets the B site of the human phosphatase and tensin homolog [PTEN] mRNA, which was previously shown to be a target for degradation using the siRNA approach[]. The fully modified duplex, which is comprised of alternating 2’-F and 2’-OMe nucleotides exhibits several desirable pharmacokinetic properties.... Consequently, 2’-F/2’-OMe siRNA showed a more than 500-fold potency versus unmodified siRNA.”

Exh. 1082 (Podbevsek 2010) at pp. 7299, 7304-05; *see also* ¶¶ 361-365 above.

370. The 2'-F/2'-OMe siRNA duplex is a fully-modified siRNA molecule having 2'-O-methyl modified nucleotides alternating with 2'-fluoro modified nucleotides on both the sense and antisense strands, including in the 3'-UU overhangs. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2. As shown above, in the siRNA duplex, each 2'-O-methyl modified ribonucleotide on the 19-nucleotide stretch of the antisense strand is base-paired with a 2'-fluoro modified ribonucleotide on the complementary 19-nucleotide stretch of the sense strand; each 2'-fluoro modified ribonucleotide on the 19-nucleotide stretch of the antisense strand is base-paired with a 2'-O-methyl modified ribonucleotide on the 19-nucleotide stretch of the sense strand, *i.e.*, there is a phase shift. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2; *see also* Exh. 1001 ('501 patent) at cols. 17:28-36, 17:56-18:2, 18:12-18, Fig. 2B. Additionally, in the 2'-F/2'-OMe siRNA duplex, the 2'-O-methyl modified ribonucleotides are located at odd nucleotide positions (1, 3, 5, 7, etc.) on the antisense strand (from the 5'- to 3'-end) and they are at even nucleotide positions (2, 4, 6, 8, etc.) on the sense strand (from the 5'- to the 3'-end).

**ii. Podbevsek 2010 meets all of the structural limitations of at least claims 1, 16, 19, 26, 27, and 29 of the '501 patent**

371. Based on the disclosures of Podbevsek 2010 described above, and in particular, the fully 2'-F/2'-OMe modified siRNA duplex, Podbevsek 2010 meets

all of the structural limitations of at least claims 1, 16, 19, 26, 27, and 29 of the '501 patent.<sup>27</sup>

**a. Podbevsek 2010 meets all of the limitations of claim 1**

372. Claim 1 of the '501 patent recites:

“A double-stranded siRNA molecule against a target nucleic acid,

wherein the double-stranded siRNA molecule comprises a first strand and a second strand,

wherein the first strand comprises a first stretch that is complementary to the target nucleic acid,

wherein the second strand comprises a second stretch that is the same length as the first stretch and is complementary to the first stretch,

wherein the first strand and the second strand form a double-stranded structure comprising the first stretch and the second stretch,

wherein the first stretch and the second stretch each comprises contiguous alternating 2'-O-alkyl ribonucleotides,

wherein the 2'-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides,

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<sup>27</sup> I note that with the exception of claims 2 and 25 (both of which require blunt ends) the fully 2'-F/2'-OMe modified siRNA duplex of Podbevsek 2010 would meet all of the structural limitations of claims 9, 10, 12, 13, 16-20, 22, and 24 for the same reasons as duplex 8 of Allerson 2005. Additionally, because the fully 2'-F/2'-OMe modified siRNA duplex has overhangs at the 3'-ends of the antisense (first) and the sense (second) strands, it would also meet the structural limitations of claims 3-5 and 7.



wherein the 2'-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2'-O-alkyl ribonucleotides of the second strand, and

wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides.”

Exh. 1001 ('501 patent) at claim 1.

**i. “A double-stranded siRNA molecule against a target nucleic acid”**

373. Claim 1 of the '501 patent recites “a double-stranded siRNA molecule against a target nucleic acid.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 366-369 above, the 2'-F/2'-OMe siRNA duplex is a double-stranded siRNA molecule that targets the nucleic acid PTEN mRNA. Exh. 1082 (Podbevsek 2010) at pp. 7299, 7304-05, Fig. 2. Thus, the 2'-F/2'-OMe siRNA duplex meets this limitation in claim 1. *See also* ¶¶ 361-365 above.

**ii. “wherein the double-stranded siRNA molecule comprises a first strand and a second strand”**

374. Claim 1 of the '501 patent recites “wherein the double-stranded siRNA molecule comprises a first strand and a second strand.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 366-369 above, the 2'-F/2'-OMe siRNA duplex meets this limitation because it is a double-stranded molecule having an antisense (first) strand and a sense (second) strand. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2.

**iii. “wherein the first strand comprises a first stretch that is complementary to the target nucleic acid”**

375. Claim 1 of the '501 patent recites “wherein the first strand comprises a first stretch that is complementary to the target nucleic acid.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 366-369 above, the antisense (first) strand of the 2'-F/2'-OMe siRNA duplex comprises a 19-nucleotide stretch that is complementary to the target nucleic acid, PTEN mRNA. Exh. 1082 (Podbevsek 2010) at pp. 7299, 7304-05, Fig. 2; *see also* Exh. 1087 (NCBI Blast Result) at p. 8. Thus, the antisense strand comprises a first stretch of 19 nucleotides that is complementary to the target nucleic acid, PTEN mRNA, and the 2'-F/2'-OMe siRNA duplex meets this limitation.

**iv. “wherein the second strand comprises a second stretch that is the same length as the first stretch and is complementary to the first stretch”**

376. Claim 1 of the '501 patent recites “wherein the second strand comprises a second stretch that is the same length as the first stretch and is complementary to the first stretch.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 366-369 above, the sense (second) strand of the 2'-F/2'-OMe siRNA duplex comprises a 19-nucleotide stretch that is complementary to the 19-nucleotide stretch of the antisense (first) strand. Exh. 1082 (Podbevsek 2010) at pp. 7299, 7304-05, Fig. 2. Thus, the sense and the antisense strand comprise

second and first stretches of equal length (19 nucleotides), respectively, that are complementary to one another and the 2'-F/2'-OMe siRNA duplex meets this limitation.

**v. “wherein the first strand and the second strand form a double-stranded structure comprising the first stretch and the second stretch”**

377. Claim 1 of the '501 patent recites “wherein the first strand and the second strand form a double-stranded structure comprising the first stretch and the second stretch.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 366-369 above, the 2'-F/2'-OMe siRNA duplex meets this limitation because the antisense and the sense strands, which comprise the first and second stretches, hybridize to form a double-stranded structure. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2; *see also* pp. 7304-05 (“The two 21-nt oligos efficiently hybridized thus forming an A-type double helix with 3' UU overhangs on both strands.”).

**vi. “wherein the first stretch and the second stretch each comprises contiguous alternating 2'-O-alkyl ribonucleotides”**

378. Claim 1 of the '501 patent recites “wherein the first stretch and the second stretch each comprises contiguous alternating 2'-O-alkyl ribonucleotides.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 366-370 above, in the 2'-F/2'-OMe siRNA duplex, the antisense (first) and the sense (second) strands, which comprise the first and second stretches, respectively, each have

contiguous alternating 2'-O-methyl modified ribonucleotides, which are a species of 2'-O-alkyl modified ribonucleotides, across both strands. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2. Thus, the 2'-F/2'-OMe siRNA duplex meets this limitation.

**vii. “wherein the 2'-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides”**

379. Claim 1 of the '501 patent recites “wherein the 2'-O-alkyl ribonucleotides alternate with unmodified or differently modified ribonucleotides.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 366-370 above, the 2'-F/2'-OMe siRNA duplex meets this limitation because the alternating 2'-O-methyl modified ribonucleotides alternate with 2'-fluoro modified ribonucleotides (*i.e.*, differently modified ribonucleotides) across both strands. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2.

**viii. “wherein the 2'-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2'-O-alkyl ribonucleotides of the second strand, and”**

380. Claim 1 of the '501 patent recites “wherein the 2'-O-alkyl ribonucleotides of the first strand are shifted by one ribonucleotide relative to the 2'-O-alkyl ribonucleotides of the second strand.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 366-370 above, the 2'-F/2'-OMe siRNA duplex has contiguous alternating 2'-O-methyl ribonucleotides that alternate with 2'-fluoro

modified ribonucleotides. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2. As also noted in paragraph 370 above, in the 2'-F/2'-OMe siRNA duplex, the alternating 2'-O-methyl modified ribonucleotides on the antisense (first) strand are shifted by one relative to the 2'-O-methyl modified ribonucleotides on the sense (second) strand, *i.e.*, are in a phase-shift. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2. Thus, the 2'-F/2'-OMe siRNA duplex meets this limitation.

**ix. “wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides”**

381. Claim 1 of the '501 patent recites “wherein each of the first stretch and the second stretch consists of at least 17 and fewer than 30 ribonucleotides.” Exh. 1001 ('501 patent) at claim 1. As discussed in paragraphs 366-369 above, the antisense (first) and the sense (second) strands of the 2'-F/2'-OMe siRNA duplex have complementary first and second stretches, respectively, having a length of 19 ribonucleotides. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2. Thus, both strands comprise first and second stretches consisting of at least 17 and fewer than 30 ribonucleotides and the 2'-F/2'-OMe siRNA duplex meets this limitation.

382. In sum, the 2'-F/2'-OMe siRNA duplex meets all of the limitations of claim 1.

**b. Podbevsek 2010 meets all of the limitations of claims 16 and 19**

383. Claim 16 is dependent on claim 1. Claim 16 additionally requires that

“the first stretch and the second stretch each comprises contiguous alternating single 2'-O-alkyl ribonucleotides, and wherein the single 2'-O-alkyl ribonucleotides alternate with the differently modified ribonucleotides.” Exh. 1001 ('501 patent) at claim 16.

384. As discussed in paragraphs 366-370 above, both the antisense (first) and sense (second) strands of the 2'-F/2'-OMe siRNA duplex contain a complementary 19-nucleotide stretch of contiguous alternating single 2'-O-methyl modified ribonucleotides, which are a species of 2'-O-alkyl modified ribonucleotides, alternating with 2'-fluoro modified ribonucleotides. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2. Thus, each of the first and second stretches comprise contiguous alternating single 2'-O-methyl modified ribonucleotides that alternate with 2'-fluoro modified ribonucleotides. Because the 2'-F/2'-OMe siRNA duplex also meets all the limitations of claim 1, as demonstrated in paragraphs 372-382 above, and because each of the first and second stretches of the 2'-F/2'-OMe siRNA duplex comprise single 2'-O-methyl modified ribonucleotides (*i.e.*, 2'-O-alkyl modified ribonucleotides) that alternate with 2'-fluoro modified ribonucleotides (*i.e.*, differently modified ribonucleotides), the 2'-F/2'-OMe siRNA duplex meets all of the limitations of claim 16.

385. Claim 19 is dependent on claim 16. Claim 19 additionally requires that “the single 2'-O-alkyl ribonucleotides are 2'-O-methyl ribonucleotides, and

the differently modified ribonucleotides are 2'-fluoro ribonucleotides.” Exh. 1001 ('501 patent) at claim 19.

386. As discussed in paragraphs 366-370 above, the 2'-F/2'-OMe siRNA duplex has contiguous alternating single 2'-O-methyl ribonucleotides (*i.e.*, a 2'-O-alkyl modified ribonucleotide) that alternate with 2'-fluoro modified ribonucleotides. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2. Because the 2'-F/2'-OMe siRNA duplex also meets all the limitations of claim 16, as demonstrated in paragraphs 383-384 above, and because each of the first and second stretches comprise contiguous alternating single 2'-O-methyl modified ribonucleotides that alternate with 2'-fluoro modified ribonucleotides, the 2'-F/2'-OMe siRNA duplex meets the limitations of claim 19.

**c. Podbevsek 2010 meets all of the limitations of claims 26, 27, and 29**

387. Claim 26 is dependent on claim 19. Claim 26 additionally requires that “at least one end of the double-stranded siRNA molecule has an overhang of to eight nucleotides.” Exh. 1001 ('501 patent) at claim 26.

388. As discussed in paragraphs 366-369 above, the 2'-F/2'-OMe siRNA duplex has overhangs of two modified uridines at the 3'-ends of the antisense and the sense strands. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2. Because the 2'-F/2'-OMe siRNA duplex meets all the limitations of claim 19, as demonstrated in paragraphs 385-386 above, and because the 2'-F/2'-OMe siRNA duplex has an

overhang of two nucleotides on both ends, and thus, is on at least one end, the 2'-F/2'-OMe siRNA duplex meets all of the limitations of claim 26.

389. Claims 27 and 29 are dependent on claim 26. Claim 27 additionally requires that “the 3'-end of the first strand has an overhang.” Claim 29 additionally requires that the “3'-end of the second strand has an overhang.” Exh. 1001 ('501 patent) at claims 27, 29.

390. As discussed in paragraphs 366-369 above, the 2'-F/2'-OMe siRNA duplex has an overhang at the 3'-end of both the antisense (first) and the sense (second) strands. Exh. 1082 (Podbevsek 2010) at p. 7299, Fig. 2. Because the 2'-F/2'-OMe siRNA duplex meets all the limitations of claim 26, as demonstrated in paragraphs 387-388 above, and because the 3'-end of the antisense strand and the 3'-end of the sense strand have an overhang, 2'-F/2'-OMe siRNA duplex meets all of the limitations of claims 27 and 29.

391. In sum, Podbevsek 2010 meets at least all of the structural limitations of claims 1, 16, 19, 26, 27, and 29 of the '501 patent.



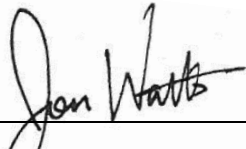
## **XV. Concluding Statements**

392. For at least the above reasons, I conclude:

- The '501 patent fails to demonstrate that the inventors were in possession of the vast genus of structurally diverse siRNA molecules of the '501 patent claims;
- The '501 patent fails to teach or provide guidance to a person of ordinary skill in the art to practice the claimed invention without undue experimentation; and
- The prior art discloses double-stranded siRNA molecules that meet all of the limitations of claims 1-5, 7, 9-27, and 29 of the '501 patent.

393. I declare that all statements made herein on my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code.

Dated: July 17, 2018

  
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Jonathan K. Watts, Ph.D.

## **APPENDIX A - LIST OF EXHIBITS CITED**

<b>Exhibit No.</b>	<b>Document</b>
1001	U.S. Patent No. 9,790,501
1003	Curriculum Vitae of Dr. Jonathan K. Watts
1004	File History of U.S. Patent No. 9,790,501
1005	U.S. Application No. 14/977,710 (continuation)
1006	European Patent Application No. 02017601
1007	U.S. Provisional Application No. 60/402,541
1008	European Patent Application No. 03008383
1009	U.S. Application No. 10/633,630 (non-provisional)
1010	U.S. Application No. 12/200,296 (continuation)
1011	U.S. Application No. 12/986,389 (continuation)
1012	U.S. Application No. 13/692,178 (continuation)
1013	U.S. Application No. 14/578,636 (continuation)
1015	U.S. Patent No. 9,695,423
1016	Allerson C.R. <i>et al.</i> , <i>Fully 2'-Modified Oligonucleotide Duplexes with Improved in Vitro Potency and Stability Compared to Unmodified Small Interfering RNA</i> , J. Med. Chem. 48:901-904 (2005)
1020	U.S. Patent No. 7,452,987
1021	U.S. Patent No. 7,893,245
1022	U.S. Patent No. 8,324,370
1023	U.S. Patent No. 8,933,215
1024	U.S. Patent No. 9,222,092
1027	Watts J.K. <i>et al.</i> , <i>Chemically Modified siRNA: Tools and Applications</i> , Drug Discovery Today 13:842-55 (2008)
1028	Czauderna F. <i>et al.</i> , <i>Structural Variations and Stabilising Modifications of Synthetic siRNAs in Mammalian Cells</i> , Nucleic Acids Res., 31(11):2705-16 (2003)
1029	Kaczmarek J.C. <i>et al.</i> , <i>Advances in the Delivery of RNA Therapeutics: From Concept to Clinical Reality</i> , Genome Medicine 9(60):1-6 (2017)
1030	Khvorova A. and Watts J.K., <i>The Chemical Evolution of Oligonucleotide Therapies of Clinical Utility</i> , Nature Biotechnology 35(3):238-48 (2017)
1031	Dolgin E., <i>The Greatest Hits of the Human Genome: A Tour Through the Most Studied Genes in Biology Reveals Some Surprises</i> , Nature, 551:427-31 (2017)

<b>Exhibit No.</b>	<b>Document</b>
1032	Gaglione M. and Messere A., <i>Recent Progress in Chemically Modified siRNAs</i> , Mini-Reviews in Medicinal Chemistry, 10(7):578-95 (2010)
1033	Nawrot B. and Sipka K., <i>Chemical and Structural Diversity of siRNA Molecules</i> , Current Topics in Medicinal Chemistry 6(9):913-25 (2006)
1034	Parrish S. <i>et al.</i> , <i>Functional Anatomy of a dsRNA Trigger: Differential Requirement for the Two Trigger Strands in RNA Interference</i> , Molecular Cell, Vol. 6:1077-87 (2000)
1035	Deleavey G.F. <i>et al.</i> , <i>Chemical Modification of siRNA</i> , Current Protocols in Nucleic Acid Chemistry, 39(suppl):16.3.1-22 (2009)
1036	Bramsen J. and Kjems J., <i>Development of Therapeutic-Grade Small Interfering RNAs by Chemical Engineering</i> , Frontiers in Genetics 3(154):1-21 (2012)
1037	Haringsma H.J. <i>et al.</i> , <i>mRNA Knockdown by Single Strand RNA is Improved by Chemical Modifications</i> , Nucleic Acids Research 40: 4125-36 (2012)
1038	Collingwood M.A. <i>et al.</i> , <i>Chemical Modification Patterns Compatible with High Potency Dicer-Substrate Small Interfering RNAs</i> , Oligonucleotides 18:187-200 (2008)
1039	Behlke M., <i>Chemical Modification of siRNAs for In Vivo Use</i> , Oligonucleotides 18:305-20 (2008)
1040	Pei Y. and Tuschl T., <i>On the Art of Identifying Effective and Specific siRNAs</i> , Nature Methods 3:670-76 (2006)
1041	Kurreck J., <i>Antisense Technologies: Improvement through Novel Chemical Modifications</i> , Eur. J. Biochem. 270:1628-44 (2003)
1042	Elbashir S.M. <i>et al.</i> , <i>Duplexes of 21-Nucleotide RNAs Mediate RNA Interference in Cultured Mammalian Cells</i> , Nature 411:494-98 (2001)
1043	Amarzguioui M. <i>et al.</i> , <i>Tolerance for Mutations and Chemical Modifications in a siRNA</i> , Nucleic Acids Research 31(2):589-95 (2003)
1044	Chiu Y.L. and Rana T.M. <i>et al.</i> , <i>siRNA Function in RNAi: A Chemical Modification Analysis</i> , RNA 9:1034-48 (2003)
1045	Patkaniowska A. and Tuschl T. <i>et al.</i> , Chapter 4, <i>Gene Silencing by Synthetic siRNA Duplexes in Mammalian Cell Culture</i> , RNA Interference (RNAi) Nuts & Bolts of RNAi Technology 71-88 (2003 ed.)
1046	Manoharan M., <i>RNA Interference and Chemically Modified Small Interfering RNAs</i> , Current Opinion in Chemical Biology 8:570-79 (2004)

<b>Exhibit No.</b>	<b>Document</b>
1047	Song J.J. <i>et al.</i> , <i>Crystal Structure of Argonaute and Its Implication for RISC Slicer Activity</i> , Science 305:1434-37 (2004)
1048	Liu J. <i>et al.</i> , <i>Argonaute2 is the Catalytic Engine of Mammalian RNAi</i> , Science 305:1437-41 (2004)
1049	Meister G., <i>Argonaute Proteins: Functional Insights and Emerging Roles</i> , Nature Reviews Genetics 14:447-56 (2013)
1050	de Fougères A.R. and Maraganore J.M., Chapter 16, <i>Discovery and Development of RNAi Therapeutics</i> , Antisense Drug Technology: Principles, Strategies and Applications (2d ed. 2008)
1052	NCBI News, <i>First Version of Human Genome Reference Sequence Debuts on DNA's 50<sup>th</sup></i> (Spring 2003)
1053	Sharp P.A., <i>RNA Interference-2001</i> , Genes & Development 15:485-90 (2001)
1054	Waterhouse P.M. <i>et al.</i> , <i>Gene Silencing as an Adaptive Defence Against Viruses</i> , Nature 411:834-42 (2001)
1055	Tuschl, T. and Borkhardt, A., <i>Small Interfering RNAs: A Revolutionary Tool for the Analysis of Gene Function and Gene Therapy</i> , Mol. Interventions 2:158-67 (2002)
1056	Klingelhoefer J.W. <i>et al.</i> , <i>Approximate Bayesian Feature Selection on a Large Meta-Dataset Offers Novel Insights on Factors That Affect siRNA Potency</i> , Bioinformatics 25(13):1594-601 (2009)
1057	Harborth J. <i>et al.</i> , <i>Identification of Essential Genes in Cultured Mammalian Cells Using Small Interfering RNAs</i> , J. Cell Sci. 114:4557-65 (2001)
1058	Fire A. <i>et al.</i> , <i>Potent and Specific Genetic Interference by Double-Stranded RNA in Caenorhabditis elegans</i> , Nature 391:806-11 (1998)
1059	Lodish H. <i>et al.</i> , Chapter 4, <i>Nucleic Acids, the Genetic Code and the Synthesis of Macromolecules</i> , Molecular Cell Biology (4th ed. 2000)
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1061	Feb. 8, 2008 Declaration of Dr. Jörg Kaufmann submitted during prosecution of U.S. Application No. 10/633,630
1062	European Patent No. EP 0 586 520
1063	European Patent No. EP 0 618 925
1064	International Publication No. WO 03/070918 by McSwiggen J. <i>et al.</i>

<b>Exhibit No.</b>	<b>Document</b>
1065	Morrissey D.V. <i>et al.</i> , <i>Activity of Stabilized Short Interfering RNA in Mouse Model of Hepatitis B Virus Replication</i> , Hepatology 41:1349-56 (2005)
1068	Pure and Applied Chemistry, 1995, 67, 1307 (Glossary of class names of organic compounds and reactivity intermediates based on structure (IUPAC Recommendations 1995))
1072	Choung S. <i>et al.</i> , <i>Chemical Modification of siRNAs to Improve Serum Stability Without Loss of Efficacy</i> , Biochemical and Biophysical Research Communications 342: 919-27 (2006)
1076	Bumcrot D. <i>et al.</i> , <i>RNAi Therapeutics: A Potential New Class of Pharmaceutical Drugs</i> , Nature Chemical Biology 2(12):711-9 (2006)
1082	Podbevsek P. <i>et al.</i> , <i>Solution-State Structure of a Fully Alternately 2'-F/2'-OMe modified 42-nt dimeric siRNA Construct</i> , Nucleic Acids Research, 38(20):7298-307 (2010)
1083	International Publication No. WO 2005/089224 by de Fougères A., <i>et al.</i>
1084	Strapps W.R. <i>et al.</i> , <i>The siRNA Sequence and Guide Strand Overhangs are Determinants of In Vivo Duration of Silencing</i> , Nucleic Acids Research, 38(14):4788-97 (2010)
1085	Kim D.H. <i>et al.</i> , <i>Synthetic dsRNA Dicer Substrates Enhance RNAi Potency and Efficacy</i> , Nature Biotechnology, 23(2):222-26 (2005)
1086	Rose S.D. <i>et al.</i> , <i>Functional Polarity is Introduced by Dicer Processing of Short Substrate RNAs</i> , Nucleic Acids Research, 33(13):4140-156 (2005)
1087	NCBI Nucleotide Blast Results for the 2'-F/2'-OMe siRNA
1088	Chang C.I., <i>et al.</i> , <i>A Structure-Activity Relationship Study of siRNAs with Structural Variations</i> , Biochem. Biophys. Res. Commun. 359(4):997-1003 (2007)
1090	Chang C. <i>et al.</i> , <i>Structural Diversity Repertoire of Gene Silencing Small Interfering RNAs</i> , Nucleic Acid Therapeutics, 21(3):125-31 (2011)

**Appendix B: List of 2'-O-methyl modified siRNA of the '501 patent**

<b>No.</b>	<b>siRNA Duplex (Seq. IDs) (Figs.)</b>	<b>Duplicates (Seq. IDs) (Figs.)</b>
1.	<b>73AB</b> (Seq. 83, 84) (Figs. 10A-C)	<b>PTENAB V2</b> (Seq. 125, 126) (Fig. 15A)
2.	<b>74AB</b> (Seq. 85, 86) (Figs. 10A-C)	<b>PTENAB V3</b> (Seq. 127, 128) (Fig. 15A)
3.	<b>75AB</b> (Seq. 87, 88) (Figs. 10A-C)	<b>PTENAB V4</b> (Seq. 129, 130) (Fig. 15A)
4.	<b>79AB</b> (Seq. 89, 90) (Figs. 10A-C)	<b>79AB</b> (Seq. 103, 104) (Fig. 12A-D); <b>PTENAB V1</b> (123, 124) (Figs. 15A-D)
5.	<b>79AB</b> (Seq. 67, 68) (Fig. 8A-B)	None
6.	<b>28A79B</b> (Seq. 80, 90) (Figs. 10A-C)	<b>PTENAB V5</b> (Seq. 131, 132) (Fig. 15A)
7.	<b>79A28B</b> (Seq. 89, 14) (Figs. 10A, 10C)	<b>PTENAB V6</b> (Seq. 133, 134) (Fig. 15A)
8.	<b>75A79B</b> (Seq. 87, 90) (Figs. 10A, 10C)	<b>75A79B</b> (Seq. 100, 90) (Figs. 11A, 11C); <b>PTENAB V11</b> (Seq. 143, 144) (Fig. 15A)
9.	<b>81AB</b> (Seq. 93, 94) (Figs. 11A, 11C)	<b>81AB</b> (Seq. 105, 106) (Figs. 12A-D); <b>PTENAB V7</b> (Seq. 135, 136) (Figs. 15A-C)
10.	<b>82AB</b> (Seq. 95, 96) (Figs. 11A-C)	<b>82AB</b> (Seq. 107, 108) (Figs. 12A-D); <b>PTENAB V8</b> (Seq. 137, 138) (Figs. 15A-C)
11.	<b>83A79B</b> (Seq. 97, 90) (Figs. 11A, 11C)	<b>PTENAB V9</b> (Seq. 139, 140) (Fig. 15A)
12.	<b>86AB</b> (Seq. 98, 99) (Figs. 11A-C)	<b>PTENAB V10</b> (Seq. 141, 142) (Figs. 15A-D)
13.	<b>86AB</b> (Seq. 109, 110) (Figs. 12A-D; 13)	None
14.	<b>94A1B1</b> (Seq. 111, 112) (Figs. 12A-D)	<b>PTENAB V13</b> (Seq. 147, 148) (Figs. 15B, 15C)
15.	<b>94A1B2</b> (Seq. 113, 114) (Figs. 12A-D; 13; 14)	<b>PTENAB V14</b> (Seq. 149, 150) (Figs. 15B-D)
16.	<b>94A2B1</b> (Seq. 115, 116) (Figs. 12A-D; 13; 14)	<b>PTENAB V15</b> (Seq. 151, 152) (Figs. 15B-D)
17.	<b>94A2B2</b> (Seq. 117, 118) (Figs. 12A-D; 13)	<b>PTENAB V12</b> (Seq. 145, 146) (Figs. 15A-D)
18.	<b>Akt1AB V3</b> (Seq. 157, 158) (Figs. 16A-B)	None
19.	<b>Akt1AB V4</b> (Seq. 159, 160) (Figs. 16A-B)	None
20.	<b>Akt1AB V5</b> (Seq. 161, 162) (Figs. 16A-B)	None

<b>No.</b>	<b>siRNA Duplex (Seq. IDs) (Figs.)</b>	<b>Duplicates (Seq. IDs) (Figs.)</b>
21.	<b>Akt1AB V6</b> (Seq. 163, 164) (Figs. 16A-B)	None
22.	<b>P110<math>\beta</math> V3</b> (Seq. 167, 168) (Fig. 16C)	None
23.	<b>P110<math>\beta</math> V4</b> (Seq. 169, 170) (Fig. 16C)	None
24.	<b>P110<math>\beta</math> V5</b> (Seq. 171, 172) (Fig. 16C)	None
25.	<b>P110<math>\beta</math> V6</b> (Seq. 173, 174) (Fig. 16C)	None