IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Hannon *et al.*	Confirmation No.:	9352
Application No:	10/997,086	Art Unit:	1635
Filed:	November 23, 2004	Examiner:	K. CHONG
Title:	METHODS AND COMPOSITIONS FOR RNA INTERFERENCE		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT AND RESPONSE TO JULY 2, 2010 OFFICE ACTION

This paper is filed in response to the July 2, 1010 Final Office Action. A reply was originally due on October 2, 2010. Applicants request a three-month extension of time to January 2, 2011, which is a Sunday. Therefore a response is due Monday, January 3, 2011 and this paper is being timely filed. The required fee for the extension accompanies this paper. The Commissioner is authorized to charge any other fees due, or to credit any overpayment in fees, to Deposit Account No. 08-0219.

AMENDMENT

In the Claims

Please amend the claims as follows, without prejudice. This listing of the claims will replace all prior versions and listings of claims in the application:

1-37. (Cancelled)

38. (Previously presented) A method for attenuating expression of a target gene in a mammalian cell, the method comprising:

introducing into a mammalian cell an expression vector comprising:

(i) an RNA polymerase promoter, and

(ii) a sequence encoding a short hairpin RNA molecule comprising a double-stranded region, wherein the double-stranded region consists of at least 20 nucleotides but not more than 29 nucleotides, [~~such that the short hairpin RNA does not trigger a protein kinase RNA-activated (PKR) response in the mammalian cell,~~]

wherein the short hairpin RNA molecule is a substrate for Dicer-dependent cleavage and does not trigger a protein kinase RNA-activated (PK) response in the mammalian cell,

wherein the double-stranded region of the short hairpin RNA molecule comprises a sequence that is complementary to a portion of the target gene, and

wherein the short hairpin RNA molecule is stably expressed in the mammalian cell in an amount sufficient to attenuate expression of the target gene in a sequence specific manner, and is expressed in the cell without use of a PK inhibitor, whereby expression of the target gene is inhibited.

39. (Cancelled)

40. (Previously presented) The method of claim 38, wherein the expression vector further comprises LTR sequences located 5' and 3' of the sequence encoding the short hairpin RNA molecule.

41. (Cancelled)

42. (Previously presented) The method of claim 38, wherein the short hairpin RNA molecule comprises a double-stranded region consisting of at least 21 nucleotides.

43. (Previously presented) The method of claim 38, wherein the short hairpin RNA molecule comprises a double-stranded region consisting of at least 22 nucleotides.

44. (Previously presented) The method of claim 38, wherein the short hairpin RNA molecule comprises a double-stranded region consisting of at least 25 nucleotides.

45. (Previously presented) The method of claim 38, wherein the short hairpin RNA molecule comprises a double-stranded region consisting of 29 nucleotides.

46. (Previously presented) The method of claim 38, wherein the short hairpin RNA molecule has a total length of about 70 nucleotides.

47. (Previously presented) The method of claim 38, wherein the RNA polymerase promoter comprises a pol II promoter or a pol III promoter.

48. (Withdrawn) The method of claim 47, wherein the pol III promoter comprises a U6, an H1, or an SRP promoter.

49. (Previously presented) The method of claim 47, wherein the pol II promoter comprises a U1 or a CMV promoter.

50. (Currently Amended) The method of claim 38, wherein the short hairpin RNA molecule attenuates expression of the target gene in the mammalian cell by at least about 60% as compared to a control cell consisting of an expression construct encoding a short hairpin RNA that does not target the target gene.

51. (Currently Amended) The method of claim 38, wherein the short hairpin RNA molecule attenuates expression of the target gene in the mammalian cell by about 60% to about 90% as compared to a control cell consisting of an expression construct encoding a short hairpin RNA that does not target the target gene.

REMARKS

I. STATUS OF THE CLAIMS

Claims 38, 40, and 42-51 are pending in this application. Claim 48 has been withdrawn. Claim 38 is amended to more particularly point out the presently claimed invention. The amendment to claim 38 raises no issue of new matter. Support for the amendments to claim 38 ("wherein the short hairpin RNA molecule is a substrate for Dicer-dependent cleavage and does not trigger a protein kinase RNA-activated (PKR) response in the mammalian cells") may be found throughout the application, for example, support may be found, *inter alia*, at Example 8 of the application entitled "dsRNA Suppression in the Absence of a PKR Response." See ¶¶ 0349-0354 of US 20080213861. Support may also be found in the originally published claims, and ¶ 0044. Support for "is expressed in the cell without use of a PK inhibitor" can be found, *inter alia*, at Example 8 and Figure 50 and ¶¶120 and 141, showing MEF cells with the PK response suppressed. This is contrasted with dsRNA in MEF cells without use of a PK inhibitor. Support for these amendments can be found throughout the present specification and in the parent application, U.S.S.N. 10/055,797. These amendments raise no issue of new matter.

II. REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 50 and 51 are rejected as being allegedly indefinite.

In response, applicants traverse the rejection. Without conceding the correctness of the Examiner's position, applicants have amended claims 50 and 51. In view of these amendments, applicants request that the Examiner reconsider and withdraw this ground of rejection.

III. DOUBLE PATENTING

The Examiner rejected the pending claims over co-pending application U.S. Serial No. 11/894,676.

In reply, applicants request that the Examiner hold this rejection in abeyance since the '676 application is not yet allowed.

IV. OBVIOUSNESS

A. Rejections

Claims 38, 40, 42-47 and 49-51 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Kreutzer *et al.*, Dietz *et al.*, and Kingsman *et al.*

Claims 38, 40, 42-47 and 49-51 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Fire *et al.*, Dietz *et al.*, and Kingsman *et al.*

In reply, applicants traverse the rejection. Before addressing the Examiner's two rejections, applicants have set out below a discussion of the state of the art as of the priority date of this application and how the inventors' claimed invention was an advance over that that art.

B. The State of the Art Prior to January 22, 2002[1]

In order to provide background to the views of a person of ordinary skill in the art, and background against which the present invention was made, Applicants provide below a brief summary of the state of the art regarding gene silencing using RNA molecules. This summary is not to be considered an admission that any reference set out below is proper prior art as to the presently claimed invention.

1. Dr. Hannon's Goal: Exploiting RNAi to Study Gene Function in Mammalian Cells

By the invention of the short hairpin technology described in the Hannon application, Dr. Hannon and his co-inventors successfully achieved an ambitious goal of exploiting RNAi as a powerful and widely applicable genetic tool to study gene function in mammalian cells. In particular, this novel approach allowed one to use RNAi to stably attenuate expression of the target gene in a sequence specific manner in a mammalian cell, without activating a non-sequence specific PK response. To achieve this goal, Dr. Hannon and his co-inventors focused on identifying and understanding the cellular machinery that mediated RNAi in the cell. A key part of their work involved identifying and characterizing the components of the RNAi pathway. Among other things, Dr. Hannon and his co-inventors isolated and described two critical

components of the RNAi machinery: the enzyme Dicer, which the inventors named and demonstrated as mediating the processing of dsRNA (Bernstein et al. Nature, 2001), and "RISC", the nuclease complex responsible for degradation of target mRNAs. Dr. Hannon concisely summarized his overall strategy in a grant proposal for the work he subsequently carried out:

> My laboratory has devoted a number of years to creating improved tools for probing gene function in cultured mammalian cells; however, our experience indicates that a facile loss-of-function tool is lacking. Unfortunately, dsRNA induces somewhat generic responses in mammalian cells. It is our hope that by understanding the mechanistic basis of dsRNA-induced silencing, we may not only unravel a mysterious and important piece of biology but also provide the means to create improved tools for analyzing gene function in diverse organisms in which traditional genetic methods are either cumbersome or unavailable. This notion that has contributed to the decision to focus substantial effort in my laboratory toward elucidating the mechanism of RNA interference…
>
> …In this application, we propose a biochemical approach to deciphering the mechanisms that underlie dsRNA-induced gene silencing. RNA-interference allows an adaptive defense against both exogenous and endogenous dsRNAs, providing something akin to a dsRNA immune response. The primary goal of the work proposed in this application is to understand the mechanisms by which a cell can raise this response. We have presented evidence that RNA interference is accomplished, at least in part, through the action of a sequence-specific nuclease that is generated in response to dsRNA. Our data, and that of others (Hamilton and Baulcombe, 1999), is consistent with a model in which dsRNAs present in a cell are converted, in a manner analogous to antigen processing, into discrete, small RNAs that guide the nuclease in the choice of substrate. We propose to purify and characterize the nuclease and to clone the protein and RNA components of the enzyme. In addition, we propose to develop approaches that may allow the use of cultured Drosophila cells as a general tool for probing gene function. The combination of these studies may lead eventually to an ability to harness RNA interference as a genetic tool in other organisms, particularly mammals, in which analogous tools are presently lacking.

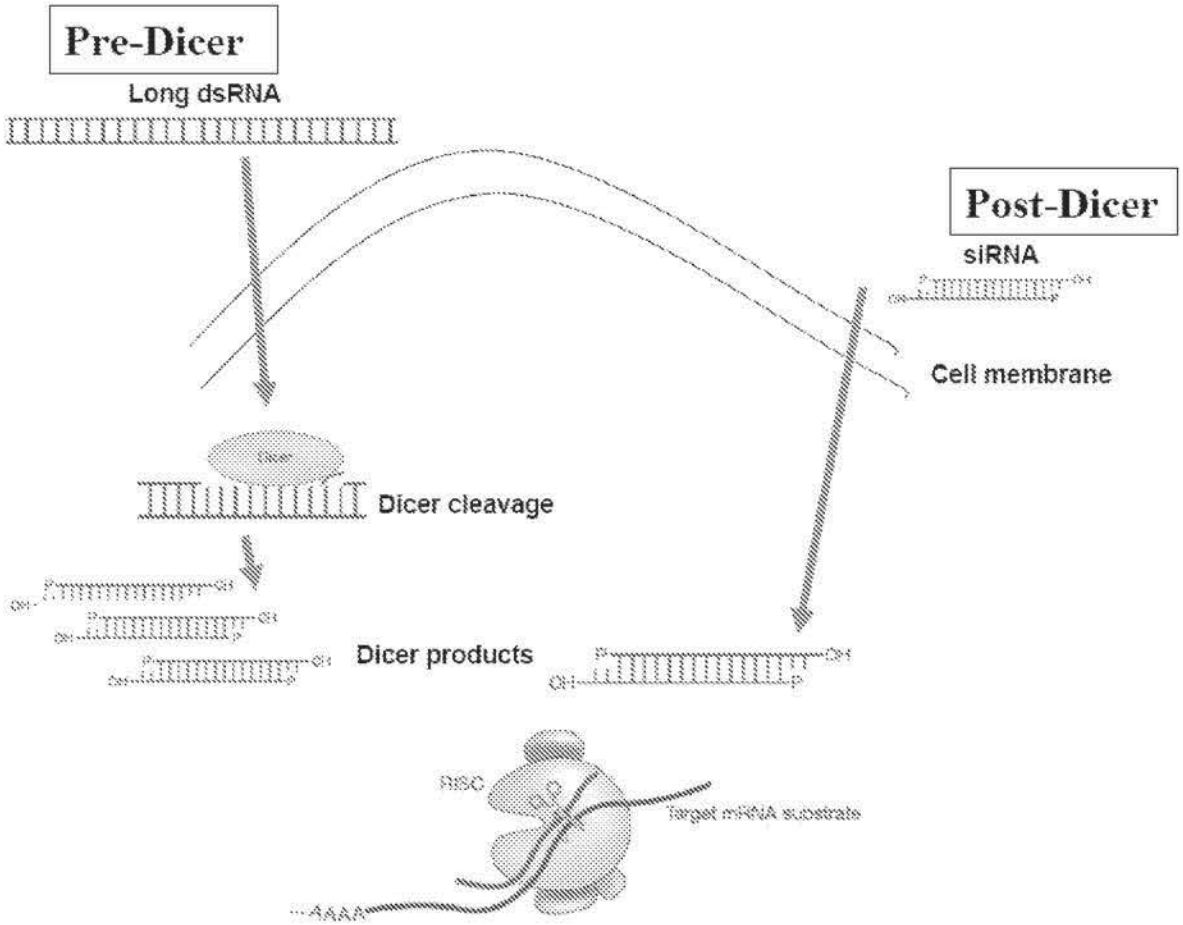
[1] January 22, 2002 is the filing date of the parent USSN 10/055,797 to which the present application claims priority.

2. Dicer Cleaves Long dsRNA to Make Guide RNAs or siRNAs

Hannon and his co-inventors demonstrated that Dicer processes long dsRNAs into short (approximately 21-25 nt) RNAs, which are referred to as short interfering RNAs (siRNAs) or "guide" RNAs, the term coined by Dr. Hannon. Bernstein et al. Nature 409: 363-366 (2001). The siRNAs are then incorporated into a protein (nuclease) complex called the RNA-induced silencing complex (RISC) Hammond et al., Nature 404:293-296 (2000). The siRNAs function to guide the RISC/siRNA complex to specific mRNAs, which are recognized through base pairing interactions by having a complementary sequence to the siRNA, and are then destroyed by RISC. Through this process, guide RNAs or siRNAs can inhibit gene expression by targeting destruction of specific mRNAs in the cell. Notably, the ability of long dsRNAs to trigger RNAi, therefore, requires Dicer to first cleave or process the long dsRNA into guide or siRNAs. Bernstein et al. Nature 409: 363-366 (2001); Hammond et al., Nature Rev. Genetics 2:110-119.

3. Pre-Dicer and Post-Dicer Strategies to Achieve RNAi

Dr. Hannon's work in discovering Dicer and the mechanism of Dicer processing demonstrated that one could potentially intervene in the RNAi pathway in two places.



(I) A **Pre-Dicer** strategy (see Pre-Dicer Pathway in above diagram) starts with long dsRNA triggers (see "Long dsRNA" in diagram above). Fire et al. demonstrated that long dsRNAs (for example, 300-500 bp) could effect gene silencing. Once introduced into a cell, these long dsRNA triggers are cleaved into siRNAs by Dicer. The siRNAs then combine with RISC to mediate specific gene silencing.

(II) A **Post-Dicer** strategy (see Post-Dicer Pathway above) uses short RNAs that mimic the siRNA products of Dicer cleavage (i.e., 21-25 nucleotide long short RNAs with 3' overhangs). Once introduced into a cell, the siRNAs bypass the Dicer enzyme altogether. The siRNAs directly combine with RISC to effect gene silencing. Elbashir SM, Lendeckel W, Tuschl T (2001) RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes Dev* 15(2):188–200. Elbashir et al. has been discussed by Dr. Hernandez in her Declaration filed in

this case. In this regard, Elbashir et al. included data showing that dsRNAs of 30 nucleotides in length or shorter were ineffective in mediating RNAi and would not work as Pre-Dicer triggers. Short RNAs would work only if they were designed to bypass Dicer processing.

4. Fire and Elbashir and Caplen Fail to Show Stable, Long Term Silencing

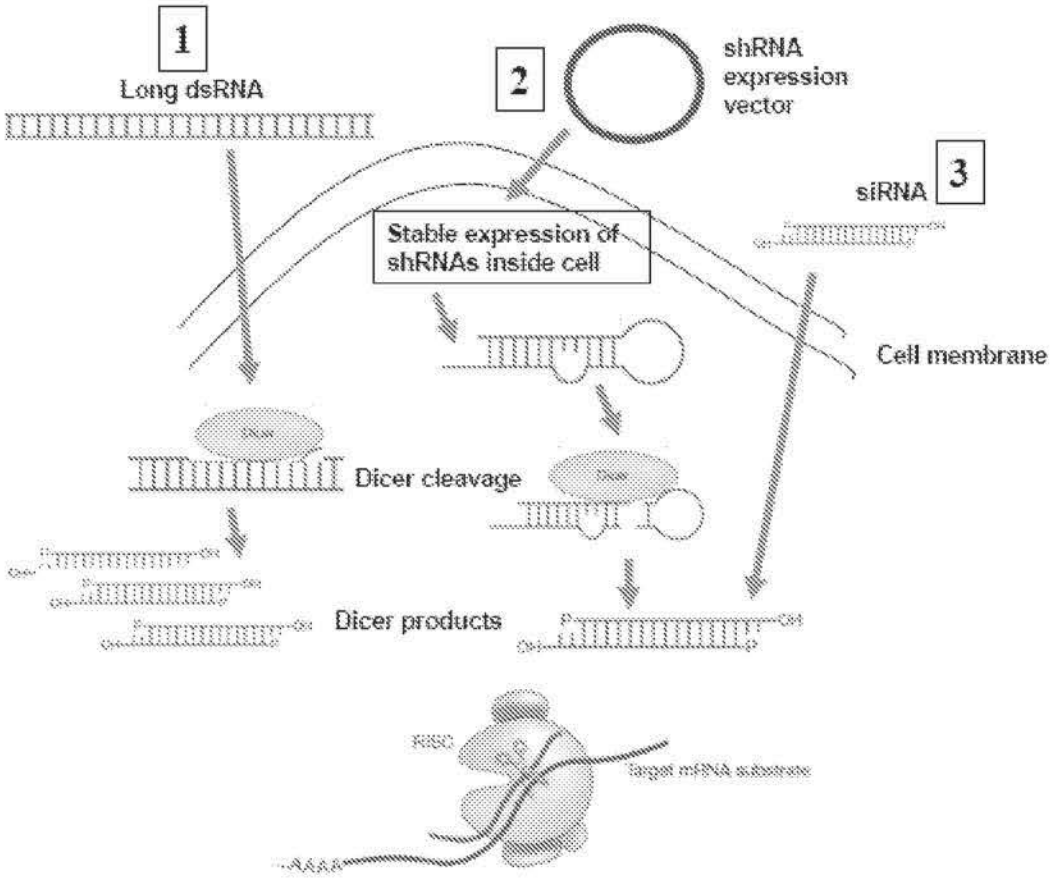
Fire's approach of using long dsRNA as a pre-Dicer trigger failed to show how one could use this strategy in mammalian cells. Of course, it was known that introducing or expressing long dsRNA in most mammalian cells would kill them by activating the anti-viral/PKR response. (Williams, B. R. Role of the double-stranded RNA-activated protein kinase (PKR) in cell regulation. *Biochem. Soc. Trans.* 25, 509–513 (1997).) This innate anti-viral pathway would have taught away from using dsRNA for silencing expression of a particular gene in a mammalian cell.

Another approach was taken by both Elbashir and Caplen -- using post-Dicer triggers, that is, siRNAs to achieve inhibition of gene expression. One primary drawback of this approach is that the effect is only transient. The application of siRNAs (see the post-Dicer pathway in the above diagram) is transitory. Once the siRNAs are applied exogenously into the cell, processed by Dicer and then complexed with RISC, there is no additional effect. This Post-Dicer approach using siRNAs will only temporarily silence genes.

These two approaches (Pre-Dicer and Post-Dicer) did not provide for stable, long term silencing in mammalian cells. Therefore, the pre-Dicer and post-Dicer approaches were of limited benefit in mammalian cells. Stable, long term silencing was necessary to carry out studies in mammalian cells to understand the genetic basis of human disease that Dr. Hannon envisioned. Before RNAi could be harnessed as a tool for silencing specific genes in mammalian systems, such as in methods claimed in the present invention, a considerable hurdle had to be overcome. The problem was how to trigger RNAi in a gene-specific manner in mammalian cells without invoking non-specific anti-viral responses to the RNAi trigger.

5. Invention of Hannon et al. Using Expressed shRNA in Mammalian Cells

Hannon demonstrated that one could actually engineer a pre-Dicer trigger that would ***not*** activate the anti-viral/PKR response, that could be ***stably expressed*** in the mammalian cell and surprisingly, would function as a potent trigger to specifically silence gene expression in mammalian cells. The presently claimed invention solves the problems of stable expression, avoidance of the PK response and sequence-specific inhibition of gene expression in mammalian cells. The diagram below illustrates the shRNA expression vector approach, which is claimed by the applicants.



The above diagram shows the introduction of shRNA expression vectors into the mammalian cell. These vectors can be ***stably expressed*** in a mammalian cell and ***don't activate the PKR response***. The vectors express a short hairpin RNA molecule which is a ***substrate for Dicer-dependent cleavage and does not activate the PKR response.*** The double-stranded region

of the short hairpin RNA molecule comprises a sequence that is complementary to a portion of the target gene.

Thus, the entirely different approach of Elbashir and Caplen - that of using **post-Dicer** triggers which could act to silence gene expression without being processed by Dicer *taught away* from Hannon's invention of using stable expression of short hairpin RNAs as **pre-Dicer** triggers to suppress mammalian gene expression.

6. Industry Acclaim

As evidenced by numerous awards and by the adoption of his short hairpin technology as a fundamental biomedical research tool, Dr. Hannon's pioneering work in the RNAi field has received widespread acclaim. In 2005, Dr. Hannon received the Award for Outstanding Achievement in Cancer Research from the American Association for Cancer Research (AACR), which honored Dr. Hannon "…for his work uncovering the biochemical mechanism of RNA interference of gene expression (RNAi) and his contributions to the discovery and development of short hairpin RNAs as tools for genetic manipulation of mammalian cells. (See **Exhibit A**). In 2007, Dr. Hannon received two more prestigious awards, the Award in Molecular Biology from the National Academy of Sciences, and the Paul Marks prize for the valuable contribution his RNAi work to cancer research from Memorial Sloan-Kettering Cancer Center. (See **Exhibits B and C**.) In granting that award, MSKCC noted how Dr. Hannon had applied his research in understanding the RNAi pathway to develop this valuable new technology, and his recognition as a leader in the field:

> Dr. Hannon is a leader in the relatively new field of RNA interference (RNAi). RNAi is a naturally occurring mechanism for regulating the expression of genes (controlling which genes are turned on and turned off in cells). In the laboratory, it is used as a tool to study the function of specific genes, and it's being investigated as a therapeutic approach for treating many different diseases, including cancer.
>
> Dr. Hannon's laboratory has elucidated key biochemical details of the components of the pathways involved in RNAi and is using these findings to develop molecular tools that can be used for gene discovery, the evaluation of gene function, and the generation of

> animal models. He has developed new techniques for using RNAi to study cancer development and is investigating possible cancer therapies that make use of small interfering RNAs (siRNAs).
>
> Dr. Hannon discovered several proteins and enzymes that are an essential part of the RNAi mechanism, including Dicer, which cleaves double-stranded RNA into siRNAs; the RISC complex, which helps regulate protein translation and is involved in the body's defense against viral infections; and Argonaute2, which cleaves messenger RNA.
>
> He also has been at the forefront of adapting RNAi techniques to study genes in mammals, and using these techniques to understand the variety of pathways that can lead to the formation of tumors.

The presently claimed invention described in the Hannon application was the basis for various shRNA libraries, which have become widely used tools for genetic analysis in mammalian cells. Reflecting the valuable contribution of this technology to biomedical research, during 2002-2006, Dr. Hannon was among the top five most highly cited scientists with the highest number of high impact papers in the field of molecular biology and genetics. The 2002 Genes & Development paper, "Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells," in which Dr. Hannon reported much of the work underlying the presently claimed invention, was cited more than 500 times, including more than 100 papers in the biotechnology field.

As further evidence of the non-obviousness of the claimed invention, Applicants previously submitted a Declaration under 37 C.F.R. § 1.132 from Professor Nouria Hernandez. (Another courtesy copy is attached as **Exhibit D**.) As Prof. Hernandez states, it would not have been obvious to one of ordinary skill in the art at the time of the invention that one could attenuate target gene expression in a mammalian cell by introducing an expression construct encoding a short hairpin RNA molecule having a double-stranded region of 20-29 nucleotides. Indeed, according to Prof. Hernandez it was unexpected that the claimed method would result in effective target gene attenuation, and one of ordinary skill at the time of the invention would have had no reasonable expectation that it would do so.

C. The Claimed Invention

The claimed invention is directed to:

> Claim 38. A method for attenuating expression of a target gene in a mammalian cell, the method comprising:
>
> introducing into a mammalian cell an expression vector comprising:
>
> (i) an RNA polymerase promoter, and
>
> (ii) a sequence encoding a short hairpin RNA molecule comprising a double-stranded region, **wherein the double-stranded region consists of at least 20 nucleotides but not more than 29 nucleotides**,
>
> **wherein the short hairpin RNA molecule is a substrate for Dicer-dependent cleavage** and does not trigger a protein kinase RNA-activated (PK) response in the mammalian cell,
>
> wherein the double-stranded region of the short hairpin RNA molecule comprises a sequence that is complementary to a portion of the target gene, and
>
> wherein the short hairpin RNA molecule is **stably expressed** in the mammalian cell in an amount sufficient to attenuate expression of the target gene in a sequence specific manner, and is expressed in the cell without use of a PK inhibitor, whereby expression of the target gene is inhibited.

Applicants note that this claim is presented as an example of the claimed invention and in order to facilitate the discussion below of certain claimed features of the invention.

D. Examiner's Comments Regarding Declaration of Professor Hernandez Under 37 C.F.R. §1.132 Are Incorrect Legally and Factually

The Examiner has taken the position the Hernandez Declaration does not provide sufficient evidence that a person of ordinary skill in the art would not have had a reasonable expectation of success at using a shRNA with a double stranded region of between 20 and 29 nucleotides in length. The Examiner takes the position that Kreutzer et al. (of record) provides evidence that a dsRNA having a double stranded region of at least 21 bp was capable of mediating RNAi in cells which is direct evidence against the data in Elbashir.

In reply, applicants respectfully traverse the Examiner's position. Applicants have identified several legal and factual errors with regard to the Examiner's discussion of the Hernandez Declaration which are important to point out. First, the Examiner erroneously discounted the "opinion" evidence provided by Professor Hernandez. The Examiner mistakenly believes Professor Hernandez is providing "expert opinion." In fact, Professor Hernandez is providing the opinion of a person of ordinary skill in the art, which is different than an expert opinions. The opinion of a person of ordinary skill in the art at the critical time is actually a fact to be considered in an obviousness analysis. Prof. Hernandez was an Investigator at the Howard Hughes Medical Institute at the time working in the area of RNA and studying RNA polymerase III. She was aware of Elbashir et al. at the time. Unlike a retrospective expert opinion, the Declaration is based on the personal knowledge of Prof. Hernandez testifying as a person of ordinary skill in the art at that time. The statement of Professor Hernandez is therefore factual evidence that must be taken into account, and not expert opinion as discussed in the passage from the MPEP relied upon by the Examiner, MPEP 716.01(c).

Second, it appears that the Examiner has impermissibly heightened the standard when carrying out a patentability assessment under 35 U.S.C. § 103. The Examiner criticizes the Hernandez Declaration because "the Declaration by Dr. Hernandez does not ***conclusively prove*** that one of ordinary skill in the art would not have expected to be able to use a dsRNA of 20-29 bps in an expression vector to mediate RNAi." (Emphasis added.) There is no requirement under the patent law that a rebuttal to obviousness "conclusively prove" that a person of ordinary skill in the art would not have an expectation. Dr. Hernandez has provided evidence that a person of ordinary skill in the art would have believed that Elbashir teaches away from the invention and that there would have been no reasonable expectation of success in carrying out the claimed invention. There is no requirement for conclusive proof, and applicants request that the Examine reconsider the evidence of the Hernandez Declaration.

Third, the Examiner has misapprehended the contents of Elbashir in her statement that "Elbashir et al. teach double stranded RNAs of 30 bp are not efficiently processed to 21 to 23 bp dsRNAs is not a true teaching of teaching away from using dsRNAs of 30 bp or less given it appeared some of the dsRNAs of less than 30 bps worked." See Office Action sentence spanning pages 9-10. Applicants direct the Examiner to the data expressed in Figure 1b of

Elbashir showing that dsRNAs of 29 bp and 30 bp in length failed to mediate RNAi (bars indicating effect of both 29bp and 30bp was equivalent to controls). The factual evidence in Elbashir et al. therefore teaches away from the claimed invention. This is conclusive evidence supporting the statements made by Prof. Hernandez that Elbashir teaches away from using dsRNAs of 29 or 30 bps, and teaches away by discouraging one of skill from pursuing the claimed invention. Moreover, there is no evidence whatsoever in Elbashir that dsRNAs shorter than 29 bp were effective as pre-Dicer triggers. Such an inference would have no scientific support.

Elbashir taught that to overcome the inability of the cellular RNAi machinery to process short dsRNA molecules into the 21-23nt (guide) siRNA mediating target gene suppression, one instead could directly introduce an RNA molecule mimicking an siRNAs into the cell. (See Figure 5 of Elbashir.) In view of Elbashir, one of skill would have expected that a 21 nucleotide long RNA could therefore serve as an RNAi trigger without the need for processing. To one of skill in the art, such a result, however, would have provided no evidence or expectation that a hairpin RNA molecule with a 21 bp double-stranded region could mediate RNAi, in particular because to mediate RNAi, the hairpin RNA would first have to be processed into an siRNA. In view of Elbashir, that a short hairpin RNA (having a double-stranded region of less than 29 bp or at least 20 base pairs) could be used as an RNAi trigger was, in fact, surprising and unexpected.

Fourth, rebuttal evidence can be submitted by way of a declaration and the entire situation regarding patentability must be reviewed in view of the new evidence. See 37 C.F.R. §1.132 and M.P.E.P. § 2141. In particular, whenever an applicant submits additional evidence, the Examiner must reconsider patentability of the claimed invention, and any decision to maintain a rejection must show it was based on the totality of the evidence. *Id.* "Facts established by the rebuttal evidence must be evaluated along with the facts on which the conclusion of obviousness was reached, not against the conclusion itself." M.P.E.P. 2142 (citing *In re Eli Lilly & Co.*, 902 F.2d 943 (Fed. Cir. 1990))(emphasis added). "Consideration of rebuttal evidence and arguments requires Office personnel to weigh the proffered evidence and arguments. Office personnel should avoid giving evidence no weight, except in rare circumstances." See *In re Alton*, 76 F.3d 1168, 1174-75, 37 USPQ2d 1578, 1582-83 (Fed. Cir. 1996).

Here, applicants have met their burden by providing rebuttal evidence via the teachings of Elbashir and the Hernandez Declaration. Instead of taking the factual evidence contained in the Hernandez Declaration into account, the Examiner characterizes the statements made by Prof. Hernandez in her Declaration and data cited therein as "opinion." Prof. Hernandez is not offering an expert opinion, but is offering the opinion of a person who was a person of ordinary skill in the art, and thus the opinion of Prof. Hernandez is ***a fact*** that must be considered in carrying out a patentability assessment as to obviousness. Indeed, Prof. Hernandez is not "arguing" but is rather presenting evidence as a person of ordinary skill in the art at that time for the Examiner to consider. It is error to consider the content of the Hernandez Declaration as "opinion." The statements therein are facts that must be taken into account.

E. The Claimed Invention Is Not Obvious In View Of the Combination of Kreutzer, Dietz and Kingsman

Claims 38, 40, 42-47 and 49-51 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Kreutzer *et al.*, Dietz *et al.*, and Kingsman *et al.*

1. Kreutzer et al. Discloses "Chemically Modified" Structures that Do Not Make Obvious the Claimed Invention

Applicants traverse the rejection. First, applicants note that the claims have been amended and now require that the "short hairpin RNA molecule is a substrate for Dicer-dependent cleavage." Kreutzer provides no evidence that the chemically modified RNA structures are even processed through the RNAi pathway. The claimed invention requires that the short hairpin RNA molecule be a substrate for Dicer and the structures described by Kreutzer, with the chemical modifications, would not be such a substrate.

The "dsRNA" that Kreutzer describes (see [0069]) is a synthetic and chemically altered RNA molecule (synthons modified by disulfide bridges) comprised of single strands linked by a disulfide bridge. Such a chemically altered species cannot be expressed within in a cell. Kreutzer would not have provided any reasonable expectation of success with regard to how an unmodified dsRNA, or a hairpin RNA molecule that is expressed within a cell, would have affected gene expression.

The sole references Kreutzer makes to RNA hairpin structures are made in the context of addressing the problem of degradation of the dsRNA in the cell. To afford protection from degradation, Kreutzer et al. suggested use of chemically altered dsRNAs, generated through "chemical modification" of the dsRNA or by chemically modifying the nucleotides in the loop region of an RNA hairpin loop. See, for example, paragraph 19 of Kreutzer et al. Here, Kreutzer states "an RNA hairpin loop, in particular when using a vector according to the invention. To afford protection from degradation, it is expedient for the nucleotides to be chemically modified in the loop region between the double-stranded structure." The vector referred to and the fact that chemical modifications are proposed both indicate that this statement refers to an RNA produced *in vitro* which is then delivered into cells. Such a synthetic structure could ***not*** be expressed in a mammalian cell from any vector.

The Examiner has relied upon Kreutzer et al. to allegedly show that a dsRNA 21 nucleotide base paired molecule was capable of efficiently reducing gene expression in mammalian cells (see Examples). The Examiner goes on to state that this is direct evidence that one of ordinary skill in the art would have expected a dsRNA of less than 29 bp or having a double stranded region of at least 20 base pairs to be capable of mediating RNAi in mammalian cells.

Applicants traverse the Examiner's position and submit that the Kreutzer et al. reference would not have made obvious the use of a short hairpin RNA structure, having a double-stranded region consisting of at least 20 nucleotides but not more than 29 nucleotides as recited in the presently claimed invention. Kreutzer et al. would not have given a person of ordinary skill in the art a reasonable expectation of success that stably expressing a short hairpin RNA having a double-stranded region consisting of at least 20 nucleotides but not more than 29 nucleotides would attenuate gene expression in mammalian cells.

Kreutzer et al. would not have provided any reasonable expectation that one could have used the presently claimed methods to successfully suppress gene expression in a mammalian cell. The Examples in Kreutzer et al. do not show expression of a short hairpin RNA in mammalian cells. Instead, the Example 1 shows *in vitro* transcription (*e.g.*, starting at paragraph 44); generation of double-stranded RNA by *in vitro* hybridization (*e.g.*, starting at paragraph 46).

Similarly, Example 2 shows transfection (not stable expression) of dsRNA having a length of 315 bp (see Seq. I.D. No. 5 and paragraph 66) and microinjection of a chemically modified, synthetic, dsRNA of 21 bp (see Seq I.D. No. 8 and paragraph 69) into a murine cell line. The 21 bp dsRNA was not a hairpin, and was chemically modified and synthesized using solid state chemistry. In paragraph 69, Kreutzer et al. state: "A dsRNA linked chemically at the 3' end of the RNA as shown in sequence listing No. 8 to the 5' end of the complementary RNA via a C18 linker group was prepared (L-dsRNA). To this end, synthons modified by disulfide bridges were used." The paragraph goes on to describe solid support chemical methods used to carry out the chemical reactions needed to obtain the L-dsRNA. The disclosure of Kreutzer et al. would not have taught or made obvious to a person of ordinary skill in the art at the time the claimed methods of Hannon et al. because the Hannon methods require *in vivo* stable expression of a construct to express a short hairpin RNA having a double-stranded region consisting of at least 20 nucleotides but not more than 29 nucleotides.

Finally, the final sentence of Kreutzer et al. clarifies the meaning of the results presented in Example 2. Kreutzer et al. state in paragraph 76 "[t]his result demonstrates that even shorter dsRNAs can be used for specifically inhibiting gene expression in mammals when the double strands are stabilized by chemically linking the single strands." A person of ordinary skill in the art would conclude that, from this statement, a person of ordinary skill in the art at the time would have understood that: (1) *in vitro* transcription of single stranded RNAs was required by the method of Kreutzer et al.; (2) that solid state chemical modification of those single strands was required by the method of Kreutzer et al. (also a set of *in vitro* chemical steps); and (3) microinjection of chemically modified dsRNAs into mammalian cells was required. None of these teachings would have made obvious the methods of Hannon et al.

Kreutzer et al. would not make obvious the presently claimed invention which requires a very different structure -- a short hairpin RNA molecule wherein the double-stranded region consists of at least 20 nucleotides but not more than 29 nucleotides and wherein the short hairpin RNA molecule is a substrate for Dicer-dependent cleavage. Kreutzer et al. do not teach or make obvious such a hairpin structure.

2. Kreutzer is Not a Proper Reference Under 102(e)

Applicants maintain their position that the '408 publication is not a proper reference under 35 U.S.C. §102(e) and thus under §103. Applicants also submit that the '408 publication in combination with Dietz and Kingsman do not render the claimed invention obvious for the reasons of record and the reasons set out below.

The '408 publication is not proper prior art under 35 U.S.C. §102(e) (and thus under §103). The '408 publication is a division of USSN 09/889,802, which was filed on September 17, 2001, which was a §371 application of PCT/DE00/00244, which was filed on January 29, 2000. January 29, 2000 is prior to November 29, 2000. Therefore, the '408 publication does not arise "from an international filing date on or after November 29, 2000" as required by MPEP § 706.02(f)(I)(C).

The Examiner does not cite to any section of the MPEP in the Final Office Action, but appears to rely upon the following statement from the MPEP :

> (c) For U.S. application publications of applications that claim the benefit under **35 U.S.C. 120** or 365(c) of an international application filed prior to November 29, 2000, apply the reference under **35 U.S.C. 102**(e) as of the actual filing date of the later-filed U.S. application that claimed the benefit of the international application.

See MPEP § 706.02(f)(I)(C)(3)(c).

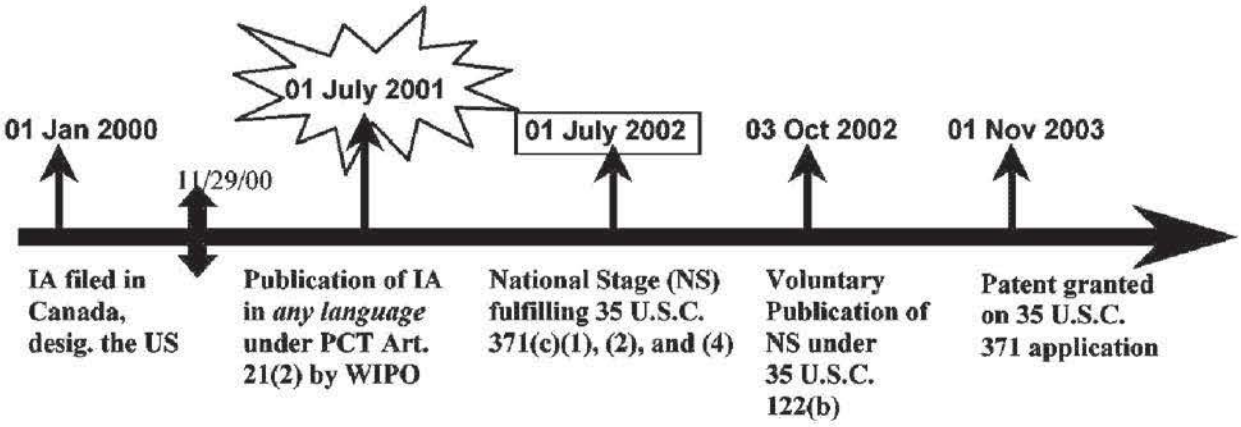
In this case, the '408 publication is not proper art under 35 U.S.C. §102(e). The '408 publication is a "U.S. application publication" that claims the benefit under § 120 of the PCT international application (the '244 PCT, filed on January 29, 2000) filed prior to November 29, 2000. The above section of the M.P.E.P. instructs Examiners to "apply the reference as of the actual filing date of the later-filed U.S. application that claimed the benefit of the international application. The '408 publication claims the benefit of the PCT under §120. (See Declaration filed in the prosecution history of the '408 publication.) Therefore, the actual filing of the U.S. application (the '408 publication) is March 6, 2003 and is the proper 102(e) date. Following this

section of the M.P.E.P., the 102(e) of the '408 publication would be March 6, 2003, and therefore, it is not proper prior art against the claims of the present application.

The '408 publication is a later-filed U.S. application publication (filed on March 6, 2003) claiming benefit of the '244 PCT, which was filed on January 29, 2000, which was filed prior to November 29, 2000. The application from which the '408 publication is a divisional, the '802 application, never published and is abandoned. Applicants previously directed the Examiner to Example 6 in the M.P.E.P. and for the Examiner's convenience reproduce it here:

Example 6 : References based on the national stage (35 U.S.C. 371) of an International Application filed prior to November 29, 2000 (language of the publication under PCT Article 21(2) is not relevant).

The reference U.S. patent issued from an international application (IA) that was filed prior to November 29, 2000 has a 35 U.S.C. 102 (e) prior art date of the date of fulfillment of the requirements of 35 U.S.C. 371 (c)(1), (2) and (4). This is the pre-AIPA 35 U.S.C. 102 (e). The application publications, both the WIPO publication and the U.S. publication, published from an international application that was filed prior to November 29, 2000, do not have any 35 U.S.C. 102 (e) prior art date. According to the effective date provisions as amended by Pub. L. 107-273, the amendments to 35 U.S.C. 102 (e) and 374 are not applicable to international applications having international filing dates prior to November 29, 2000. The application publications can be applied under 35 U.S.C. 102 (a) or (b) as of their publication dates.



The 35 U.S.C. 102(e)(1) date for the IA Publication by WIPO is: None. The 35 U.S.C. 102(e)(1) date for the Publication by USPTO is: None. The 35 U.S.C. 102(e) date for the Patent is: 01 July 2002.

The IA publication by WIPO can be applied under 35 U.S.C. 102 (a) or (b) as of its publication date (01 July 2001).

Additional * Benefit Claims :

If the IA properly claimed ** > the benefit of < any earlier-filed U.S. application (whether provisional or nonprovisional), there would still be no 35 U.S.C. 102 (e)(1) date for the U.S. and WIPO application publications, and the 35 U.S.C. 102 (e) date for the patent will still be 01 July 2002 (the date of fulfillment of the requirements under 35 U.S.C. 371 (c)(1), (2) and (4)).

If a later-filed U.S. nonprovisional (35 U.S.C. 111 (a)) application claimed the benefit of the IA in the example above, the 35 U.S.C. 102 (e)(1) date of the application publication of the later-filed U.S.

application would be the actual filing date of the later-filed U.S. application, and the 35 U.S.C. 102 (e) date of the patent of the later-filed U.S. application would be 01 July 2002 (the date that the earlier-filed IA fulfilled the requirements of 35 U.S.C. 371 (c)(1), (2) and (4)).

If the patent was based on a later-filed U.S. application that claimed the benefit of the international application and the later filed U.S. application's filing date is before the date the requirements of 35 U.S.C. 371 (c)(1), (2) and (4) were fulfilled (if fulfilled at all), the 35 U.S.C. 102 (e) date of the patent would be the filing date of the later-filed U.S. application that claimed the benefit of the international application.

The parent of the '408 publication was U.S. application Serial No. 09/889,802 ("the '802 application), which is now abandoned. For purposes of Example 6, the '408 publication is a "later-filed U.S. non-provisional (35 U.S.C. 111 (a)) application claim[ing] the benefit of" an international application filed before November 29, 2000. Therefore, the '408 publication falls within the category of applications treated in the box of Example 6 entitled "Additional Benefit Claims." As the emphasized section states, the publication of a later-filed application has a 102(e)(1) date of its actual filing date, not its effective filing date. Therefore, the 102(e) date of the '408 publication is its actual filing date, March 6, 2003, which is later than the January 22, 2002 priority date of the present application.

The Examiner argues that the '408 publication has a 102(e) date which is the effective filing date of its direct parent, the '802 application. However, the '802 never published and would not be "an application publication" as referred to in M.P.E.P. Example 6 and therefore would not be eligible for a 102(e) date. Accordingly, the '408 publication is not a proper reference under Section 102(e) and the rejection should be withdrawn. Should the Examiner insist that Kreutzer is prior art, Applicants request supervisory review of her interpretation of Section 102(e) in view of Example 6 of M.P.E.P. § 706.02(f)(1).

Finally, as discussed in the recent interview, the priority date of the '408 publication cannot be confirmed because the PCT application is published in German. Even if the '408 publication was prior art, it does not render the claimed invention obvious alone or in combination with Dietz and Kingsman as discussed below.

3. The '408 Publication Combined with Dietz and Kingsman Do Not Make Obvious the Presently Claimed Invention

The Examiner's rejection ignores several elements of the present claims and fails to articulate any rational basis for why these missing elements would have been obvious in view of the '408 publication and other prior art cited in the office action. The '408 publication cannot make the claimed invention obvious because, for example, it does not contemplate stable expression in a mammalian cell of short hairpin RNAs comprising a double-stranded region wherein the double-stranded region consists of at least 20 nucleotides but not more than 29 nucleotides as required by the present claims. There is no motivation to combine the '408 publication with Dietz and Kingsman and to do so requires hindsight in view of the claimed invention.

Applicants point out that the only mention of a hairpin in the '408 publication is in ¶ [0019], which refers to *chemically modifying* the loop region, which is a context wherein a hairpin could *never* be expressed from a vector in a mammalian cell containing the target gene, as required by the claims. The presently claimed invention requires that the vector be expressed stably in mammalian cells and this could not be the case based on the description in the '408 publication. The Examiner does not dispute the context of ¶ [0019], but instead responds by referring to dsRNA "that is formed by a single auto complementary RNA comprising a loop", which the Examiner interprets as a hairpin RNA. The Examiner provides no citation, but presumably relies on ¶ [0017], which refers in part to "[a] region II which is complementary within the double-stranded structure is formed by…autocomplementary regions of a topologically closed RNA single strand which is preferably in circular form." An encoded hairpin RNA molecule expressed within a cell (as presently claimed) has a 5' and 3' end, is therefore topologically open. This expressed RNA is not rendered obvious by a single reference in the '408 publication to an entirely distinct molecule, a topologically closed (preferably circular) RNA. Again, the disclosure in the '408 publication cannot render obvious the claimed invention. The two other references which the Examiner cites (Dietz and Kingsman) cannot remedy these deficiencies. The Examiner's interpretation of the '408 publication as referring to an expressed hairpin RNA molecule is clear factual error.

For example, further missing from the '408 publication is any disclosure of a size range of the double-stranded region as presently claimed, that is, consisting of at least 20 nucleotides but not more than 29 nucleotides. Also missing from the '408 publication is any disclosure about a requirement that the double-stranded region is complementary to the target gene, or that the short hairpin RNA molecule is stably expressed in the mammalian cell.

Dietz and Kingsman do not remedy the shortcomings of the '408 publication. The Examiner states that Dietz teach "the routine nature of stable expression of RNA inhibitory molecules." Dietz does not teach the short hairpin RNA approach that is a feature of the claimed invention here. Indeed, the structures that are depicted in the Dietz patent are complicated stem loop structures and are not hairpins. Furthermore, there is no teaching that these complicated structures would be a substrate for Dicer-dependent cleavage as is required by the claimed invention. The Examiner states that Kingsman "teach expression vectors capable of delivering ribozyme nucleic acid sequences." Again, this disclosure of Kingsman does not remedy the many shortcomings of Kreutzer and Dietz. At least, the combination of Kreutzer, Dietz and Kingsman still fail to teach or make obvious: the size requirement of the double-stranded region of the short hairpin in the claimed invention, the aspect of the invention that requires stable expression of the vector encoding the short hairpin RNA and the requirement that the short hairpin RNA be a substrate for Dicer. Therefore, the combination of Kreutzer, Dietz and Kingsman do not make obvious the claimed invention.

In failing to take these missing elements into account, the Examiner has not made out a *prima facie* case, and the rejection cannot possibly articulate a rational basis for a finding of obviousness based on the '408 publication. Such a finding constitutes clear factual error. The Examiner seems to have used hindsight to supplement her rejection with piecemeal references to attempt to find all of the claimed elements of the present claims. Applicants maintain that the combination does not render obvious the claimed invention and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

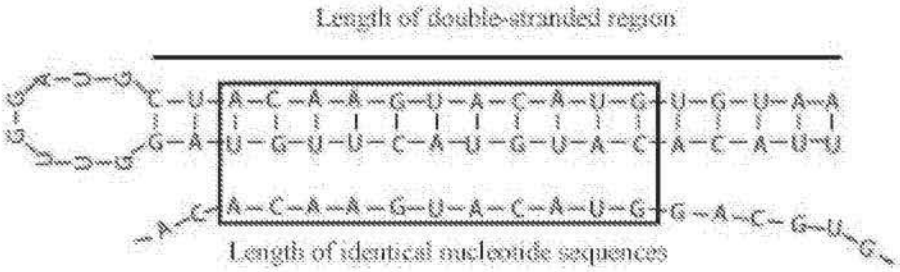
F. Claimed Invention Not Obvious in View of Fire, Dietz and Kingsman

Applicants respectfully traverse the rejection over Fire, Dietz and Kingsman. This combination would not make the claimed invention obvious to one of ordinary skill in the art at the time. These three references are not sufficient to make a *prima facie* case of obviousness for the reasons set out below. None of the cited references, alone or in any combination, disclose or suggest a method for attenuating target gene expression in a mammalian cell by introducing an expression vector encoding a short hairpin RNA. The claimed invention provides a solution to the problem of inhibiting gene expression in mammalian cells without provoking PKR-mediated apoptosis, overcoming technical difficulties that are not encountered or addressed using the prior art methods in non-mammalian cells.

Applicants claims require the double-stranded region to consist of at least 20 but not more than 29 nucleotides. The Examiner's § 103(a) rejection over Fire relies on the erroneous factual finding that Fire discloses a dsRNA of 25 bases in length. Here, the Examiner improperly argues that the disclosure in Fire et al. of a range of lengths ("the length of the identical nucleotide sequences may be at least 25…") constitutes a disclosure of the endpoint of that range (25 bases) as a species. This constitutes clear legal error in view of *Atofina* as "may be at least 25…" discloses only a range and not any endpoint. See M.P.E.P. § 2163.03(II), *Atofina v. Great Lakes Chem. Corp*, 441 F.3d, 991, 1000 (Fed. Cir. 2006) ("[T]he disclosure of a range is no more a disclosure of the end points of the range than it is each of the intermediate points.") Therefore, the disclosure in Fire of "at least 25…" is a range and does not, as per the Federal Circuit in *Atofina*, disclose the end point 25 as a single species.

Moreover, Fire lacks any disclosure of a short hairpin RNA molecule as presently claimed, that is, a single-stranded RNA molecule comprising a double-stranded region having a length of at least 20 nucleotides but not more than 29 nucleotides. The Examiner erroneously alleges that Fire discloses the length of the dsRNA region "to be at least 25 bases in length." However, the language to which the Examiner expressly refers states only that "the length of *the identical nucleotide sequences* may be at least 25, 50, 100, 200, 300 or 400 bases." (Fire, 8:5-6, emphasis added.) The language makes no reference whatsoever to the length of the double-stranded region. The italicized phrase above refers back to sentence at 7:53-54 which recites "nucleotide sequences identical to a portion of the target gene…" This sentence does not refer to

the length of the double-stranded region of a hairpin, but rather refers to the sequence that is identical to a portion of the target gene. These are two different things. Applicants illustrate this point with the diagram below. The length of the sequence identical to a portion of the target gene does not disclose the length of the double-stranded region, as exemplified below:



In stating "there is no mention in the Fire et al. disclosure that the two strands of the hairpin RNA can be very different lengths as argued by Applicant," the Examiner entirely mischaracterizes Applicants' argument, which merely points out that the Fire specification never discloses the length of the duplex portion of a hairpin RNA molecule.

Moreover, Claim 15 of Fire cannot provide any basis for the Examiner's erroneous contention that Fire et al describes a short hairpin RNA molecule comprising a double-stranded region having a length of 25 base pairs. The Examiner asserts that Fire claim 15 discloses a hairpin RNA because "Fire clearly states in ¶ (07) that 'the double-stranded structure may be formed by a single self-complementary strand [can be a hairpin] or two complementary RNA strands [or comprised of two strands].'" The specific text the Examiner cites as describing a hairpin says that RNA is formed by a single strand. Fire claim 15 depends on claim 12, which expressly states that the claimed RNA is (and is limited to) a double-stranded molecule, and specifies it is a double-stranded molecule with a first strand…and a second strand. In other words it is made up of two strands and therefore cannot be a single strand, or a hairpin. The Examiner's contention that it can be is clear factual error.

The rejection omits another critical factual finding in failing to set forth any evidence establishing a reasonable expectation of success. On the contrary, the evidence in the record instead establishes that there was no reasonable expectation of success. The failure of the Examiner to provide any evidence otherwise precludes a finding of obviousness. Taking into account the record as a whole, including the Hernandez Declaration and Elbashir et al. (2001)

Genes Dev. 15:188-200 (Elbashir 2001(a)) cited by Dr. Hernandez as additional evidence, there was no reasonable expectation that the presently claimed methods would be successful for attenuating expression of a target gene in a sequence specific manner. As one of ordinary skill, who at the time of the invention was working in the field of RNA expression, the sworn testimony of Prof. Hernandez constitutes evidence of the state of the art, including the reasonable expectation of those of ordinary skill at the time.

In particular, Prof. Hernandez presents evidence:

> (1) that one of skill would have understood the process of RNAi to be mediated by 21- and 22- nucleotide small interfering RNAs (siRNAs) generated from longer double-stranded RNA (dsRNA) precursors;
>
> (2) given this fact, that expressed hairpin molecules, in order to mediate RNAi, similarly had to be processed in the cell to 21- and 22-nt siRNAs;
>
> (3) that Elbashir (2001a) reports, using an established *in vitro* system, dsRNA precursors of 29-36 bp failed to produce an RNAi response;
>
> (4) that the failure of 29-36 bp dsRNA precursors to do so could be explained by the fact that such dsRNA precursors were not effectively processed into the 21- and 22- nucleotide siRNAs mediating the RNAi response,
>
> (5) that, for this same reason, the data of Elbashir (2001a) would have caused one of skill to expect that a short hairpin RNA with a double-stranded region consisting of 20-29 bp region would also be ineffective in mediating RNAi, and
>
> (6) that in view of these data, there would have been no reasonable expectation that one could successfully use an RNA molecule comprising a double-stranded region consisting of 20-29 bp, such as the short hairpin RNA molecule recited in the present claims, to mediate RNAi.

Subsequent to a recent interview, the SPE has stated that "Elbashir's teaching clearly indicated that short 30 bp dsRNAs are processed to 21 and 22-nt RNA's, albeit inefficiently; thereby not constituting a teaching away." (See Interview Summary, May 4, 2010.) This view ignores the factual evidence in Elbashir itself, and the facts set out by Prof. Hernandez (*e.g.*, that

that dsRNAs of 29-36 bp failed to mediate any RNAi response. See Decl. ¶11, Elbashir Fig. 1. The authors of Elbashir state on page 189, first column, that "[s]pecific inhibition of target RNA expression was detected for dsRNAs as short as 38 bp, but dsRNAs of 29–36 bp were not effective in this process." In Fig. 2 of Elbashir, the authors observe that processing of a 29 bp dsRNA into 21-23nt siRNAs was dramatically delayed and decreased, as compared to the processing of dsRNA 39bp in length or longer. Interpreting Figure 2, the authors state: "This observation is consistent with a role of 21–23-nt fragments in guiding mRNA cleavage and provides an explanation ***for the lack of RNAi by 30-bp dsRNAs.*** The length dependence of 21–23 mer formation is likely to reflect ***a mechanism to prevent the undesired activation of RNAi by short intramolecular base-paired structures of cellular RNAs***." (See Elbashir et al. Genes & Development, 2001, p. 189, 2nd col. first partial ¶, emphasis added.) The authors themselves conclude that RNAi is not achieved with 30-bp dsRNAs. This conclusion is echoed by Prof. Hernandez's reading of Elbashir as set out in her sworn Declaration.

Here, Prof. Hernandez states that Elbashir "discourages" one from using short hairpin RNAs with a ds region of less than 38 bp (*see* ¶11). Prof. Hernandez declares that one would have been taught away from using short hairpins, as claimed, in view of Elbashir (see ¶ 16) and that Elbashir "expressly teaches away" from using shRNAs of less than 30 bp (see ¶ 14). See also Hernandez Declaration ¶¶ 7, 9-13, 16 and 19. Prof. Hernandez states that in view of Elbashir, there "would have been no expectation of success…" (see ¶ 19). The SPE's view that Elbashir does "not constitute[ing] a true teaching away" is not supported by any evidence which would rebut the evidence filed by Applicants. Again, the Patent Office is improperly choosing to ignore the factual record set out by applicants including the sworn testimony of Prof. Hernandez, one of ordinary skill in the art at the time. There is no evidence proffered by the Examiner or the SPE otherwise. The small, significantly delayed processing of dsRNAs of 29 bp in length does not provide any expectation of success (as per Prof. Hernandez, Declaration ¶ 19). The Examiner's unsubstantiated conclusion that data such as in Fig. 2 would have provided a reasonable expectation that RNA precursors having a double-stranded region of 29 base pairs or less would successfully mediate RNAi expressly contradicts the evidence set forth in the Declaration, including the contemporaneous interpretation by Elbashir et al. of their own data.

Fire does not disclose or make obvious that "the short hairpin RNA molecule is **stably expressed** in the mammalian cell in an amount sufficient to attenuate expression of the target gene in a sequence specific manner…" as required by applicants' claims. Fire does not make obvious stably expression short hairpin RNA molecules as claimed.

Combining Fire with Dietz and Kingsman does not remedy the deficiencies set out above regarding Fire. The Examiner states that Dietz teach allegedly "stable" expression of RNA inhibitory molecules. Dietz does not suggest any motivation to be combined with Fire. The mere teaching of stable expression does not remedy the issues applicants discuss above as to Fire. The Examiner also points to Kingsman. There is no disclosure of the many other claimed characteristics of the present invention in either Dietz or Kingsman. Furthermore, there is no motivation to combine the Kingsman document specifically with Fire or Dietz. The Examiner is using hindsight to fill in the missing gaps in Fire, namely **stable expression in a mammalian cell** sufficient to attenuate expression of the target gene.

In sum, applicants assert that the claims are not rendered obvious by the combination Fire, Dietz and Kingsman, that there is no motivation to combine these references, and that the evidence provided in the Declaration from Prof. Hernandez supports a finding of non-obviousness. Applicants respectfully request the Examiner to reconsider and withdraw this ground of rejection.

G. The Examiner Applies Improper Standards In Considering Evidence from the Declaration of Professor Hernandez

First, the Examiner has stated that the applicants rebuttal fails to overcome the rejection based on Fire because applicants "have provided no evidence that one of ordinary skill in the art, following the methods as set forth in the Fire '559 [sic] patent, ***would be unsuccessful*** at mediating RNAi in mammalian cells." (Emphasis added.) See Office Action top of page 9. There is no requirement under the patent law to show methods "would be unsuccessful" in order to rebut an obviousness rejection. On the contrary, applicants have provided evidence that a person of ordinary skill in the art, namely Prof. Hernandez, would not have found the claimed invention obvious in view of the cited art, and indeed, have found that the state of the art taught

away from the claimed invention. This is sufficient evidence of non-obviousness under the law and applicants request that the Examiner reconsider and withdraw this ground of rejection.

Second, the Examiner appears to require the Declaration of Prof. Hernandez to "***conclusively prove*** that one of ordinary skill in the art would not have expected to be able to use a dsRNA of 20-29 bps in an expression vector to mediate RNAi." (Emphasis added.) See page 10 of the Office Action. There is no such requirement in the patent law. The Declaration of Prof. Hernandez is submitted as evidence and should be considered as such. The Declaration is evidence of the view of a person of ordinary skill in the art at the time in view of the references cited by the Examiner and that make up the state of the art. The opinions of Prof. Hernandez are evidence in that she was a person of ordinary skill in the art at the time. There is not a requirement to "conclusively prove" anything, but rather a consideration of whether a person of ordinary skill in the art at the time would have had a reasonable expectation of success of carrying out the claimed invention. In this case, Prof. Hernandez has provided evidence that there was no reasonable expectation of success of carrying out the invention claimed here by Prof. Hannon and the other co-inventors. Indeed, it is the opinion of Prof. Hernandez that the references relied upon by the Examiner would have taught away from the claimed invention.

H. The Examiner Has Not Presented a *Prima Facie* Case of Obviousness

In making an obviousness rejection, the M.P.E.P. instructs that "Office personnel must first obtain a thorough understanding of the invention disclosed and claimed in the application under examination." M.P.E.P. § 2141.II.A. The Examiner has not done so, as evidenced from statements in the January 27, 2010 Office Action for child application Serial No. 11/894,676. Specifically, with respect to the Declaration of Professor Nouria Hernandez (attached and discussed below), the Examiner states: "Professor Hernandez argues that Elbashir *et al.* discourage the use of precursors, however the claims are not limited to the use of precursor RNA." This statement reflects a critical misunderstanding of the claimed invention.

The claimed method involves the attenuation of gene expression by introducing into a mammalian cell an expression vector encoding an shRNA. The shRNA comprises a double-stranded region of at least 20 but not more than 29 nucleotides; within the double-stranded region is region that is complementary to a portion of a target gene. According to the claimed

method, the vector encoding the shRNA is introduced into the mammalian cell, where it is stably expressed. As disclosed in the present application, the expressed short hairpin is then processed by Dicer in the cell to yield a small interfering RNA (siRNA). This concept is explained in various passages throughout the specification. See, for example, Paragraphs [0135], [0144], [0203], and Example, 12, which discusses the function of Dicer in shRNA processing. An shRNA is necessarily a precursor because it is cleaved by the cell's endogenous Dicer enzyme before it effects target gene attenuation. In other words, to trigger RNAi, the expressed short hairpin RNAs of the invention must first be processed to the siRNA forms that directly mediate gene silencing by acting as guide RNAs for sequence specific mRNA degradation. As discussed below, at the time of the invention, that such processing would take place and that expressed short hairpins could be used to achieve sequence-specific gene silencing was surprising and unexpected.

In *KSR International Co. v. Teleflex, Inc.*, 550 U.S. 398 (2007), the Supreme Court explicitly left undisturbed the framework for determining whether an invention is obvious as set forth by the Court in *Graham*. *KSR*, 550 U.S. at 415. *Graham* set forth several factual inquiries that form the background of an obviousness evaluation: (1) "the scope and content of the prior art;" (2) "differences between the prior art and the claims at issue;" and (3) "the level of ordinary skill in the pertinent art." *Graham*, 383 U.S. at 17. In addition to these factors, secondary factors such as unexpected results, failure of others, long-felt need, and commercial success can serve as objective criteria of non-obviousness. *Graham*, 383 U.S. at 17-18; *see* M.P.E.P. § 2141.II.

In determining the scope and content of the prior art, legal precedent dictates that one "cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." *In re Fine*, 837 F.2d 1071, 1075 (Fed. Cir. 1988). The Supreme Court recently reiterated the concept that hindsight analysis must be avoided in considering the issue of obviousness. *KSR*, 550 U.S. at 421 ("A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon ex post reasoning." (citing *Graham v. John Deere Co.*, 383 U.S. 1, 36 (1966))).

The M.P.E.P. directs that the focus in an obviousness determination "should be on what a person of ordinary skill in the pertinent art would have known at the time of the invention, and on what such a person would have reasonably expected to have been able to do in view of that

knowledge." M.P.E.P. § 2141.II. An examiner must support a rejection under 35 U.S.C. § 103 with a "clear articulation of the reason(s) why the claimed invention would have been obvious." M.P.E.P. § 2142. The examiner must provide factual support, not "mere conclusory statements." *Id.* (quoting *In re Kahn*, 441 F.3d 977, 978 (Fed. Cir. 2006).

In the present case, all the Examiner has provided is mere conclusory statements. Page 12 of the August 26, 2009 Office Action provides a laundry list of elements that Fire supposedly discloses or does not disclose, followed by a reference to two pieces of art that allegedly supply the missing elements. In ascertaining the differences between the prior art and the claims at issue, the fact finder must consider whether the invention as a whole would have been obvious. *See* M.P.E.P. § 2141.02.I. But the Examiner has not considered the invention as a whole; instead, she has picked and chosen elements in isolation and alleges that their combination renders obvious a method that is not taught or contemplated by the cited references. However, the Examiner has not articulated findings of fact sufficient to support the allegation that one of ordinary skill in the art would have understood from the teachings of Fire in combination with Dietz *et al.* and Kingsman *et al.* that target gene expression could be attenuated in mammalian cells in a sequence-specific manner by introducing an expression vector encoding an shRNA of a length that would not elicit a PKR response in the cell, which shRNA would be stably expressed in the cell and would effect RNAi.

Moreover, for a claimed invention to be obvious, one of ordinary skill in the art must have a reasonable expectation of success in making the allegedly obvious modifications to the prior art. *See* M.P.E.P. § 2143.02; *In re O'Farrell*, 853 F.2d 894, 904 (Fed. Cir. 1988); *Medichem, S.A. v. Rolabo, S.L.*, 437 F.3d 1157, 1165 (Fed. Cir. 2006). Here, the obviousness rejection relies on the notion that "Fire *et al.* disclose a method of attenuating expression of a target gene in mammalian cells." Office Action at 12 (Aug. 26, 2009). Notably, in a 1999 publication (attached), Fire himself expressed uncertainty as to whether the RNAi machinery was present in higher organisms, and if so, whether RNAi would be possible in mammalian cells in view of the PKR response:

> From a technical perspective, one could certainly hope that RNA-triggered silencing would exist in vertebrates: this would facilitate functional genomics and might allow medical application involving targeted silencing of "renegade" genes. Although this hope is not ruled out by any current data, the simple protocols used for invertebrate and plant systems are unlikely to be effective.

> Mammals have a vehement response to dsRNA, the best-characterized component of which is a protein kinase (PKR) that responds to dsRNA by phosphorylating (and inactivating) translation factor EIF2a. . . . Nonetheless, a recent report of co-suppression in mammalian cells, and the implication of RNA triggers with potentially double-stranded character in a number of natural genetic interference processes (X inactivation and imprinting) suggest the possibility that some components of RNA-triggered silencing machinery could be conserved from lower organisms.
>
> Even if the underlying mechanisms are absent in mammals, it is possible that RNA-triggered silencing will have clinical applications. In particular, the ability to silence essential parasite genes (thereby limiting a parasite infection) could be of great value. Of course, the dsRNA would have to be delivered so as to avoid harming the host. The PKR system (although non-essential for survival in mouse models) is sufficiently ubiquitous that interfering with it might be counterproductive. An alternative would be to find chemical modifications to the dsRNA that would still enable it to function in gene-specific interference (*e.g.* in a parasite), while not inducing the PKR response in the host.

Andrew Fire, "RNA-Triggered Gene Silencing," *Trends in Genetics* 15(9): 358, 363 (Sept. 1999) (internal citations omitted). Fire's own commentary demonstrates that at the time he filed his patent application, he had no reasonable expectation that the skilled practitioner would be able to successfully exploit use of RNAi in mammalian cells: Fire did not know whether RNAi-based gene silencing was generally possible in mammalian cells or how to achieve gene silencing without triggering a PKR response in those cells. The assertion that Fire renders obvious the achievement of the presently claimed invention merely because the patent provides a catalog of separate elements is untenable, in view of the actual disclosure in the cited references.

I. The Claims Are Not *Prima Facie* Obvious

Even if the Examiner were to make a *prima facie* case of obviousness, objective evidence of the knowledge of one of ordinary skill in the art at the time of the invention rebuts the allegation of obviousness. As additional evidence of the non-obviousness of the claimed invention, and in particular, as evidence that before the present invention, the skilled practitioner would have had no reasonable expectation of success in carrying out the presently claimed

methods, Applicants submit the attached Declaration by Professor Nouria Hernandez under 37 C.F.R. § 1.132 ("the Declaration").

At the time of the present invention, one of ordinary skill in the art would have understood from the teachings of Elbashir *et al.*, (2001) *Nature* 411:494-98, that RNAi is a process mediated by 21- and 22-nucleotide siRNAs generated from longer dsRNAs by a processing step within the cell. *See* Declaration of Professor Nouria Hernandez, ¶¶ 8-10. One of ordinary skill would have also known from the literature that in cases where the RNAi response was initiated by dsRNA, the ability of that dsRNA to be processed to an siRNA within the cell and allow that dsRNA to trigger an RNAi response was sharply dependent on the length of the dsRNA region. *See id.* at ¶¶ 9-10. For example, both *in vitro* and *in vivo* analysis of the length requirements of dsRNA had revealed that dsRNAs of fewer than 150 base pairs in length appeared less effective than longer dsRNAs, and in some cases ineffective, in their ability to degrade target mRNA. In particular, dsRNAs that were potentially short enough to avoid a PKR response in mammalian cells were observed to be ineffective in mediating RNAi.. *See* Elbashir *et al.* (2001) *Genes Dev.* 15:188-200; Bernstein *et al.* (2001) *Nature* 409:363-66; Declaration of Professor Nouria Hernandez, ¶¶ 9-11.

In view of the state of the art at the time, the skilled artisan would have had no motivation to employ RNA molecules comprising short dsRNA structures that must be processed within the cell to activate RNAi. There was no realization that the ineffectiveness of such molecules in mediating RNAi could have been overcome by expressing RNA molecules within the cell in the form of a hairpin structure, as taught and claimed in the present application. In particular, the skilled artisan would not have expected that an RNA hairpin having a double-stranded region of 20 to 29 nucleotides in length would undergo processing to an siRNA or would be effective in triggering sequence specific gene attenuation through RNAi. That solution, provided only by the present invention, was not suggested or addressed by the finding of Elbashir et al., which suggested that one could achieve gene silencing by using synthetic 21- and 22-nucleotide siRNA duplexes (mimicking a Dicer processed product) to bypass the dsRNA processing step. In fact, Elbashir et al. expressly suggests that short RNA hairpins would not trigger an RNAi response, reflecting a mechanism to avoid inadvertent activation of the RNAi by short hairpin structures formed within cellular RNAs: "the length dependence of 21-23 mer formation is likely to reflect

a mechanism to prevent the undesired activation of RNAi by short intramolecular base-paired structures of cellular RNAs." *See* Elbashir *et al.* (2001) *Genes Dev.* at 189.

As Dr. Hernandez states in Paragraph 19, it would not have been obvious to one of ordinary skill in the art at the time of the invention that one could attenuate target gene expression in a mammalian cell by introducing an expression construct encoding a short hairpin RNA molecule having a double-stranded region of 20-29 nucleotides. Indeed, according to Dr. Hernandez it was unexpected that the claimed method would result in effective target gene attenuation, and one of ordinary skill at the time of the invention would have had no reasonable expectation that it would do so. In fact, the prior art taught away from the method discovered and now claimed by the Applicants.

An examiner must consider evidence supporting non-obviousness. *See* M.P.E.P. §§ 2141.II, 2142, 2145. To that end, the Applicants emphasize that the Declaration is evidence, not argument, regarding how one of ordinary skill in the art would have viewed the state of the art based on the literature at the time of the invention. The Examiner must reconsider the obviousness rejection anew in the face of this evidence, as instructed by M.P.E.P. § 2145 ("Office personnel should not evaluate rebuttal evidence for its 'knockdown' value against the *prima facie* case, or summarily dismiss it as not compelling or insufficient." (citation omitted)), and by the courts:

> When prima facie obviousness is established and evidence is submitted in rebuttal, the decision-maker must start over. . . . Prima facie obviousness is a legal conclusion, not a fact. Facts established by rebuttal evidence must be evaluated along with the facts on which an earlier conclusion was reached, not against the conclusion itself.

In re Rinehart, 531 F.2d 1048, 1052 (C.C.P.A. 1976).

Here, the Declaration by one of ordinary skill in the art at the time of the present invention is evidence that one of ordinary skill in the art would have not expected to be able to use a short hairpin RNA comprising a double-stranded region consisting of 20-29 base pairs to attenuate target gene expression in mammalian cells, while avoiding a PKR response. As evidenced by the Declaration, that an expressed short hairpin could do so was unexpected in view of the state of the art at the time. "Usually, a showing of unexpected results is sufficient to overcome a *prima facie* case of obviousness." M.P.E.P. § 2145 (citing *In re Albrecht*, 514 F.2d 1389 (C.C.P.A. 1975). If the Examiner considers the rebuttal evidence insufficient, she must

"set forth the reasoning and facts that justify this conclusion." M.P.E.P. § 2145. In other words, the Examiner must do more than disagree with the declarant—she must provide factual evidence as to why the statements made by a person of ordinary skill in the art are incorrect.

In view of the foregoing, Applicants request reconsideration and withdrawal of the obviousness rejections.

CONCLUSION

Consideration of this paper, and early allowance of this application, is respectfully requested. If it would advance prosecution, the Examiner is invited to contact the undersigned to discuss the contents of this paper.

Dated: January 3, 2011

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Hannon et al.	Confirmation No.:	9352
Application No:	10/997,086	Art Unit:	1635
Filed:	November 23, 2004	Examiner:	K. CHONG
Title:	METHODS AND COMPOSITIONS FOR RNA INTERFERENCE		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT IN RESPONSE TO OCTOBER 28, 2011 NON-FINAL OFFICE ACTION

This Amendment is filed in response to the October 28, 2011 Non-Final Office Action for which a response was due January 28, 2012. Applicants request a two-month extension of time to March 28, 2012. Accordingly, this paper is being timely filed. The Commissioner is authorized to charge any fees due, or to credit any overpayment in fees, to Deposit Account No. 08-0219.

Claim Amendments begin on page 2.
Remarks begin on page 4.

AMENDMENT

In the Claims

Please amend the claims as follows, without prejudice. This listing of the claims will replace all prior versions and listings of claims in the application:

1-37. (Cancelled)

38. (Previously presented) A method for attenuating expression of a target gene in a mammalian cell, the method comprising:

introducing into a mammalian cell an expression vector comprising:

(i) an RNA polymerase promoter, and

(ii) a sequence encoding a short hairpin RNA molecule comprising a double-stranded region, wherein the double-stranded region consists of at least 20 nucleotides but not more than 29 nucleotides,

wherein the short hairpin RNA molecule is a substrate for Dicer-dependent cleavage and does not trigger a protein kinase RNA-activated (PK) response in the mammalian cell,

wherein the double-stranded region of the short hairpin RNA molecule comprises a sequence that is complementary to a portion of the target gene, and

wherein the short hairpin RNA molecule is stably expressed in the mammalian cell in an amount sufficient to attenuate expression of the target gene in a sequence specific manner, and is expressed in the cell without use of a PK inhibitor, whereby expression of the target gene is inhibited.

39. (Cancelled)

40. (Previously presented) The method of claim 38, wherein the expression vector further comprises LTR sequences located 5' and 3' of the sequence encoding the short hairpin RNA molecule.

41. (Cancelled)

42. (Previously presented) The method of claim 38, wherein the short hairpin RNA molecule comprises a double-stranded region consisting of at least 21 nucleotides.

43. (Previously presented) The method of claim 38, wherein the short hairpin RNA molecule comprises a double-stranded region consisting of at least 22 nucleotides.

44. (Previously presented) The method of claim 38, wherein the short hairpin RNA molecule comprises a double-stranded region consisting of at least 25 nucleotides.

45. (Previously presented) The method of claim 38, wherein the short hairpin RNA molecule comprises a double-stranded region consisting of 29 nucleotides.

46. (Currently amended) The method of claim 38, wherein the short hairpin RNA molecule has a total length of ~~about~~ 70 nucleotides.

47. (Previously presented) The method of claim 38, wherein the RNA polymerase promoter comprises a pol II promoter or a pol III promoter.

48. (Withdrawn) The method of claim 47, wherein the pol III promoter comprises a U6, an H1, or an SRP promoter.

49. (Previously presented) The method of claim 47, wherein the pol II promoter comprises a U1 or a CMV promoter.

50. (Cancelled)

51. (Cancelled)

REMARKS

I. STATUS OF THE CLAIMS AND FORMAL MATTERS

Claims 38, 40, and 42-49 are pending in this application. Claim 48 is withdrawn from consideration. Upon allowability of generic claim 38, Applicants request rejoinder of claim 48. Claim 46 is amended; claims 50 and 51 are cancelled. No new matter is added.

An Interview Summary regarding the in-person interview held on December 20, 2011 to discuss the outstanding Office Action was mailed by the Patent Office on January 9, 2012. The present paper further addresses the issues discussed at the in-person interview of December 20, 2011.

II. DOUBLE PATENTING REJECTION

The Examiner provisionally rejected claims 38, 40, 42-47, and 49-51 under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 50, 52, 54-60, and 62-64 of co-pending application Serial No. 11/894,676 ("the '676 application"). A Terminal Disclaimer accompanies this paper, obviating the double patenting rejection.

III. REJECTIONS UNDER 35 U.S.C. §112

The Examiner rejected claim 46 as allegedly being indefinite. Applicants have amended the claim to remove the word "about" in order to expedite prosecution of this application and without prejudice to pursue the subject matter in another application. Accordingly, Applicants request the Examiner to withdraw this ground of rejection.

IV. REJECTION OF CLAIMS UNDER 35 U.S.C. §103

The Examiner rejected claims 38, 40, and 42-51 as allegedly being obvious over Zamore ('995 patent), Symonds et al. (2002), Elbashir (2001), Good et al. (1997) and Noonberg et al. ('803 patent). This rejection was discussed in detail at the interview at the USPTO on December 20, 2011. The rebuttal points raised at the interview are summarized here. In particular, the Examiner relies on Zamore (USPN 7,691,995, issued from USSN 10/195,034 filed July 12, 2002) as a primary prior art reference under 35 U.S.C. 103(a).

A. Applicants Response

In response, Applicants traverse. The Zamore '995 patent itself, which has a filing date after the January 22, 2002 filing date of the present application, is not prior art under 35 U.S.C. §102(e) and therefore under 35 U.S.C. §103. The only disclosure of Zamore that is potentially available as prior art is limited to subject matter that is disclosed in the underlying Zamore '185 provisional application in compliance with 35 U.S.C. § 112, first paragraph. *See* MPEP 2136.03, III. Priority from Provisional Application Under 35 U.S.C. § 119(e)(emphasis added); *see also*, *Ex parte Yamaguchi*, 88 U.S.P.Q.2d 1606 (B.P.A.I. 2008). The Office Action fails to set forth factual findings identifying any such subject matter that would support a conclusion that the claimed invention as a whole would have been obvious, nor does it articulate a reasoned rationale for such a conclusion. *See* MPEP 2141, 2141.02.

As discussed below, the presently claimed method requires a number of critical elements: "a short hairpin RNA molecule comprising a double-stranded region wherein the double-stranded region consists of at least 20 nucleotides but not more than 29 nucleotides," which is "expressed in the cell without use of a PK inhibitor," and attenuation of target gene expression "in a sequence specific manner." None of these critical aspects is described or taught in the '185 provisional, either alone or in combination with any of the secondary references upon which the Office Action relies.

In particular, the Office Action relies upon four secondary references for the rejection under 35 U.S.C. § 103. All of these references were already considered in detail, along with rebuttal evidence, in one or both of the Declarations Under 37 C.F.R. §1.132 submitted in this case by Prof. Nouria Hernandez. This evidence was also discussed by Prof. Hernandez with the three participants from the USPTO (Examiner Chong, SPE Calamita and Ex. Celsa) in two prior interviews in connection with this application. Dr. Hernandez provided her written and oral statements as a person of skill in the art as of the effective filing date who, at that time, was familiar with the state of the art. Among other things, this evidence demonstrated that the skilled practitioner would have had no reasonable expectation of success in using the presently claimed methods to achieve sequence specific inhibition of a target gene without use of a PK inhibitor. *See* Declarations of Dr. Hernandez dated October 29, 2009 and January 4, 2011.

As discussed below, nothing in the '185 provisional provides any teaching or suggestion missing from the secondary references (either by itself or in combination) that would have

rendered the presently claimed methods obvious, including any teaching or suggestion that would have provided the skilled practitioner with a reasonable expectation of the success of the claimed methods. Thus, the Examiner cannot now continue to rely upon those references, and conclude the opposite—that they instead would have provided a reasonable expectation of success.

1. The Invention Claimed in the Present Application

Applicants previously provided a detailed discussion of the presently claimed methods as contrasted with the state of the art, and for more detail we refer the Examiner to previously filed papers, including the Amendment and Response to the July 2, 2010 Final Office Action, dated January 3, 2011, in particular, pages 5-12. The presently pending claims are directed to methods for achieving stable, long-term silencing of genes in mammalian cells by expressing a pre-Dicer RNAi trigger (precursor) without the use of a PK inhibitor. In particular, the pre-Dicer RNAi trigger is engineered in such a way (*i.e.*, in the form of a short hairpin RNA where the double stranded region consists of no more than 29 base pairs) that its expression in the cell does not invoke non-specific anti-viral responses, and therefore it is able to silence gene expression in a sequence-specific manner.

Before the invention of the presently claimed methods, the use of RNA interference to suppress expression of specific genes in mammalian cells having a PK response was limited. Use of post-Dicer triggers (siRNA as described in Elbashir (2001)) achieved only transient suppression. *See* Amendment and Response to the July 2, 2010 Final Office Action, dated January 3, 2011 at 8-9. On the other hand, expression of long hairpins required continued use of a PK inhibitor to inhibit general antiviral responses against double-stranded RNA. *See id.* at 8; U.S. Publication 2003/0084471 ¶¶ 0106, 0254, Example 8. The deleterious effects of these potent antiviral responses, including ultimately cell death via apoptosis, placed a significant limitation on the utility of this approach in mammalian cells. *See, e.g.*, U.S. Publication 2003/0084471 ¶¶ 0254; Paddison et al. at 948-49.

In contrast, by expressing a short hairpin RNA as presently claimed, that is, having a double-stranded region consisting of not more than 29 nucleotides, one could achieve sequence-specific suppression without use of PK inhibitors and at the same time avoid these deleterious effects. *See, e.g.*, U.S. Publication 2003/0084471 Example 6: "Generation of Short Hairpin dsRNA and Suppression of Gene Expression Using Such Short Hairpins;" Example 7: "Encoded

Short Hairpins Function in vivo" ("The specific suppression observed in HeLa cells in the presence of short dsRNAs is contrary to the non-specific effects observed when HeLa cells were treated with long dsRNAs and demonstrate that short dsRNAs do not provoke a non-specific PKR or PKR-like response.").

Evidencing the substantial advance that the presently claimed methods represented over the prior art, the Paddison et al. paper, in which the inventors published these methods, was after its publication among the most cited "high impact" papers in molecular biology and genetics. *See* Declaration Under 37 C.F.R. §1.131 of Gregory J. Hannon dated January 31, 2011 ¶¶ 33-36, Exhibits M-N. Commercial recognition of the value of Dr. Hannon's shRNA invention is further demonstrated by numerous prestigious awards Dr. Hannon received for his development of short hairpin RNA as a genetic tool. *See id.* at ¶¶ 37-38, Exhibits O-Q.

2. The Zamore '185 Provisional Application Does Not Disclose or Suggest Critical Aspects of the Invention

As referred to above, the '185 provisional application fails to describe or teach a number of critical elements required by the presently claimed method: "a short hairpin RNA molecule comprising a double-stranded region wherein the double-stranded region consists of at least 20 nucleotides but not more than 29 nucleotides," which is "expressed in the cell without use of a PK inhibitor" and attenuation of target gene expression "in a sequence specific manner."

The '185 provisional is instead directed to use of certain engineered RNA precursors (pre-siRNAs) to silence target genes in mammalian and other cells. These engineered precursors are expressly defined as molecules that are altered or modified from naturally occurring wild-type stRNA precursors (pre-stRNAs) by modifying or replacing portions of the nucleotide sequence of the wild-type stRNA. *See* '185 provisional, page 7, lines 5-7. ("Engineered RNA precursors (pre-siRNAs) are similar to naturally occurring pre-stRNAs, but are altered from the wild-type precursor sequences to promote their processing into duplex siRNAs rather than single-stranded stRNAs in vitro and in vivo.")

The same page explains specifically what such altering entails. Namely, one selects a desired 21 nucleotide sequence corresponding to a sequence that will hopefully be processed into a desired siRNA. To make the engineered precursor, this 21 nucleotide sequence is then used in place of (*i.e.*, it replaces) a 21 or 22 nucleotide portion of a duplex stem of the naturally occurring stRNA. *See* '185 provisional, page 7, line 23 - page 8, line 1. The resulting stem of

the engineered precursor will therefore be longer and consist of two portions, a 21 or 22 nucleotide stem portion that has been replaced with a selected siRNA sequence and a stem portion in the stRNA that has not been replaced. As discussed during the interview, the reference on page 7 at line 9 to a "stem portion" of 21 nucleotides in length therefore does not mean the total length of the duplex stem in the engineered precursor, which, as taught on page 7, must be longer.

Nowhere does the '185 provisional teach or suggest that one should shorten the length of the resulting duplex stem (or double-stranded region). In fact, the '185 provisional only teaches the opposite, *i.e.*, "introducing additional base-paired nucleotides to one or both of the stem portions of the natural pre-stRNA." '185 provisional, page 7, lines 11-15. (emphasis added). Indeed, the '185 provisional never describes the critical element of the presently claimed method that is essential for avoiding a non-sequence specific (PK) response in mammalian cells, *i.e.*, limiting the double-stranded region to no more than 29 base pairs.

The '185 provisional provides no guidance or suggestion as to how the engineered precursor should be designed to avoid such a response. The '185 provisional merely hypothesizes (it includes no data) that the engineered RNA precursors "as a defining feature" would not induce, or would induce a lower level of such sequence non-specific response "as a consequence of their length, sequence and/or structure." '185 provisional, page 8, lines 11-15. There is no description as to what such a defining feature is. The '185 disclosure provides no guidance as to which one (or more) of these variables (length, sequence and/or structure) would need to be manipulated, or how, to avoid a non-sequence specific (PK) response.

In this regard, the "19 to 22 nucleotide sequence" referred to on page 2 again does not mean the total length of the stem. It refers to the portion of the wild type stRNA stem that has been replaced with a selected siRNA sequence and is included in the entire stem of the engineered RNA precursor. *See* '185 provisional, page 2, lines 11-18 (An engineered RNA precursor includes "a first stem portion including a 19 to 22 nucleotide long sequence (although the portion can be longer) that is identical to a specific targeted gene . . . .")(emphasis added). The reference to "19" logically follows from the statement on page 7 that the "last two nucleotides of the 21 nucleotide sequence [of the selected siRNA sequence] will typically be UU." '185 provisional, page 7, line 30. These last two nucleotides would therefore not necessarily be identical to the target gene, in which case the selected siRNA sequence identical

to the target gene would be 19 nucleotides long (21 minus 2). In short, the description of the engineered precursor on page 2 does not describe or suggest any limit on the length of the stem or double stranded region.

Moreover, the '185 provisional does not disclose a method of attenuating gene expression in a sequence-specific manner without the use of a PK inhibitor. It instead teaches away from such a method. Since the '185 provisional teaches that the engineered precursors would in some cases induce a sequence non-specific response (and therefore would not achieve sequence-specific attenuation), to achieve sequence specific attenuation by following the '185 disclosure, the only evident approach would have been to use a PK inhibitor.

In sum, the '185 provisional fails to describe two crucial limitations of Hannon's method as presently claimed—use of a short hairpin RNA having a double-stranded region consisting of no more than 29 base pairs, and expressing that short hairpin RNA in a mammalian cell without use of a PK inhibitor. Accordingly, the '185 provisional does not reasonably convey to one skilled in the relevant art that the Applicants had possession of the invention as presently claimed (*i.e.*, described and enabled in compliance with 35 U.S.C. § 112, first paragraph). As such, neither the '995 patent, nor its corresponding published application, nor the underlying '185 provisional can qualify as prior art under § 102(e), or under § 103 as allegedly describing or suggesting these critical elements or rendering the presently claimed invention obvious.

3. The '185 Provisional Does Not Teach Use of "shRNA"

The Office Action contends that "Zamore et al. teach the use of shRNA for attenuating expression of a target gene wherein the shRNA consists of stem portions that are about 18 to about 40 or more nucleotides in length." Office Action at 5 There is no such language or range disclosed in the cited pages of the '185 provisional. Moreover, as discussed above, the "engineered RNA precursors (pre-siRNAs)" disclosed in the '185 provisional are not shRNAs as claimed in the present application. Indeed, the '185 provisional never describes the critical element of the presently claimed method, *i.e.*, a double-stranded region of no more than 29 base pairs. The Office Action improperly imports the language of the presently pending claims—shRNA—into the '185 provisional. The Office Action repeats the erroneous reference to "shRNA" on page 6, namely "Zamore et al. further teach the shRNA vector . . . and teach methods of mediating RNAi in cells using the shRNA constructs . . . ." The '185 provisional has no such disclosure.

4. The Critical Aspects of the Presently Claimed Method Were Added to the Zamore Application Only After the Publication of Paddison et al.

The Paddison et al. *Genes & Development* paper published in April 2002. In that paper, Dr. Hannon et al. reported much of the work underlying the presently claimed invention, including the unexpected and surprising finding, in view of the prior art, that short hairpins with a double-stranded region under 30 base pairs in length could mediate suppression of genes in mammalian cells and avoid inducing a harmful non-specific / anti-viral (PK) response. (*See, e.g.*, Amendment filed January 3, 2011 at 8-11; Second Declaration of Professor Nouria Hernandez Under 35 U.S.C. §1.132 ¶¶ 8-15).

Three months after the Paddison paper was published, and seven months after the effective filing date of the present application, Zamore et al. filed the '034 application. Only then did the Zamore applicants revise the definition of an engineered precursor and include key aspects of the presently claimed method that can be found in Paddison et al. For example, the '034 application added that "[w]hen used in mammalian cells, the length of the stem portions should be less than about 30 nucleotides to avoid provoking non-specific responses like the interferon pathway." *See* '995 patent at col. 6:45-49. The '185 provisional lacks any such disclosure.

In this regard, the conclusion that the Zamore application would have rendered the presently claimed methods obvious lacks a factual underpinning. While the '185 provisional expressly identifies reducing or eliminating the PK response as advantageous, it does not teach how to do so. It only hypothesizes (and states prophetically, without any data) that some feature of the engineered precursors would do so, but without any guidance as to whether that would involve its length, sequence or structure or instead some combination of these features.

Nonetheless, the Office Action contends that what that key feature was, how to modify that key feature to avoid the PK response, and how to achieve sequence-specific attenuation without using a PK inhibitor, would have all been obvious. Yet, despite the fact that the Zamore applicants at the time were among the most experienced scientists in the RNAi field, they failed to identify or disclose these key aspects in the '185 provisional. Rather, they disclosed them only in the '034 application and after Dr. Hannon, in Paddison et al., demonstrated that short hairpin RNAs with a double-stranded region under 30 base pairs in length could in fact

successfully mediate sequence-specific suppression of genes in mammalian cells, and without inducing a PK response.

5. Factual Findings that Improperly Rely on Incomplete Quotations from the '185 Provisional

In several places, the Office Action makes factual findings as to subject matter purportedly disclosed in the '185 provisional regarding the PK response, where the findings rely on partial quotes from the '185 provisional. However, omitting the full sentences substantively changes the meaning of the quoted sentences and renders the findings incorrect.

Among these, a partial quote on page 7 refers to the "…defining feature of these engineered RNA precursors is that as a consequence of their length, sequence, and/or structure, they do not induce sequence non-specific responses, such as induction of the interferon response…" The incomplete quote erroneously implies that the '185 provisional provided guidance as to how the engineered precursor should be designed to avoid such a response without use of a PK inhibitor. Instead, the omitted language ". . . or that they induce a lower level of such sequence specific responses than long, double-stranded RNA (> 150 bp) currently used to induce RNAi," illustrates that the Zamore applicants did not know how to modify the engineered precursor to avoid such a response, and thus allow sequence-specific attenuation without use of a PK inhibitor. The teaching, namely, that "[w]hen used in mammalian cells, the length of the stem portions should be less than about 30 nucleotides to avoid provoking non-specific responses like the interferon pathway" was only added after the publication of Paddison et al., demonstrating use of shRNA to achieve sequence specific attenuation in mammalian cells. *See* ''995 patent at col. 6:45-49.

6. The Evidence of Record Demonstrates That There was No Reasonable Expectation of Success and the Art Taught Away from the Claimed Invention as a Whole

A conclusion that a claimed invention as a whole would have been obvious cannot be sustained if the evidence of record demonstrates that there was no reasonable expectation of success. *See* MPEP 2143.02. "The question under 35 U.S.C. 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious. " MPEP 2141.02. If the record demonstrates there was no reasonable expectation of success in carrying out that invention, there can be no reasoned basis to modify the prior art to do so. *See* MPEP 2143.02.

The presently claimed method, as a whole, is directed to expressing an RNA precursor having a double-stranded region of 29 base pairs or less to specifically silence a target gene in a mammalian cell, without using a PK inhibitor. Here, the record includes substantial evidence addressing whether one of ordinary skill, before Hannon's invention, would have had a reasonable expectation that this method would work. It demonstrates there was no such expectation. Among this evidence, Prof. Hernandez, as a person of ordinary skill and familiar with the state of the art at the time of the invention, provided testimony, two declarations and other direct evidence regarding the state of the art, including published data in the field of the invention.

Taking into account all of the secondary references now cited in the Office Action, *i.e.*, Symonds, Elbashir, Good and Noonberg, Prof. Hernandez stated that a person of ordinary skill in the art would not have had a reasonable expectation of success. *See, e.g.*, entire First Declaration and Second Declaration ¶¶ 8-15 and 26-27. Prof. Hernandez explained in detail how the art at that time would have taught away and discouraged one of skill from using the presently claimed invention.

Among this literature, Prof. Hernandez referred to detailed experimental data in Elbashir et al. that "provide a factual basis for my conclusion as a person of ordinary skill in the art as of January 22, 2002." Notably, "Elbashir et al. disclosed negative results that would have caused one to expect that a short hairpin RNA with a double-stranded region consisting of 20-29bp in length (a) would not be processed to the 21 and 22-nt siRNA structures necessary to mediate RNAi and (b) would consequently be ineffective in mediating RNA" Second Declaration ¶ 10.

> In particular, among these results, the data in Elbashir et al. demonstrate a distinct negative linear correlation between the length of a dsRNA (from 500bp to 29bp) and its ability to act as an RNAi trigger (see Elbashir et al. Fig. 1). . . . Decreasing the length of the dsRNA to below 100bp resulted in a marked, approximately linear decrease in the effectiveness of the dsRNA as an RNAi trigger. In particular, as referred to in my first Declaration, shortening the length of the dsRNA to 30 or 29 bp completely eliminated the ability of the dsRNA to serve as an RNAi trigger. (Elbashir et al., Fig. 1). Notably, this lack of any RNAi activity for 29 and 30bp dsRNA was observed even under optimized conditions, using a 100:1 molar ration of dsRNA to target. (Elbashir et al., Fig. 1 and page 189, first column). In this regard, dsRNA of 39-bp in length or longer all appeared to be efficiently processed into the 21 and 22-nt (guide) siRNAs ultimately

> responsible for mediating cleavage of the target RNA (see Elbashir et al., Figs. 2 and 7). In contrast, 29 bp RNA was only slowly processed to such guide fragments, strongly suggesting that without efficient processing to yield sufficient siRNA product, the dsRNA would fail to act as an RNAi trigger.

Second Declaration ¶11

Regarding Elbashir, Prof. Hernandez emphasized, "it would have been backwards and contrary to the Elbashir paper's text for a person of ordinary skill in the art to interpret the negative results of Elbashir as providing any reasonable expectation that one could have achieved gene silencing by stably expressing a short hairpin RNA in mammalian cells." Second Declaration ¶15. Rather, "one of skill at the time would have understood these data to indicate that there was a critical minimal length requirement for dsRNA to be able to serve as RNAi triggers. The dsRNA would have to be long enough, i.e., over 30 bp in length to provide for enough production of guide RNAs to result in degradation of the target mRNA." Second Declaration ¶12.

Regarding Symonds, Prof. Hernandez explained how Symonds, including its two priority applications (the '731 and '733), not only fails to describe or suggest the presently claimed methods, or make such methods obvious (itself or in combination), it is directed to approaches (using Tat type or ribozyme-type structures) that are entirely different from and would have taught away from the presently claimed methods. *See* Second Declaration ¶¶28-43, in particular ¶¶30-31.

The '185 provisional application provides no data or experimental results. The examples are entirely prophetic. As an entirely prophetic document, the '185 provisional would not have altered the reasonable expectation of the skilled scientist, where that expectation was based on actual experimental data that taught away from the claimed invention and indicated that expressing a double-stranded RNA with a double-stranded region of 30 base pairs or less would fail to serve as an RNAi trigger. With nothing to change this expectation, one of skill would not have had any motivation to modify the methods taught in the '185 provisional to express an shRNA as presently claimed to attenuate target gene expression in a mammalian cell.

In this regard, the '185 provisional includes only a single, entirely prophetic, example relating to expressing an engineered precursor in a mammalian cell. Nothing in this example teaches or suggests modifying the engineered precursor to avoid a PK response. In fact, the

specific precursor the method teaches (that of Example 1 and Figure 2B) depicts a double-stranded region consisting of 31 base pairs, outside the range required by the instant Hannon claims and teaching away from the presently claimed methods.

B. In Asserting a Prima Facie Case of Obviousness, the Office Action Has Failed to Consider the Evidence of Record

1. The PTO Is Required To Consider All of the Evidence of Record

The MPEP instructs that "rejections on obviousness cannot be sustained with mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." MPEP 2142 (citations omitted). Regarding rejections under 35 U.S.C. § 103, the examiner bears the initial burden of factually supporting any prima facie conclusion of obviousness. To establish a *prima facie* case of obviousness, the PTO:

> (1) must consider any evidence supporting the patentability of the claimed invention, such as any evidence in the specification or any other evidence submitted by the applicant,
>
> (2) must provide sufficient evidence, based on the record as a whole, including evidence submitted by the applicant, to establish a *prima facie* case of obviousness by a preponderance of evidence,
>
> (3) must clearly articulate of the reason(s) why the claimed invention would have been obvious, making explicit the analysis supporting the rejection.

MPEP 2142.

The Examiner should consider all rebuttal arguments and evidence of record presented by applicants, including declarations and all evidence relating to secondary considerations of non-obviousness. *See* MPEP 2145, 716.01(a).

2. The PTO Provides No Countervailing Evidence and Zamore Provides No Actual Data

In alleging a *prima facie* case of obviousness, the Office Action improperly does not consider or discuss any of the above evidence (including declarations and evidence relating to secondary considerations of non-obviousness), which in the previous allowance was found persuasive in demonstrating the non-obviousness of the presently claimed methods. *See* MPEP 2145, 716.01(a). Moreover, the present Office Action has not provided any countervailing evidence or basis to discount the evidence provided by Prof. Hernandez of the lack of any

reasonable expectation of success. The '185 provisional application provides no data or experimental results and does not add to this evidentiary record.

In sum, Applicants assert that the claims are not rendered obvious by the combination of Zamore, Symonds, Elbashir, Good and Noonberg. The evidence provided in the First and Second Declarations from Prof. Hernandez supports a finding of non-obviousness. Applicants request the Examiner reconsider and withdraw this ground of rejection.

C. Rebuttal to Specific Factual Findings and Statements in the Office Action

In traverse of the pending rejection, Applicants respectfully include the following additional comments regarding specific factual findings made in the Office Action. *See* MPEP 2141.

1. The '185 Priority Application Does Not Disclose Using an Engineered Precursor with Less Than 30 Base Pairs to Avoid an Antiviral/PK Response

The Office Action, on page 5, includes a purported quote from the '185 provisional allegedly indicating that it teaches use of an engineered precursor with a double stranded region of less than 30 base pairs to avoid a PK response. However, the critical language quoted here, "[f]or example, the interferon response is triggered by dsRNA longer than 30 base pairs," does not appear in the '185 provisional. It was only added to the '034 non-provisional Zamore application after the Paddison et al. *Genes & Development* paper was published. As discussed above, there is no guidance in the '185 provisional for how to modify or alter the described engineered RNA precursor to avoid a non-sequence specific (PK) response in mammalian cells.

Regarding the stem of the engineered RNA precursor, the '185 provisional teaches only to lengthen the stem by "introducing additional base-paired nucleotides to one or both of the stem portions of the natural pre-stRNA." '185 provisional, page 7, lines 11-15 (emphasis added). However, the '185 provisional never refers to the length of the stem. The concept was added to the '034 application only after the Paddison et al. Genes & Development paper was published.

2. The Office Action in Alleging a Prima Facie Case of Obviousness Did Not Consider Rebuttal Evidence Already of Record

The Office Action appears to allege that "because it was well known in the art at the time of filing of the instant application that dsRNAs greater than 30 base pairs activated an unwanted

PKR response in cells" one of skill would have found it obvious to use an shRNA in a mammalian cell without use of a PK inhibitor, and therefore "in the absence of evidence to the contrary, the invention as a whole would have been prima facie [obvious]." Office Action at 7.

As discussed above, Applicants have already submitted extensive evidence on this very issue into the record, including two Rule 132 Declarations of Dr. Hernandez and published data reflecting the state of the art. As discussed above, such evidence demonstrated the opposite. For example: "As of January 22, 2002, a person of ordinary skill in the art would have had no reasonable expectation of success in carrying out sequence specific gene silencing by using an expression vector encoding a short hairpin RNA molecule having a double-stranded region consisting of 20-29 base pairs (bp). As discussed below, the references cited by the Examiner (along with the leading literature in the field) would have taught away from using an expressed short hairpin molecule, which to have gene silencing activity must first be processed in the cell." Second Declaration ¶¶ 8, 11-13. Nowhere, however, does the Office Action take this evidence into account.

In sum, the elements of the presently claimed invention, including the claimed structural features, describe a method one can use to achieve sequence specific attenuation of the target gene in a mammalian cell without use of a PK inhibitor. There is no disclosure of the claimed invention, or suggestion or motivation to practice the claimed invention, in the cited prior art references, either singly or in any combination. Applicants request reconsideration and withdrawal of the obviousness rejection.

CONCLUSION

Consideration of this paper and allowance of this application are requested. If it would advance prosecution, the Examiner is invited to contact the undersigned to discuss the contents of this paper.

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Respectfully submitted,

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