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(88) **Date of publication of the international search report**:
1 May 2014

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(54) **Title**: METHODS AND COMPOSITIONS FOR MODULATING GENE EXPRESSION USING COMPONENTS THAT SELF ASSEMBLE IN CELLS AND PRODUCE RNAI ACTIVITY

(57) **Abstract**: Compositions and methods for downmodulating expression of target nucleic acids are disclosed.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/34595

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/11; C12N 15/00 (2012.01)
USPC - 514/44A
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 514/44A

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/44A; 435/455
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,USOC,EPAB,JPAB); Google; PubMed
Search terms: AUUAAAU, PLS3-1657, seed, siRNA, RNAi, miRNA, sequential, administration, single-stranded, intracellular, duplex, boranophosphate, hairpin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2006/0069050 A1 (RANA) 30 March 2006 (30.03.2006) para [0008], [0042], [0033], [0044], [0054], [0056], [0058]-[0059], [0063], [0075], [0081], [0095], [0130]-[0132]; Fig. 2	1-5, 7-8, 10, 35-36
Y	Ui-TEI et al. Thermodynamic stability and Watson-Crick base pairing in the seed duplex are major determinants of the efficiency of the siRNA-based off-target effect. Nucleic Acids Res. December 2008 (12.2008), Vol. 36, No. 22, pages 7100-7109; pg 7100, para 1; pg 7102, para 2; pg 7105, para 2-3; Supplementary Table S1	1-5, 7-8, 10, 35-36
Y	US 2004/0014957 A1 (ELDRUP et al.) 22 January 2004 (22.01.2004) para [0111], [0119]	9, 36/9
A	MATRANGA et al. Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. Cell. 18 November 2005 (18.11.2005), Vol. 123, No. 4, pages 607-620	1-5, 7-10, 35-36

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 September 2012 (24.09.2012)	16 OCT 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/34595

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 7-10, and 35-36

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/34595

Continuation of:
Box NO III. Observations where unity of invention is lacking

Group I+: claims 1-25 and 35-41, drawn to a nucleic acid based compound which inhibits expression of a target sequence in a cell within a mammal, said nucleic acid based compound comprising at least one single stranded oligonucleotide modified to
i) increase nuclease resistance;
ii) bioavailability; and
iii) inhibitory activity within said cell;
said compound being deliverable in vivo in single stranded form in a vehicle, wherein said compound is selected from the group consisting of:
a) compounds shown in the Figures or
b) duplexes of the compounds of a),
said duplexes being formed intracellularly following sequential administration of single strand oligonucleotides in vivo, said duplexes being effective cause RNAi dependent silencing of expression of said gene product upon intracellular duplex formation;
said compounds exhibiting enhanced silencing activity in said target cell in vivo relative to nucleic acid compounds which lack said modifications. The first invention is restricted to the first compound of Fig. 2 comprising seqMiR PLS3-1657 seed sequence AUUAAAU (Claims 1-5, 7-10 and 35-36). Should an additional fee(s) be paid, Applicant is invited to elect an additional compound(s) or duplex(es) of the compounds to be searched. The exact claims searched will depend on Applicant's election. For example, the duplex of AUUAAAU can be selected as the second invention. Alternatively, Sequence with Seed Sequence Target Site that was Inserted into the Expression Vector of seqMiR PLS3-1657 UCGUGCUGAAAGUAUUUAAUUGA can be selected as the second invention.

Group II: claims 26-41 and 50-51, drawn to the use of a compound as claimed in claim 1, comprising a first and a second oligonucleotide for use in inhibiting expression of a target gene in a method of medical treatment, said method comprising the sequential administration of the first and second oligonucleotides such that the first oligonucleotide is taken up by a cell expressing the target gene before the second oligonucleotide, such that the first and the second oligonucleotides form a duplex intracellularly thereby triggering inhibition of gene target expression; wherein said first and/or said second oligonucleotides have been adapted to increase nuclease resistance.

Group III+: claims 42-49, drawn to an in vitro method of improving an RNAi effect in vivo against a target gene, said method comprising;
(i) obtaining a first and a second oligonucleotide sequence capable of forming a duplex intracellularly;
(ii) modifying said first and/or said second oligonucleotide sequence to increase its nuclease resistance;
(iii) contacting said first oligonucleotide sequence with a cell expressing the target gene;
(iv) following step (iii) contacting said second oligonucleotide sequence with a said cell and;
(v) determine the expression of the target gene as compare to the expression of the target gene without step (ii). The first invention is restricted to the first compound of Fig. 2 comprising seqMiR PLS3-1657 seed sequence AUUAAAU. Should an additional fee(s) be paid, Applicant is invited to elect an additional compound(s) or duplex(es) of the compounds to be searched. The exact claims searched will depend on Applicant's election. For example, the duplex of AUUAAAU can be selected as the second invention. Alternatively, Sequence with Seed Sequence Target Site that was Inserted into the Expression Vector of seqMiR PLS3-1657 UCGUGCUGAAAGUAUUUAAUUGA can be selected as the second invention.

The inventions listed as Groups I+ through III+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups I+-II do not include the inventive concept of an in vitro method of improving an RNAi effect in vivo against a target gene, as required by Group III.

The inventions of Group I+ and III+ do not include the inventive concept of the use of a compound as claimed in Claim 1, in a method of medical treatment, as required by Group II.

The inventions of Groups I-III share the technical feature of a compound claimed in Claim 1. However, this shared technical feature does not represent a contribution over prior art as being obvious over US 2006/0069050 A1 (Rana). Rana discloses a nucleic acid based compound which inhibits expression of a target sequence in a cell within a mammal (para [0008], the invention provides for the separate and temporal administration of single-stranded nucleic acids that are as effective as canonical (duplexed and annealed) siRNA agents for carrying out RNAi/gene silencing; para [0063]) said nucleic acid based compound comprising at least one single stranded oligonucleotide modified to
i) increase nuclease resistance (para [0075], modification to increase resistance of the siRNA molecules to nuclease);
ii) bioavailability (para [0075]; modification to improve the uptake of the siRNA by a cell) and
iii) inhibitory activity within said cell (para [0054], the first strand administered can also function as a priming agent and enhance the level of RISC or RNAi responsiveness of the cell, cell extract, or organism such that the second strand, when introduced, has improved effect; para [0042]),
said compound being deliverable in vivo in single stranded form in a vehicle (para [0056], liposome), wherein following sequential administration said duplexes being effective cause RNAi dependent silencing of expression of said gene product (para [0008], [0130]-[0132] and Fig. 2, these results indicate that double stranded siRNA agents can be administered separately as single-strands and over a period of time and still be substantially effective in RNAi/gene silencing of given target gene);
said compounds exhibiting enhanced silencing activity in said target cell in vivo relative to nucleic acid compounds which lack said modifications (para [0075], modification such that target efficiency is enhanced compared to a corresponding unmodified siRNA), but does not expressly teach that duplexes of the compounds being formed intracellularly following sequential administration of single strand oligonucleotides in vivo. However, one of ordinary skill in the art would have recognized that sequential administration of each strand of a double stranded siRNA agent into cells as taught by Rana (para [0131]) will result in duplex formation intracellularly due to the inherent properties of said sense and antisense strands which comprises complimentary nucleotide sequences. As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

----------continued in next Supplemental Box----------

Continuation of:
Box NO III. Observations where unity of invention is lacking

Another special technical feature of the inventions listed as Groups I+ and III+ is the specific compounds or duplexes of the compounds recited therein. The inventions do not share a special technical feature, because no significant structural similarities can readily be ascertained among compound or duplexes of the compounds. Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ through III+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.