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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

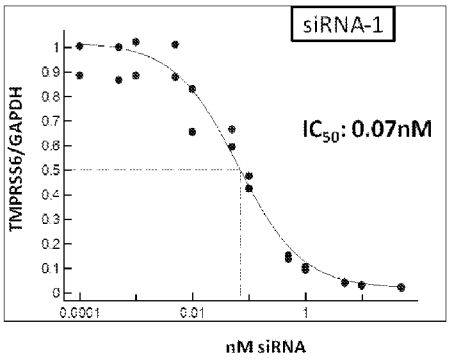
Published:

— *with international search report (Art. 21(3))*

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

(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF TMPRSS6 GENE

FIG. 2A



(57) Abstract: The disclosure relates to double-stranded ribonucleic acid (dsRNA) compositions targeting the TMPRSS6 (Transmembrane Protease, Serine 6) gene, and methods of using such dsRNA compositions to inhibit expression of TMPRSS6. Further disclosed are dsRNA sequences and pharmaceutical compositions comprising lipid formulation of the dsRNA and methods of using the dsRNA compositions for treating disorders associated with an increased expression of TMPRSS6.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/30786

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 48/00; C12N 15/11; A61K 9/127 (2012.01)
USPC - 514/44R; 514/44A; 424/450
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)
IPC(8) - A61K 48/00; C12N 15/11; A61K 9/127; C07H 21/04; C12N 15/00; C12N 5/00 (2012.01)
USPC - 514/44R; 514/44A; 424/450; 536/23.1, 536/24.5; 435/320.1; 435/325

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - A61K 48/00; C12N 15/11; A61K 9/127; C07H 21/04; C12N 15/00; C12N 5/00 (2012.01) - see keyword below
USPC - 514/44R; 514/44A; 424/450; 536/23.1, 536/24.5; 435/320.1; 435/325 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: TMPRSS6, transmembrane serine protease 6, Transmembrane Protease, Serine 6, mosaic serine proteinase, matriptase-2, siRNA, miRNA, dsRNA, siNA, dsNA, RNAi, double-stranded, antisence, modified, complementary, inhibit, expression, overhang, 2'-O-methyl, 5'-phosphorothioate, cholesteryl, 2'-deoxy-2'-fluo

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LAKHAL et al. Regulation of type II transmembrane serine proteinase TMPRSS6 by hypoxia-inducible factors: new link between hypoxia signaling and iron homeostasis. J Biol Chem. 2011 Feb, Vol. 286(6), p. 4090-7. Epub 2010 Oct 21. Entire documentation, especially Abstract; pg 4092, col 1, para 2; pg 4093, col 2, para 4; and pg 4094, col 2, para 3; and Fig 3C	1-10, 13-14, 31
Y	US 2009/0192104 A1 (MCSWIGGEN et al.) 30 July 2009 (30.07.2009), para [0006], [0008], [0024], [0033], [0034], [0035], [0037], [0053], [0058], [0062], [0063], [0079], [0081], [0090], [0092], [0118], [0198], [0276], [0294], [0308], [0312], and [0313]	1-10, 13-14, 31
Y	US 2007/0093443 A1 (MADISON et al.) 26 April 2007 (26.04.2007), pg 44, Table 14, SEQ ID NO: 283	1-10, 13-14, 31
A	NCBI_NM_153609, Homo sapiens transmembrane protease, serine 6 (TMPRSS6), mRNA. 18 November 2006 [online]. [Retrieved on 2012.05.30]. Retrieved from the Internet: <URL: http://www.ncbi.nlm.nih.gov/nuccore/56682967?sat=11&satkey=8411313> Definition; and Origin	1-10, 13-14, 31
A	US 2009/0191263 A1 (REICH et al.) 30 July 2009 (30.07.2009), para [0007], [0020], [0021], [0038], and [0040]	1-10, 13-14, 31

☐ 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
20 August 2012 (20.08.2012)	04 SEP 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 12/30786

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.: 21-26
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+, claims 1-15, 31, drawn to a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of TMPRSS6, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a TMPRSS6 transcript which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense sequences listed in Tables 2, 3 or 4. The first named invention (claims 1-10, 13-14, 31) is limited to wherein said dsRNA comprises at least one modified nucleotide. Applicant is invited to elect (an) additional modification(s) inclusing the dsRN A further comprising a ligand (claims 11-12), and/or a cell containing the dsRNA (claim 15) to be searched by paying additional fee for each election.

Group II, claims 16-18, drawn to a pharmaceutical composition for inhibiting expression of a TMPRSS6 gene comprising the dsRNA of claim 1, wherein the pharmaceutical composition further comprising a lipid formulation.
**********Continued in the extra sheet**********

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.:
1-10, 13-14, 31

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.
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Continuation of:
Box No III (unity of invention is lacking)

Group III, claims 19-20, drawn to a method of inhibiting TMPRSS6 expression in a cell, the method comprising: (a) introducing into the cell the dsRNA of claim 1; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TMPRSS6 gene, thereby inhibiting expression of the TMPRSS6 gene in the cell.

Group IV, claims 27-30, drawn to a vector encoding at least one strand of a dsRNA, wherein said dsRNA comprises a region of complementarity to at least a part of an mRNA encoding TMPRSS6, wherein said dsRNA is 30 base pairs or less in length, and wherein said dsRNA targets said mRNA for cleavage.

The inventions listed as Groups I+-IV 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:

Groups I- III do not include the inventive concept of a vector encoding at least one strand of a dsRNA, wherein said dsRNA targets said mRNA for cleavage, as required by Group IV.

Groups I-II and IV do not include the inventive concept of inhibiting TMPRSS6 expression in a cell comprising introducing into the cell a dsRNA, as required by Group III.

Groups I and III- IV do not include the inventive concept of a pharmaceutical composition for inhibiting expression of a TMPRSS6 gene comprising a dsRNA, as required by Group II.

Among Group I+, claims 1-10, 13-15 and 31 do not include the inventive concept of a dsRNA further comprising a ligand, as required by the claims 11-12; and claims 1-14 and 31 do not nclude the inventive concept of a cell comprising a dsRNA, as required by claim 15.

The inventions of Groups I+-IV share the technical feature of a dsRNA comprises a region of complementarity to at least a part of an mRNA encoding TMPRSS6 or a TMPRSS6 transcript; and Groups I+-III further share the technical feature of a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of TMPRSS6, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a TMPRSS6 transcript which comprises at least contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense sequences listed in Tables 2, 3 or 4. However, these shared technical feature do not represent a contribution over prior art as being obvious over an article entitled 'Regulation of type II transmembrane serine proteinase TMPRSS6 by hypoxia-inducible factors: new link between hypoxia signaling and iron homeostasis' by LAKHAL et al. (hereinafter 'Lakhal'; J Biol Chem. 2011 Feb, Vol. 286(6), p. 4090-7), in view of US 2009/0191263 A1 to REICH et al. (hereinafter 'Reich'), and further in view of US 2007/0093443 A1 to MADISON et al. (hereinafter 'Madison') as follows:

Lakhal discloses a siRNA for inhibiting expression of TMPRSS6 (pg 4093, col 2, para 4 - 'When cells were pretransfected with ... TMPRSS6 siRNA prior to hypoxia, the decrease in membrane HJV was abolished', indicating 'TMPRSS6 siRNA inhibits the expression of TMPRSS6', which leads to 'the decrease in membrane HJV was abolished' as 'hypoxia' increases TMPRSS6 expression; pg 4092, col 1, para 2 - 'Real-time PCR showed a significant increase in TMPRSS6 mRNA under hypoxia (Fig. 1A)'; Fig 1A - 'quantitative real-time PCR showing TMPRSS6 mRNA expression'; Abstract - 'TMPRSS6 is up-regulated ...by hypoxia ...This HIF-dependent up-regulation of TMPRSS6 increases membrane HJV shedding'; pg 4094, Fig 3, C).

Although Lakhal does not expressively teach wherein the siRNA is a double-stranded ribonucleic acid (dsRNA), wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a TMPRSS6 transcript, this is determined by the inherent property of siRNA for inhibiting target gene expression (please see Reich: para [0020] - 'isolated siRNA comprising short double-stranded RNA...The siRNA comprise a sense RNA strand and a complementary antisense RNA strand...the sense strand comprises a nucleic acid sequence which is substantially identical to a target sequence contained within the target mRNA', wherein the target mRNA can be TMPRSS6 transcript when the siRNA is for inhibiting TMPRSS6 expression; para [0038] - ' the antisense strand ...complementary to position 2 of the 23-nt sense strand ... is generally complementary to the targeted sequence'), and the inhibitory effect of TMPRSS6 siRNA disclosed by Lakhal (pg 4093, col 2, para 4; and pg 4092, col 1, para 2, as discussed above).

Lakhal does not specifically teach the dsRNA comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense sequences listed in Tables 2, 3 or 4. Reich discloses a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of a target gene (para [0020] - 'provides isolated siRNA comprising short double-stranded RNA'; para [0007] - 'siRNA-mediated RNAi is ... for inhibiting expression of a target gene'), wherein said dsRNA comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides for the target mRNA (para [0040] - 'The siRNA ... can be targeted to any stretch of approximately 19-25 contiguous nucleotides in any of the target mRNA sequences'; para [0021] - 'an siRNA ... comprise a sense strand comprise nucleic acid sequences which differ from a target sequence by one, two or three ... nucleotides, as long as RNAi-mediated degradation of the target mRNA is induced by the siRNA').

Reich also does not specifically teach one of the antisense sequences listed in Tables 2, 3 or 4. Madison discloses a TMPRSS6 transcript sequence (pg 44, Table 14, - Nucl AC NO: - NM_153609; SEQ ID NO: 283; please see NCBI Accession Number NM_153609: 'Homo sapiens transmembrane protease, serine 6 (TMPRSS6), mRNA', 18 November 2006 , Retrieved on 2012.05.30 from the Internet: URL: http://www.ncbi.nlm.nih.gov/nuccore/56682967?sat=11&satkey=8411313; see attachment: NCBI_NM_153609), which is 100% identical to SEQ ID NO: 1 of the application, and comprises all antisense sequences listed in Table 2 (Specification: pg 106-110, Table 2), such as wherein the sequence comprises a region between nucleotides 54-36, that is 100% identical to the claimed SEQ ID NO: 10 with the substitution of T (DNA form) with U (RNA form), the first antisense strand of a dsRNA listed in the Table 2.

**********Continued in the next extra sheet**********

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/30786

Continuation of:
Box No III (unity of invention is lacking)

(Continuation of the discussion for Groups I+-IV)
It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Lakhal, Reich, and Madison, to obtain a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of TMPRSS6, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a TMPRSS6 transcript, based on the teaching of Lakhal and the inherent property of siRNA, wherein the dsRNA comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense sequences listed in Tables 2, based on the combination of Madison, Reich, and Lakhal, in order to include different regions of TMPRSS6 mRNA as targets for generating dsRNA for inhibiting TMPRSS6 expression for achieving a desired effect on inhibiting upregulated TMPRSS6 mRNA expression associated with different disorders. Without a shared special technical feature, the inventions lack unity with one another.

The inventions of claim 15 of Groups I+ and claim 30 of Group IV further share the technical feature of a cell comprising a dsRNA comprising a region of complementarity to a TMPRSS6 transcript. However, this shared technical feature does not represent a contribution over prior art. Specifically, Lakhal further discloses a cell comprising dsRNA comprising a region of complementarity to a TMPRSS6 transcript (pg 4093, col 2, para 4 - 'When cells were pretransfected with ... TMPRSS6 siRNA', wherein siRNA is dsRNA and 'TMPRSS6 siRNA ' must 'comprising a region of complementarity to a TMPRSS6 transcript' for inhibiting TMPRSS6 expression, as discussed above; pg 4094, col 2, para 3 - 'the inhibition of these hypoxic effects by ... TMPRSS6 siRNA were also observed in wt-Hep3B cells'; pg 4092, col 1, para 2; Fig 1A; Please also see Reich: para [0007],[0020], [0038]). Without a shared special technical feature, the inventions lack unity with one another.

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

Note re item 4: Claims 21-26 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.