

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
7 October 2010 (07.10.2010)

PCT

(10) International Publication Number  
WO 2010/115202 A3

- (51) International Patent Classification:  
C07H 21/02 (2006.01)
- (21) International Application Number:  
PCT/US2010/029986
- (22) International Filing Date:  
5 April 2010 (05.04.2010)
- (25) Filing Language:  
English
- (26) Publication Language:  
English
- (30) Priority Data:  
61/166,578 3 April 2009 (03.04.2009) US  
61/166,559 3 April 2009 (03.04.2009) US  
61/174,306 30 April 2009 (30.04.2009) US  
61/174,279 30 April 2009 (30.04.2009) US  
61/257,820 3 November 2009 (03.11.2009) US  
61/257,810 3 November 2009 (03.11.2009) US

(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, RO, RS, RU, 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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(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

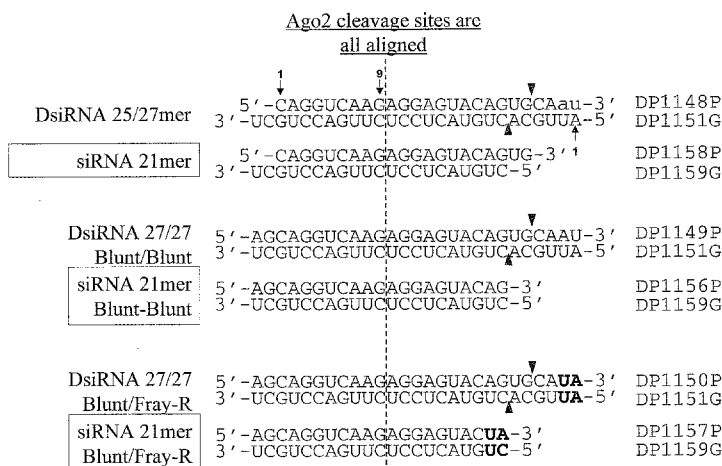
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Published:  
— with international search report (Art. 21(3))  
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(88) Date of publication of the international search report:  
9 December 2010

(54) Title: METHODS AND COMPOSITIONS FOR THE SPECIFIC INHIBITION OF KRAS BY BLUNT ENDED DOUBLE-STRANDED RNA

Figure 1



(57) Abstract: This invention relates to compounds, compositions, and methods useful for reducing KRAS target RNA and protein levels via use of blunt-ended Dicer substrate siRNA (DsiRNA) agents.

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## INTERNATIONAL SEARCH REPORT

PCT/US 10/29986

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - 07H 21/02 (2010.01)

USPC - 536/23.1, 514/44R

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) - 07H 21/02 (2010.01)

USPC - 536/23.1, 514/44R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST - DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ; Google Scholar

search terms: k-ras, kras, k-ras1, k-ras2, kras1, kras2, ki-ras1, ki-ras2, ki-ras, rask2, k-ras2a, k-ras2b, ma, interfer\$, double, duplex, loop, silenc\$, short, hairpin, administ\$, dose, dosage, treat\$, therap\$, effec\$, division, divid\$, prolifer\$, growth, apopto\$, death, huma

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---	US 2007/0088152 A1 (KHVOROVA et al.) 19 April 2007 (19.04.2007) para [0012]; [0069]; [0079]; [0088]; [0111]-[0121]; [0124]; [0128]; [0149]; [0221]; [0230]; [0231]; [0255]; [0259]-[0265]; [0271]; [0273]; [0282]-[0288]; [0338]; [0339]; Fig. 8a.	1-5, 9-27, 54-57, 66, 67, 70, 71
Y		6, 7, 28-32, 35-53, 58-65, 68, 69, 72, 73
Y	US 2002/0076696 A1 (KAWAGUCHI et al.) 20 June 2002 (20.06.2002) para [0040]; SEQ ID NO: 7.	6, 28-32, 35-53, 73
Y	US 2004/0063111 A1 (SHIBA et al.) 01 April 2004 (01.04.2004) para [0184]; SEQ ID NO: 1.	7
Y	US 2007/0254850 A1 (LIEBERMAN et al.) 01 November 2007 (01.11.2007) para [0007]; [0049]; [0078]; [0079]; [0162]; [0224]; Table 1.	58-65, 8, 69, 72

 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

"&amp;" document member of the same patent family

Date of the actual completion of the international search

29 September 2010 (29.09.2010)

Date of mailing of the international search report

13 OCT 2010

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

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PCT Helpdesk: 571-272-4300  
PCT OSP: 571-272-7774

**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:

Group I: Claims 1-7, 9-32 and 35-73, drawn to an isolated double stranded RNA comprising first and second nucleic acid strands, wherein the second strand is complementary to a target KRAS of SEQ ID NO:141, wherein the first strand comprises a sequence of SEQ ID NO:6, and wherein the second strand comprises a sequence of SEQ ID NO:11.

Group II+: Claims 1-32 and 35-73, drawn to an isolated double stranded RNA comprising first and second nucleic acid strands, wherein the second strand is complementary to a target KRAS sequence selected from the group consisting of SEQ ID NO:142-186, wherein the first strand comprises a sequence the group consisting of SEQ ID NO:9, 187, and 189-231, and wherein the second strand comprises a sequence selected from the group consisting of SEQ ID NO:12-50 and 135-140. If Applicant elects to have this group searched, Applicant must specify the specific sequences to be searched. Each set of sequences (the first and second strands of the double stranded RNA, and the targeted KRAS sequence) is an inventive concept. Please note that claim 8 will be search if the sequences that comprise each claimed pair of first strand/second strand sequences are selected as sequences to be searched.

---please see 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.: 1-7, 9-32 and 35-73, limited to SEQ ID NOs: 141, 6 and 11

**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.

Continuation of:

Box No. III Observations where unity of invention is lacking

Group III+: Claims 1-6 and 9-73, drawn to an isolated double stranded RNA comprising first and second nucleic acid strands, wherein the second strand is complementary to a target KRAS sequence selected from the group consisting of SEQ ID NO:141-186, wherein the first strand comprises a sequence the group consisting of SEQ ID NO:7, 10, 91-131, 133-134, and 188, and wherein the second strand comprises a sequence selected from the group consisting of SEQ ID NO:11-50 and 135-140. If Applicant elects to have this group searched, Applicant must specify the specific sequences to be searched. Each set of sequences is an inventive concept. Please note that claim 34 will be search if the sequences that comprise each claimed pair of first strand/second strand sequences are selected as sequences to be searched.

Group IV+: Claims 74-146, drawn to an isolated double stranded RNA comprising first and second nucleic acid strands, wherein the second strand is complementary to a target KRAS sequence selected from the group consisting of SEQ ID NO:1638-2341 and 4450-5152, wherein the first strand comprises a sequence the group consisting of SEQ ID NO:935-1637, 2341-3043, 3747-4449, and 5153-5855, and wherein the second strand comprises a sequence selected from the group consisting of SEQ ID NO:232-934 and 3044-3746. If Applicant elects to have this group searched, Applicant must specify the specific sequences to be searched. Each set of sequences is an inventive concept. Please note that claims 81 and 107 will be search if the sequences that comprise each claimed pair of first strand/second strand sequences are selected as sequences to be searched.

The inventions listed as Groups I, II+, III+, and 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:

The technical features shared by the groups listed as Groups I, II+, III+, and IV+ is a dsRNA comprising a first and second nucleic acid strand and a duplex region of 25 base pairs, wherein said dsRNA comprises blunt ends, wherein each of said first and second oligonucleotide strands consists of the same number of nucleotide residues, wherein said second oligonucleotide strand is sufficiently complementary to a target KRAS cDNA along at least 19 nucleotides and at most 35 nucleotides of said second oligonucleotide strand length to reduce KRAS target gene expression when said double stranded nucleic acid is introduced into a mammalian cell. This shared technical feature fails to provide a contribution over the prior art, as evidenced by US 2007/0088152 A1 to Khvorova et al. (published 19 April 2007). Khvorova et al. teaches a double stranded ribonucleic acid (dsRNA) comprising first and second nucleic acid strands and a duplex region of at least 25 base pairs (para [0271] - between 18-30 base pairs), wherein said dsRNA comprises blunt ends (para [0271] - overhangs are not necessarily present), wherein each of said first and second oligonucleotide strands consists of the same number of nucleotide residues (para [0291] - "The first nucleotide of the 5'-end of the sense strand is position 1, which corresponds to position 19 of the antisense strand for a 19-mer."), wherein said second oligonucleotide strand is sufficiently complementary to a target sequence along at least 19 nucleotides and at most 35 nucleotides (para [0271] - anti-sense strand is 100% complementary to 19 contiguous bases) of said second oligonucleotide strand length to reduce target gene expression (para [0112]) when said double stranded nucleic acid is introduced (para [0128]) into a mammalian cell (para [0069] - mammalian systems). Khvorova et al. also specifically teaches double stranded RNA directed against KRAS (Example XVIII, para [0339]) and teaches that mismatches may be present in the duplex region (para [0111]). In the absence of a contribution over the prior art, the shared technical feature is not a shared special technical feature. Without a shared special technical feature, the inventions lack unity with one another.

The special technical feature of each of the inventions listed as Groups I, II+, III+, and IV+ is the specific target KRAS cDNA sequence and first and second nucleic acid strands recited therein. Significant structural similarities cannot readily be ascertained among the sequences. Without significant structural similarities, the sequences do not have a shared special technical feature. In the absence of a shared special technical feature, the inventions lack unity with one another. In this case, the first named sequences that will be searched without additional fees are SEQ ID NOs: 141, 6 and 11. In order for more sequences to be examined, the appropriate additional examination fees must be paid and the desired sequences to be searched clearly identified.