

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
29 April 2010 (29.04.2010)

(10) International Publication Number  
**WO 2010/048228 A3**

PCT

(51) International Patent Classification:

*C12N 15/113* (2010.01) *C12N 15/11* (2006.01)  
*A61K 31/713* (2006.01) *C07H 21/02* (2006.01)

(21) International Application Number:

PCT/US2009/061381

(22) International Filing Date:

20 October 2009 (20.10.2009)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/106,956	20 October 2008 (20.10.2008)	US
61/115,738	18 November 2008 (18.11.2008)	US
61/156,670	2 March 2009 (02.03.2009)	US
61/185,545	9 June 2009 (09.06.2009)	US
61/242,783	15 September 2009 (15.09.2009)	US
61/244,794	22 September 2009 (22.09.2009)	US

(71) Applicant (for all designated States except US): **ALNYLAM PHARMACEUTICALS, INC.** [US/US]; 300 Third Street, Third Floor, Cambridge, MA 02142 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SAH, Dinah, Wen-ye** [US/US]; C/o Alnylam Pharmaceuticals, Inc., 300 Third Street, Third Floor, Cambridge, MA 02142 (US). **HINKLE, Gregory** [US/US]; C/o Alnylam Pharmaceuticals, Inc., 300 Third Street, Third Floor, Cambridge, MA 02142 (US). **ALVAREZ, Rene** [US/US]; C/o Alnylam Pharmaceuticals, Inc., 300 Third Street, Third Floor, Cambridge, MA 02142 (US). **MILSTEIN, Stuart** [US/US]; C/o Alnylam Pharmaceuticals, Inc., 300 Third Street, Third Floor, Cambridge, MA 02142 (US). **CHEN, Qingmin** [CN/US]; C/o Alnylam Pharmaceuticals, Inc., 300 Third Street, Third Floor, Cambridge, MA 02142 (US).

(74) Agents: **ULLSPERGER, Chris** et al.; Fenwick & West LLP, Silicon Valley Center, 801 California Street, Mountain View, CA 94041 (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, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, 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).

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:

16 September 2010

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

(57) Abstract: The invention relates to a double-stranded ribonucleic acid (dsRNA) targeting a transthyretin (TTR) gene, and methods of using the dsRNA to inhibit expression of TTR.



WO 2010/048228 A3

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2009/061381

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. C12N15/113 A61K31/713 C12N15/11  
ADD. C07H21/02

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)  
C12N

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 practical, search terms used)

EPO-Internal, WPI Data, Sequence Search

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>STEIN THOR D ET AL: "Neutralization of transthyretin reverses the neuroprotective effects of secreted amyloid precursor protein (APP) in APPSW mice resulting in tau phosphorylation and loss of hippocampal neurons: support for the amyloid hypothesis." THE JOURNAL OF NEUROSCIENCE : THE OFFICIAL JOURNAL OF THE SOCIETY FOR NEUROSCIENCE 1 SEP 2004 LNKD- PUBMED:15342738, vol. 24, no. 35, 1 September 2004 (2004-09-01), pages 7707-7717, XP002577897 ISSN: 1529-2401 figure 5</p> <p style="text-align: center;">----- -/--</p>	1-71

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

☐ See patent family annex.

\* 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 document 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

15 April 2010

Date of mailing of the international search report

26/07/2010

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040,  
Fax: (+31-70) 340-3016

Authorized officer

Romano, Alper

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2009/061381

**C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>KUROSAWA ET AL: "Selective silencing of a mutant transthyretin allele by small interfering RNAs"</p> <p>BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US LNKD-DOI:10.1016/J.BBRC.2005.09.142, vol. 337, no. 3, 25 November 2005 (2005-11-25), pages 1012-1018, XP005165808 ISSN: 0006-291X figures 1-5</p>	1-71
A	<p>ZIMMERMANN TRACY S ET AL: "RNAi-mediated gene silencing in non-human primates."</p> <p>NATURE 4 MAY 2006 LNKD- PUBMED:16565705, vol. 441, no. 7089, 4 May 2006 (2006-05-04), pages 111-114, XP002577898 ISSN: 1476-4687 the whole document</p>	19-71
A	<p>AKINC AKIN ET AL: "A combinatorial library of lipid-like materials for delivery of RNAi therapeutics."</p> <p>NATURE BIOTECHNOLOGY MAY 2008 LNKD-PUBMED:18438401, vol. 26, no. 5, May 2008 (2008-05), pages 561-569, XP002577899 ISSN: 1546-1696 the whole document</p>	19-71

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2009/061381

### 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:

see additional 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 an 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, 8, 22, 46(completely); 6, 7, 9-21, 23-45, 47-71(partially)

#### 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.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention: 1; Claims: 1-5, 8, 22, 46(completely); 6, 7, 9-21, 23-45, 47-71(partially)

A dsRNA targeting transthyretin (TTR) positions 518-536, its modified forms and lipid compositions thereof

---

Inventions: 2-166; Claims: 6, 7, 9-21, 23-45, 47-71(all partially)

A dsRNA targeting transthyretin (TTR) at other positions such as described in claims 6,11-13, its modified forms and lipid compositions thereof

---