



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/11, A61K 31/70, C07H 21/04	A3	(11) International Publication Number: WO 98/11211 (43) International Publication Date: 19 March 1998 (19.03.98)
(21) International Application Number: PCT/US97/16017 (22) International Filing Date: 10 September 1997 (10.09.97) (30) Priority Data: 08/711,568 10 September 1996 (10.09.96) US (71) Applicant: HYBRIDON, INC. [US/US]; 620 Memorial Drive, Cambridge, MA 02139 (US). (72) Inventor: AGRAWAL, Sudhir; 61 Lamplighter Drive, Shrews- bury, MA 01545 (US). (74) Agents: KEOWN, Wayne, A. et al.; Hale and Dorr LLP, 60 State Street, Boston, MA 02109 (US).	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims</i> <i>and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 16 April 1998 (16.04.98)	
(54) Title: METHOD FOR USING OLIGONUCLEOTIDES HAVING MODIFIED CpG DINUCLEOSIDES		
(57) Abstract		
<p>The invention relates to modified oligonucleotides that are useful for studies of gene expression and for the antisense therapeutic approach. The invention provides modified oligonucleotides that inhibit gene expression and that produce fewer side effects than conventional phosphorothioate oligonucleotides. In particular, the invention provides modified CpG-containing oligonucleotides that result in reduced splenomegaly and platelet depletion when administered to a mammal, relative to conventional CpG-containing phosphorothioate oligonucleotides. The invention further provides methods for using such oligonucleotides to modulate gene expression <i>in vivo</i>, including such use for therapeutic treatment of diseases caused by aberrant gene expression.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/16017

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 A61K31/70 C07H21/04

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 6 C12N A61K C07H

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 02555 A (UNIV IOWA RES FOUND) 1 February 1996 see page 8, line 18 - line 24 see page 10, line 22 - line 30 see table 1	1-4
Y	see page 21, line 28 - page 24, line 25 see claim 22	1-4
Y	---	
	ZHAO Q ET AL: "EFFECT OF DIFFERENT CHEMICALLY MODIFIED OLIGODEOXYNUCLEOTIDES ON IMMUNE STIMULATION" BIOCHEMICAL PHARMACOLOGY, vol. 51, no. 2, 26 January 1996, pages 173-182, XP000610208 cited in the application see the whole document ---	1-4
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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

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- *E* earlier document but published on or after the international filing date
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- *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

27 January 1998

Date of mailing of the international search report

05.03.98

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/16017

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 19572 A (HYBRIDON INC ;TANG JINYAN (US); ROSKEY ALLYSEN M (US); AGRAWAL SUD) 27 June 1996 see page 8, line 1 - line 11 see claims ---	2
X	KRIEG A M ET AL: "CPG MOTIFS IN BACTERIAL DNA TRIGGER DIRECT B-CELL ACTIVATION" NATURE, vol. 374, 6 April 1995, pages 546-549, XP000197060 cited in the application see the whole document ---	1-4
X	STEIN C A: "Phosphorothioate antisense oligodeoxynucleotides: questions of specificity" TRENDS IN BIOTECHNOLOGY, vol. 14, no. 5, May 1996, page 147-149 XP004035783 see page 149, left-hand column, paragraph 2 - right-hand column ---	1-4
A	BALLAS, Z. ET AL.: "Induction of NK activity in murine and human cells by CpG motifs in oligodeoxynucleotides and bacterial DNA" JOURNAL OF IMMUNOLOGY., vol. 157, 1 September 1996, BALTIMORE US, pages 1840-1845, XP002053416 see the whole document ---	1-4
A	SPROAT B S: "Chemistry and applications of oligonucleotide analogues" JOURNAL OF BIOTECHNOLOGY, vol. 41, no. 2, 31 July 1995, page 221-238 XP004036938 see the whole document ---	2
A	TORRENCE P F ET AL: "TARGETING RNA FOR DEGRADATION WITH A (2'-5')OLIGOADENYLATE-ANTISENSE CHIMERA" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 90, no. 4, February 1993, pages 1300-1304, XP000644528 see the whole document ---	2

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/16017

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	<p>AGRAWAL S: "Antisense oligonucleotides: towards clinical trials" TRENDS IN BIOTECHNOLOGY, vol. 14, no. 10, October 1996, page 376-387 XP004035728 see page 384, left-hand column, line 2 - page 386 see figures 6,7</p>	1-4
T	<p>--- ZHAO, Q. ET AL.: "Pattern and kinetics of cytokine production following administration of phosphorothioate oligonucleotides in mice" ANTISENSE & NUCLEIC ACID DRUG DEVELOPMENT., vol. 7, October 1997, NEW YORK US, pages 495-502, XP002053417 see the whole document</p>	1-4
T	<p>--- BOGGS, R. ET AL.: "Characterization and modulation of immune stimulation by modified oligonucleotides" ANTISENSE & NUCLEIC ACID DRUG DEVELOPMENT., vol. 7, October 1997, NEW YORK US, pages 461-471, XP002053418 see the whole document</p> <p>-----</p>	1-4

INTERNATIONAL SEARCH REPORT

Int. l. application No.
PCT/US 97/16017

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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:
see FURTHER INFORMATION sheet PCT/ISA/210

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 II Observations where unity of invention is lacking (Continuation of item 2 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 fee, this Authority did not invite payment of any additional fee.

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.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-4 (all partially)

A composition for inhibiting gene expression with reduced side-effects comprising an alkylphosphonate CpG, and therapeutic methods using it.

2. Claims: 1-4 (all partially)

A composition for inhibiting gene expression with reduced side-effects comprising an inverted CpG, and therapeutic methods using it.

3. Claims: 1-4 (all partially)

A composition for inhibiting gene expression with reduced side-effects comprising a 2'-O-substituted CpG, and therapeutic methods using it.

4. Claims: 1-4 (all partially)

A composition for inhibiting gene expression with reduced side-effects comprising a 5-methylcytosine CpG, and therapeutic methods using it.

5. Claims: 1-4 (all partially)

A composition for inhibiting gene expression with reduced side-effects comprising a stereospecific phosphorothioate CpG, and therapeutic methods using it.

6. Claims: 1-4 (all partially)

A composition for inhibiting gene expression with reduced side-effects comprising a phosphotriester CpG, and therapeutic methods using it.

7. Claims: 1-4 (all partially)

A composition for inhibiting gene expression with reduced side-effects comprising a phosphoramidate CpG, and therapeutic methods using it.

8. Claims: 1-4 (all partially)

A composition for inhibiting gene expression with reduced side-effects comprising a 2'-5' CpG, and therapeutic methods using it.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/16017

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 3 and 4 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/16017

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9602555 A	01-02-96	AU 1912795 A	16-02-96
		EP 0772619 A	14-05-97

WO 9619572 A	27-06-96	AU 4514696 A	10-07-96
		CA 2208528 A	27-06-96
		EP 0807171 A	19-11-97
