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(71) Applicant *(for all designated States except US)*: **NASTECH PHARMACEUTICAL COMPANY INC.** [US/US]; 3450 Monte Villa Parkway, Bothell, WA 98021-8906 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CHEN, Lishan** [US/—]; 13620 S.E. 43rd Place, Bellevue, WA 98006 (US). **CUI, Kunyuan** [US/US]; 3224-189th Street SE, Bothell, WA 98012 (US). **CHEN, Yuching** [—/US]; 13620 S.E. 43rd Place, Bellevue, WA 98006 (US). **MAYER, Sasha, J.** [CA/US]; 6011 174th St SE, Snohomish, WA 98296 (US).

(74) Agents: **KNUDSEN, Peter, J.** et al.; Nastech Pharmaceutical Company Inc., 3450 Monte Villa Parkway, Bothell, WA 98021-8909 (US).

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

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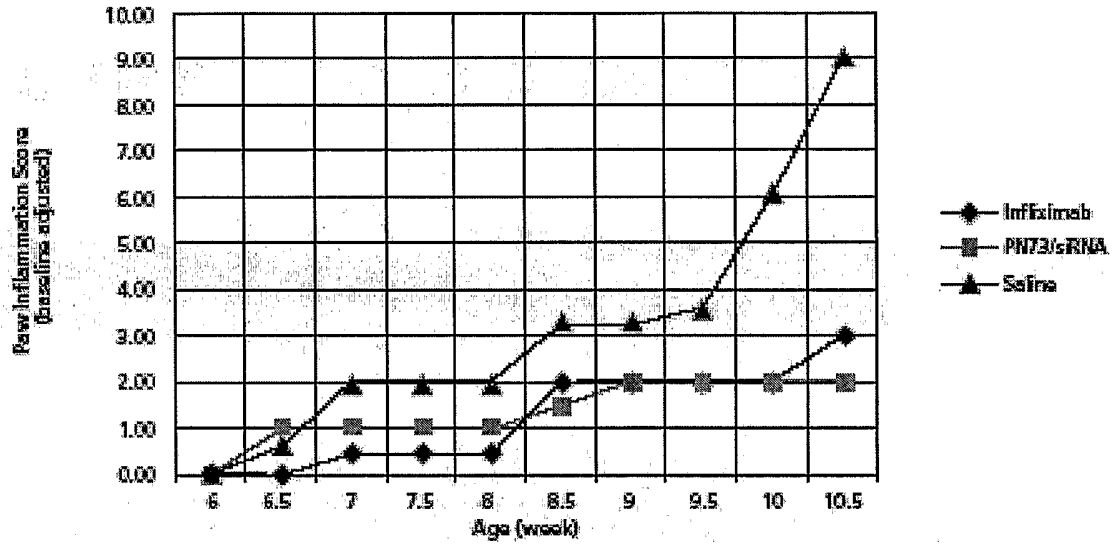
[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR ENHANCING DELIVERY OF NUCLEIC ACIDS INTO CELLS AND FOR MODIFYING EXPRESSION OF TARGET GENES IN CELLS



(57) Abstract: Polynucleotide delivery-enhancing polypeptides are admixed or complexed with, or conjugated to, nucleic acids for enhancing delivery the nucleic acids into cells. The transported nucleic acids are active in target cells as small inhibitory nucleic acids (siNAs) that modulate expression of target genes, mediated at least in part by RNA interference (RNAi). The siNA/polypeptide compositions and methods of the invention provide effective tools to modulate gene expression and alter phenotype in mammalian cells, including by altering phenotype in a manner that eliminates disease symptoms or alters disease potential in targeted cells or subject individuals to which the siNA/polypeptide compositions are administered.

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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)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

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

— *of inventorship (Rule 4.17(iv)) for US only*

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— *with international search report*

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/015574

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/87 C12N15/11

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, Sequence Search, BIOSIS, CHEM ABS Data, EMBASE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PUEBLA I ET AL: "A RECOMBINANT H1 HISTONE-BASED SYSTEM FOR EFFICIENT DELIVERY OF NUCLEIC ACIDS" JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 105, no. 3, 2003, pages 215-226, XP009057520 ISSN: 0168-1656 figures 1-6	1-6, 11-21, 26-36, 41-53, 58-70, 75-87
X	WO 2004/007721 A (UNIVERSITY OF OTAGO; ECCLES, ROGER, MICHAEL; MURATOVSKA, ALEXANDRA) 22 January 2004 (2004-01-22) the whole document figure 1	1-6, 11-21, 26-36, 41-53, 58-70, 75-87

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[X] Further documents are listed in the continuation of Box C.

[X] 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	Date of mailing of the international search report
8 September 2006	20/10/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Petri, Bernhard

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/019008 A1 (LEWIS DAVID L ET AL) 29 January 2004 (2004-01-29) the whole document paragraph [0012]	1-6, 11-21, 26-36, 41-53, 58-70, 75-87
X	MURATOVSKA A ET AL: "Conjugate for efficient delivery of short interfering RNA (siRNA) into mammalian cells" FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 558, no. 1-3, 30 January 2004 (2004-01-30), pages 63-68, XP004488270 ISSN: 0014-5793 the whole document figure 1	1-6, 11-21, 26-36, 41-53, 58-70, 75-87
A	WO 03/070897 A (RIBOZYME PHARMACEUTICALS, INCORPORATED; MCSWIGGEN, JAMES; BEIGELMAN, L) 28 August 2003 (2003-08-28) the whole document page 83, line 10 - line 11	1-6, 11-21, 26-36, 41-53, 58-70, 75-87
A	SORENSEN D R ET AL: "Gene Silencing by Systemic Delivery of Synthetic siRNAs in Adult Mice" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 327, no. 4, 4 April 2003 (2003-04-04), pages 761-766, XP004454177 ISSN: 0022-2836 the whole document figure 3	1-6, 11-21, 26-36, 41-53, 58-70, 75-87
A	HEBERT ERIC: "Improvement of exogenous DNA nuclear importation by nuclear localization signal-bearing vectors: A promising way for non-viral gene therapy?" BIOLOGY OF THE CELL (PARIS), vol. 95, no. 2, 2003, pages 59-68, XP002358850 ISSN: 0248-4900 the whole document	1-6, 11-21, 26-36, 41-53, 58-70, 75-87

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/015574

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEN J ET AL: "GALACTOSYLATED HISTONE-MEDIATED GENE TRANSFER AND EXPRESSION" HUMAN GENE THERAPY, vol. 5, 1994, pages 429-435, XP000199161 ISSN: 1043-0342 the whole document table 1	1-6, 11-21, 26-36, 41-53, 58-70, 75-87
X	WO 03/106491 A (CEPEP AB; HAELLBRINK, MATTIAS; POOGA, MARGUS; METSIS, MADIS; KOGERMAN,) 24 December 2003 (2003-12-24) the whole document example 15 page 85, line 4 - line 5 figure 37 page 85, line 17 - line 24 page 36	1-7, 11-22, 26-37, 41-54, 58-71, 75-87
X	FERNANDEZ-CARNEADO JIMENA ET AL: "Amphipathic peptides and drug delivery" BIOPOLYMERS, vol. 76, no. 2, 8 March 2004 (2004-03-08), pages 196-203, XP002398047 ISSN: 0006-3525 the whole document page 197, right-hand column, paragraph 3	7,22,37, 54,71
X	SIMEONI F ET AL: "Insight into the mechanism of the peptide-based gene delivery system MPG: implications for delivery of siRNA into mammalian cells" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 31, no. 11, 13 March 2003 (2003-03-13), pages 2717-2724, XP002984580 ISSN: 0305-1048 the whole document page 2718, left-hand column, paragraph 2 page 2718, left-hand column, paragraph 4 - right-hand column, paragraph 1 figure 3 page 2720, right-hand column, last paragraph - page 2721, right-hand column, paragraph 1	7,22,37, 54,71
P,X	WO 2004/048545 A (UNIVERSITY OF MASSACHUSETTS; RANA, TARIQ, M) 10 June 2004 (2004-06-10) page 12 the whole document	7,22,37, 54,71

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/015574

Box 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. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 64-79 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.

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 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 fee, this Authority did not invite payment of any additional fee.

3. [X] 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.:

Invention 1: Claims 1-6, 11 (in part), 12 (in part), 13-21, 26 (in part) 27 (in part), 28-36, 41 (in part), 42 (in part), 43-53, 58 (in part) 59 (in part), 60-70, 75 (in part), 76 (in part), 77-87; Invention 6: 7, 22 37, 54, 71

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.

[X] 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 1-6, 11 (in part), 12 (in part), 13-21, 26 (in part), 27 (in part), 28-36, 41 (in part), 42 (in part), 43-53, 58 (in part), 59 (in part), 60-70, 75 (in part), 76 (in part), 77-87

Methods and compositions comprising a polynucleotide delivery-enhancing polypeptide and a double stranded nucleic acid, wherein the polynucleotide delivery-enhancing peptide comprises Histone H2B, compositions mixtures, and methods relating thereto.

Invention 2: Claims 11-12 (in part), 26-27 (in part), 41-42 (in part), 58-59 (in part), 75-76 (in part)

Methods and compositions comprising a polynucleotide delivery-enhancing polypeptide and a double stranded nucleic acid, wherein the polynucleotide delivery-enhancing peptide comprises Histone H3, compositions mixtures, and methods relating thereto.

Invention 3: Claims 11-12 (in part), 26-27 (in part), 41-42 (in part), 58-59 (in part), 75-76 (in part)

Methods and compositions comprising a polynucleotide delivery-enhancing polypeptide and a double stranded nucleic acid, wherein the polynucleotide delivery-enhancing peptide comprises Histone H4, compositions mixtures, and methods relating thereto.

Invention 4: Claims 11-12 (in part), 26-27 (in part), 41-42 (in part), 58-59 (in part), 75-76 (in part)

Methods and compositions comprising a polynucleotide delivery-enhancing polypeptide and a double stranded nucleic acid, wherein the polynucleotide delivery-enhancing peptide comprises Histone H4, compositions mixtures, and methods relating thereto.

Invention 5: Claims 11-12 (in part), 26-27 (in part), 41-42 (in part), 58-59 (in part), 75-76 (in part)

Methods and compositions comprising a polynucleotide delivery-enhancing polypeptide and a double stranded nucleic acid, wherein the polynucleotide delivery-enhancing peptide comprises Histone H1, compositions mixtures, and methods relating thereto.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention 6: Claims 7, 22, 37, 54, 71

Methods and compositions comprising a polynucleotide delivery-enhancing polypeptide and a double stranded nucleic acid, wherein the polynucleotide delivery-enhancing peptide comprises an amphipathic amino acid, compositions mixtures, and methods relating thereto.

Invention 7: Claims 8, 23, 38, 55, 72

Methods and compositions comprising a polynucleotide delivery-enhancing polypeptide and a double stranded nucleic acid, wherein the polynucleotide delivery-enhancing peptide comprises a transduction domain or motif, compositions mixtures, and methods relating thereto.

Invention 8: Claims 9, 24, 39, 56, 73

Methods and compositions comprising a polynucleotide delivery-enhancing polypeptide and a double stranded nucleic acid, wherein the polynucleotide delivery-enhancing peptide comprises a fusogenic peptide domain or motif, compositions mixtures, and methods relating thereto.

Invention 9: Claims 10, 25, 40, 57, 74

Methods and compositions comprising a polynucleotide delivery-enhancing polypeptide and a double stranded nucleic acid, wherein the polynucleotide delivery-enhancing peptide comprises a DNA-binding domain or motif, compositions mixtures, and methods relating thereto.

Invention 10-127: Claim 11 (in part), 26 (in part), 41 (in part), 58 (in part), 75 (in part)

as invention 1, however wherein the polynucleotide delivery enhancing polypeptide is the 4th peptide in table 2, the 5th peptide in table 2, the 12th peptide in table 2, the 14th peptide in table 2 ... , the last peptide in table 8, respectively.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2005/015574

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004007721 A	22-01-2004	AU 2003281078 A1	02-02-2004
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