



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| (51) International Patent Classification ⁵ : C12N 15/11, 9/00, A61K 31/70 C12Q 1/68 // A61K 48/00 | A3 | (11) International Publication Number: WO 93/22434 (43) International Publication Date: 11 November 1993 (11.11.93) |
| (21) International Application Number: PCT/US93/03961 (22) International Filing Date: 28 April 1993 (28.04.93) (30) Priority data: 875,099 28 April 1992 (28.04.92) US 931,937 18 August 1992 (18.08.92) US (71) Applicant: YALE UNIVERSITY [US/US]; 451 College Street, New Haven, CT 06511 (US). (72) Inventors: YUAN, Yan ; 550 Whitney Avenue, Apartment 2, New Haven, CT 06511 (US). GUERRIER-TAKADA, Cecilia, L. ; 57 Ramsdell Street, New Haven, CT 06515 (US). ALTMAN, Sidney ; 71 Blake Road, Hamden, CT 06517 (US). | (74) Agents: PABST, Patrea, L. et al.; Kilpatrick & Cody, 1100 Peachtree Street, Suite 2800, Atlanta, GA 30309-4530 (US). (81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 23 December 1993 (23.12.93) | |
| (54) Title: TARGETED CLEAVAGE OF RNA USING EUKARYOTIC RIBONUCLEASE P AND EXTERNAL GUIDE SEQUENCE | | |
| (57) Abstract <p>It has been discovered that any RNA can be targeted for cleavage by RNAase P from eukaryotic cells, for example, human HeLa cells, using a suitably designed oligoribonucleotide ("external guide sequence", or EGS) to form a hybrid with the target RNA, thereby creating a substrate for cleavage by RNAase P <i>in vitro</i>. There are two classes of EGS that can target RNA for cleavage by human RNAase P. These are prepared by transcription from a small DNA fragment that contains a sequence coding for an RNA that can assume a tRNA-like secondary structure. In the first class, the structure lacks at least the first thirteen nucleotides from the 5' terminus of the tRNA-like sequence, the anticodon stem and loop, the variable loop or part of the variable loop. The most efficient EGS with human RNAase P is the EGS in which the anticodon stem and loop was deleted. In the second class, the EGS has changes in both the equivalent of the T-loop and the anticodon stem of the tRNA-like segment of the EGS. Methods are also disclosed to randomly select and to express a suitable EGS <i>in vivo</i> to make a selected RNA a target for cleavage by the host cell RNAase P, thus preventing expression of the function of the target RNA. The methods and compositions should be useful to prevent the expression of disease-causing genes <i>in vivo</i>.</p> | | |

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/03961

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| I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶ | |
| According to International Patent Classification (IPC) or to both National Classification and IPC | |
| Int.Cl. 5 C12N15/11; //A61K48/00 | C12N9/00; A61K31/70; |
| | C12Q1/68 |
| II. FIELDS SEARCHED | |
| Minimum Documentation Searched ⁷ | |
| Classification System | Classification Symbols |
| Int.Cl. 5 | C12N ; A61K ; C12Q |
| Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸ | |
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| III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ | | |
|---|---|-------------------------------------|
| Category ¹⁰ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
| X | WO,A,9 203 566 (YALE UNIVERSITY) 5 March 1992 cited in the application see page 10, line 6 - page 11, line 11 see example 2 see page 21, line 13 - page 23, line 7 see claims; figures --- | 1, 4, 9-13, 19-24 |
| A | SCIENCE vol. 249, 17 August 1990, LANCASTER, PA US pages 783 - 786 FORSTER, A. & ALTMAN, S. 'External guide sequences for an RNA enzyme' see the whole document --- | 1-24 |
| | -/-- | |

¹⁰ Special categories of cited documents:

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| <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> | <p>"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</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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.</p> <p>"A" document member of the same patent family</p> |
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| IV. CERTIFICATION | |
|---|---|
| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
| 08 SEPTEMBER 1993 | 12 -11- 1993 |
| International Searching Authority | Signature of Authorized Officer |
| EUROPEAN PATENT OFFICE | ANDRES S.M. |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) | | |
|--|--|-----------------------|
| Category ° | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
| A | PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 89, 15 April 1992, WASHINGTON US pages 3185 - 3189 LI, Y. ET AL. 'Targeted cleavage of mRNA in vitro by RNase P from Escherichia coli' cited in the application see Discussion | 1-24 |
| P,X | ----- PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 89, September 1992, WASHINGTON US pages 8006 - 8010 YUAN, Y. ET AL. 'Targeted cleavage of mRNA by human RNase P' see the whole document ----- | 1-9 |

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:
Remark : Although claim 13-24 as far as in vivo methods are concerned 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 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:

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.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9303961
SA 73826

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
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08/09/93

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|--|------------------|-------------------------|------------------|
| WO-A-9203566 | 05-03-92 | US-A- 5168053 | 01-12-92 |
| | | AU-A- 8627391 | 17-03-92 |
| | | CA-A- 2088917 | 18-02-92 |
| | | EP-A- 0546092 | 16-06-93 |
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