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(71) Applicant: **BOEHRINGER INGELHEIM INTERNATIONAL GMBH** [DE/DE]; Binger Strasse 173, 55216 Ingelheim Am Rhein (DE).

(72) Inventors: **FLORIN, Lore**; Boehringer Ingelheim GmbH, Corporate Patents, Binger Strasse 173, 55216 Ingelheim Am Rhein (DE). **KAUFMANN, Hitto**; Boehringen Ingelheim GmbH, Corporate Patents, Binger Strasse 173, 55216 Ingelheim am Rhein (DE). **HAUSSER, Angelika**; Universitaet Stuttgart, Institut fuer Zellbiologie und Immunologie, Keplerstrasse 7, 70174 Stuttgart (DE). **OLAYIOYE, Monilola**; Universitaet Stuttgart, Institut fuer Zellbiologie und Immunologie, Keplerstrasse 7, 70174 Stuttgart (DE). **STROTBEK, Michaela**; Universitaet Stuttgart, Institut fuer Zellbiologie und Immunologie, Keplerstrasse 7, 70174 Stuttgart (DE).

(74) Agent: **DÖRRIES, Ulrich, H.**; Fünf Höfe, Theatinerstraße 16, 80333 Munich (DE).

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— *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))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
30 January 2014

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(54) Title: CELL ENGINEERING USING RNAs

(57) Abstract: The invention concerns the field of cell culture technology. It concerns RNA having a specific sequence, expression vectors encoding said RNA, production host cell lines comprising said RNA, and methods of producing recombinant biopharmaceutical products using engineered host cell with altered levels of said RNAs, such as small non-coding RNAs, preferably micro RNAs (mi RNAs). The invention also relates to engineered host cells with altered levels in one or more of said RNAs. Those cell lines have improved secretion and/or growth characteristics in comparison to control cell lines.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/061465

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 C12N15/11 C12P21/02
ADD.

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 C12P

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)
EPO-Internal, BIOSIS, EMBASE, 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	WO 2010/124231 A2 (THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM [US] COREY; YUE) 28 October 2010 (2010-10-28) page 7 - page 8; figure 8a page 19 - paragraph 69 page 27 - page 28 page 64; sequence 12 ----- -/--	1-31, 39-45, 48-50, 53,55-57

[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 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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 August 2013	05/12/2013

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, Fax: (+31-70) 340-3016	Macchia, Giovanni

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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.
A	BOUDREAU RYAN L. ET AL.: "Rapid Cloning and Validation of MicroRNA Shuttle Vectors: A Practical Guide", 2011, RNA INTERFERENCE TECHNIQUES HUMANA PRESS INC, 999 RIVERVIEW DR, STE 208, TOTOWA, NJ 07512-1165 USA SERIES : NEUROMETHODS (ISSN 0893-2336 (PRINT)), XP008164332, ISSN: null pages 19-37, the whole document -----	1-30, 39-45
A	WO 2008/015662 A1 (DUBLIN CITY UNIVERSITY [IE]; GAMMELL P [IE]; BARRON N. [IE]; CLYNES M.) 7 February 2008 (2008-02-07) the whole document -----	1-57
A	BARRON N.; KUMAR N.; SANCHEZ N.; DOOLAN P.; CLARKE C.; MELEADY P.; O'SULLIVAN F.; CLYNES M.: "Engineering CHO cell growth and recombinant protein productivity by overexpression of miR-7", J BIOTECHNOL., vol. 151, no. 2, 20 January 2011 (2011-01-20), pages 204-11, XP028171088, cited in the application the whole document -----	1-57

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/061465

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010124231 A2	28-10-2010	EP 2421972 A2	29-02-2012
		US 2010273863 A1	28-10-2010
		WO 2010124231 A2	28-10-2010
WO 2008015662 A1	07-02-2008	AU 2007280004 A1	07-02-2008
		CA 2658301 A1	07-02-2008
		CN 101541972 A	23-09-2009
		EP 2046970 A1	15-04-2009
		JP 2009545309 A	24-12-2009
		US 2010190258 A1	29-07-2010
		WO 2008015662 A1	07-02-2008

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/061465

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   [X] on paper

   [X] in electronic form

   b. (time)

   [X] in the international application as filed

   [X] together with the international application in electronic form

   [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/061465

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.:

1-57(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:

1. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:6, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

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2. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:1, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

---

3. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:2, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

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4. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:3, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

---

5. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:4, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

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6. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:5, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

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7. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:7, wherein said RNA leads to an increase in the production and/or secretion of a

therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

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8. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:8, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

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9. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:9, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

---

10. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:10, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

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11. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:11, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

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12. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:12, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

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13. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:13, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.

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14. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:14, wherein said

RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.
---

15. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:15, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.
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16. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:16, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.
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17. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:17, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.
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18. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:18, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.
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19. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:19, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.
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20. claims: 1-57(partially)

A ribonucleic acid (RNA) as in SEQ ID NO:20, wherein said RNA leads to an increase in the production and/or secretion of a therapeutic protein of interest from a mammalian cell. Expression vectors, cells, methods and uses related thereto.
