Title: MIRNA FOR TREATMENT OF BREAST CANCER

Abstract: The presently-disclosed subject matter relates to RNA-based composition and method to treat breast cancer in a subject. More particularly, the presently disclosed subject matter relates to a RNA nanostructure and composition containing a multiple branched RNA nanoparticle, a breast cancer targeting module, and an effective amount of a breast cancer therapeutic agent. Further, the presently disclosed subject matter relates to a method of using the RNA nanoparticle composition to treat breast cancer in a subject having or at risk of having breast cancer.
###INTERNATIONAL SEARCH REPORT

**International application No.:** PCT/US16/21451

####A. CLASSIFICATION OF SUBJECT MATTER

**1Pc**(8) - C12N 15/11 15, 15/13; A61K, 47/26, 47/48 (2016.01)

**CPC** - A61K 47/4892, 31/704; C12N 15/11, 15/11 1, 15/11 137

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(8) Classifications: C12N 15/1 15, 15/1 13; A61K 49/00, 47/26, 47/46; C12P 19/30; C07H 1/00; A61K 47/4892, 31/704; C12N 15/11, 15/11 1, 15/11 137

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)
PatisPeer (US, EP, WO); USPTO Web Page; Google; Google Scholar; EBSCO: Entrez Pubmed; NCBI BLAST; Lens.org, ENA; Search terms - 'branched RNA', nanostructure, ligand, aptamer, miRNA, siRNA, 'zeta potential', Her2, EGFR, folicule, mirR-21, anti-mir-R-21

####C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 2014/0179578 A1 (GUO, P.) June 24, 2014; abstract; paragraphs [0018], [0025], [0067], [0096], [0101], [0110], [0113], [0121], [0173J. [0145], [0154], [0155], [0162], [0173], [0194], [0195], [0236], [0238], [0315], [0335])</td>
<td>1-6, 12-16, 22-42</td>
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<td>Y</td>
<td>US 2008/0213177 A1 (RADEMACHER, TW et al.) September 4, 2008; abstract; paragraphs [0001], [0021], [0038], [0045], [0046], [0063], [0065], [0069], [0074]-[0077])</td>
<td>1-6, 12-16, 22-42</td>
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<tr>
<td>Y</td>
<td>US 2010/0203142 A1 (ZHANG, L et al.) August 12, 2010; paragraphs [0014], [0050], [0156])</td>
<td>15, 16</td>
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<td>Y</td>
<td>US 2009/0143262 A1 (OBAD, S et al.) June 4, 2009; paragraphs [0005], [0231], [0271], [0272], [0329], [0384], [0436], [0468], [0479]) SEQ ID NO: 2</td>
<td>35-40</td>
</tr>
</tbody>
</table>

- 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 the cited document. "L" means "Longer" and "S" means "Shorter"
  - "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 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.
  - "V" document of particular relevance; the claimed invention cannot be considered novel or 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: 25 July 2016 (25.07.2016)
- Date of mailing of the international search report: 26 AUG 2016
- Name and mailing address of the ISA/Authorized officer: Shane Thomas
  - Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
  - P.O. Box 1450, Alexandria, Virginia 22313-1450
  - Facsimile No: 571-273-8300
  - Authorized officer: Shane Thomas
  - PCT Helpdesk: 571-272-4300
  - PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)
**INTERNATIONAL SEARCH REPORT**

International application No. PCT/US16/21451

<table>
<thead>
<tr>
<th>Box No. II</th>
<th>Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)</th>
</tr>
</thead>
</table>

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.: 43-51** because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

<table>
<thead>
<tr>
<th>Box No. III</th>
<th>Observations where unit of invention is lacking (Continuation of item 3 of first sheet)</th>
</tr>
</thead>
</table>

This International Searching Authority found multiple inventions in this international application, as follows:

- "Continued Within the Next Supplemental Box."-

| 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 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.: 1-7, 12-16, 22-42; SEQ ID Nos: 5, 10, 14

**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.

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2015)
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups Ia, Claims 1-42 and SEQ ID NOs: 5, 10 and 11 are directed toward an artificial RNA nanostructure molecule, comprising: a multiple branched RNA junction motif comprising at least one RNA oligonucleotides, and a breast cancer targeting module coupled to the RNA junction motif.

The artificial RNA nanostructure will be searched to the extent that it encompasses SEQ ID NO: 5 (first exemplary branched RNA sequence); a ligand encompassing SEQ ID NO: 10 (first exemplary ligand sequence), and an anti-miRNA encompassing SEQ ID NO: 11 (first exemplary anti-miRNA sequence). Applicant is invited to elect additional branched RNA and/or anti-miRNA sequence(s), with specified SEQ ID NO: for each, to be searched. Additional branched RNA and/or anti-miRNA sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1-7, 12-16, 22-39, 40 (in-part), 41 and 42 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 5 (branched RNA sequence); SEQ ID NO: 10 (ligand sequence), and SEQ ID NO: 11 (anti-miRNA sequence). Applicants must specify the claims that encompass any additionally elected branched RNA and/or anti-miRNA sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "a" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a branched RNA sequence encompassing SEQ ID NO: 1 (first exemplary elected branched RNA sequence).

No technical features are shared between the branched RNA sequences of Groups Ia and, accordingly, these groups lack unity a priori. No technical features are shared between the anti-miRNA sequences of Groups Ia and, accordingly, these groups lack unity a priori.

Groups Ia share the technical features including: an artificial RNA nanostructure molecule, comprising: a multiple branched RNA junction motif comprising at least one RNA oligonucleotides, and a breast cancer targeting module coupled to the RNA junction motif.

However, these shared technical features are previously disclosed by WO 2010/148085 A1 to The United States of America, as Represented by the Secretary, Department of Health and Human Services et al. (hereinafter "HHS") in view of US 2013/0202679 A1 to Petrenko et al. (hereinafter "Petrenko").

HHS discloses an artificial RNA nanostructure molecule (an artificial RNA nanostructure molecule; abstract, page 4, lines 22-26), comprising: a multiple branched RNA junction motif (comprising: a multiple branched RNA junction motif; page 4, lines 22-26) comprising at least one RNA oligonucleotide (comprising at least one RNA oligonucleotide; page 4, lines 22-30, Figure 8), a targeting module coupled to the RNA junction motif (a targeting agent (module) coupled to the RNA junction motif; page 33, lines 24-26); and treatment of breast cancer (treatment of breast cancer; page 12, line 30 - page 13, line 3; page 39, line 26 - page 40, line 4).

HHS does not disclose a breast cancer targeting module.

Petrenko discloses targeted particles (targeted particles; abstract), targeted to breast cancer cells (targeted to breast cancer cells; paragraph [0006]).

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of HHS to have provided a targeting module, as disclosed by HHS, for targeting nanoparticles to breast cancer cells, as disclosed by Petrenko, in order to better enable specific delivery of a therapeutic agent to the target cells in order to provide effective treatment for breast cancer, as disclosed by HHS.

Since none of the special technical features of the Groups Ia inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the HHS and Petrenko references, unity of invention is lacking.