Abstract: Methods for treating systemic lupus erythematosus and other autoimmune conditions by using microRNA-31 compositions are disclosed. Methods of modifying interleukin-2 (IL-2) expression are also provided, comprising administering a subject a composition that modifies microRNA-31 or RhoA expression.
**INTERNATIONAL SEARCH REPORT**

**INTERNATIONAL APPLICATION**

International application No. PCT/US 12/44197

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(8) - A61K 31/7088; C12N 15/1 13 (2012.01)

USPC - 514/44A

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) - A61K 31/7088; C12N 15/1 13 (2012.01)

USPC - 514/44A

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)

PatBase; Google Scholar

Search terms: systemic lupus erythematosus, lupus, T cell, T lymphocyte, interleukin-2, IL-2, IL2, miRNA-31, miRNA31, microRNA-31, microRNA31, miR-31, miR31, RhoA, Ras homolog gene family member A, flaire

**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>X</td>
<td>CRISPIN et al., Transcriptional regulation of IL-2 in health and autoimmunity prevailing. Autoimmun. Rev.; January 2009; Vol. 8, No. 3; pages 190-5. Especially p 190, col 1, para 2; p 191, Table 1; p 193, col 2, para 6</td>
<td>1-2, 6-7, 9, 17</td>
</tr>
<tr>
<td>A</td>
<td>CRISPIN et al., &quot;Novel molecular targets in the treatment of systemic lupus erythematosus&quot; Autoimmun. Rev.; January 2008 (published online 4 December 2007); Vol. 7, No. 3; pages 256-61</td>
<td>1-2, 6-7, 9, 17</td>
</tr>
<tr>
<td>A</td>
<td>W O 03/070744 A1 (MCOSWIGGEN et al.) 28 August 2003 (28.08.2003) para [0017], [0165], [0229]</td>
<td>1-2, 6-7, 9, 17</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

* 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 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
- "a" document member of the same patent family

Date of the actual completion of the international search

20 November 2012 (201.1.2012)

Date of mailing of the international search report

Π DEC 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer: Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/2 10 (second sheet) (July 2009)
INTERNATIONAL SEARCH REPORT

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.: 8, 10-16 and 18-25
   - 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:

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.

Group I: Claims 1-2, 6-7, 9, and 17, drawn to methods of increasing IL-2 expression.

Group II: Claims 3 and 6-7, drawn to methods of treating systemic lupus erythematosus comprising administering a composition that increases expression of microRNA-31 or decreases expression of RhoA.

Group III: Claims 4-7 and 9, drawn to methods of decreasing RhoA expression.

Group IV: Claims 26-35, drawn to methods of diagnosing and treating systemic lupus erythematosus comprising determining the expression of microRNA-31 or RhoA —please see extra 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 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.: 1-2, 6-7, 9, and 17

Remarks 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.
Observations where unity of invention is lacking

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The shared technical feature of the inventions listed as Groups I-IV is systemic lupus erythematosus and its association with IL-2 expression in T cells, wherein said IL-2 expression is modulated by microRNA-31 or RhoA. This shared technical feature fails to provide a contribution over the prior art, as evidenced by the article entitled Transcriptional regulation of IL-2 in health and autoimmunity* by Crispin et al. (published in Autoimmun Rev, January 2009, Vol 8, No 3, pages 190-5; hereinafter ‘Crispin’). Crispin discloses systemic lupus erythematosus as an autoimmune disease that is marked by T cells that fail to produce normal amounts of IL-2 upon activation (p190, col 1, para 1 - "failure to produce normal amounts of IL-2 upon activation is considered a hallmark of T cells from patients with systemic lupus erythematosus (SLE), a chronic autoimmune disease"). Crispin further teaches that IL-2 expression in T cells is inhibited by RhoA (p 191, col 2, para 2 - "RhoA, a GTPase known by its role in cytoskeletal rearrangement, was shown to inhibit IL-2 production in Jurkat and primary T cells by interfering with NFAT activity"); p 191, Table 1 - "RhoA"). In the absence of a contribution over the prior art, the shared technical feature is not a shared special technical feature.

The shared technical feature of the inventions listed as Groups I-III is the administration of a composition that increases expression of microRNA-31 or decreases expression of RhoA. The shared technical feature of the inventions listed as Groups II and IV is the treatment of systemic lupus erythematosus. These shared technical features are obvious over Crispin, which discloses that RhoA inhibits the expression of IL-2 expression in T cells (p 191, col 2, para 2; p 191, Table 1) and that abnormally low IL-2 expression in T cells is a hallmark of systemic lupus erythematosus (p 190, col 1, para 1). It would have been obvious to one of ordinary skill in the art in view of Crispin’s teachings to administer a composition to decrease expression of RhoA in order to minimize the inhibitory effect of RhoA on IL-2 expression and thereby treat a subject who has systemic lupus erythematosus. In the absence of a contribution over the prior art, the shared technical features are not shared special technical features.

Further, the special technical feature of the inventions listed as Group I is the method step of increasing IL-2 expression. This special technical feature is not shared by the inventions of Groups I-II-IV. The special technical feature of the inventions listed as Group II is the method step of treating systemic lupus erythematosus by administering a composition that increases expression of microRNA-31 or decreases expression of Rho A. This special technical feature is not shared by the inventions of Groups I and III-IV. The special technical feature of the inventions listed as Group III is the method step of decreasing RhoA expression by increasing expression of microRNA-31. This special technical feature is not shared by the inventions of Groups I-II and IV. The special technical feature of the inventions listed as Group IV is the method step of determining the expression of microRNA-31 or RhoA. This special technical feature is not shared by the inventions of Groups I-III.

Unity of invention exists only when the same or corresponding technical feature is shared by the claimed inventions. Without a shared special technical feature, the inventions of Groups I-IV lack unity with one another.