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(54) Title: COMPOSITIONS AND METHODS FOR ANTIBODIES TARGETING STAPHYLOCOCCAL LEUKOTOXINS

(57) Abstract: The disclosure is directed to antibodies that bind to *Staphylococcus aureus* bi-component leukotoxins and/or gamma-he-
molysins. Other aspects of the disclosure are directed to pharmaceutical compositions and diagnostic kits containing the leukotoxin and
hemolysin antibodies, and therapeutic and diagnostic methods utilizing the leukotoxin and hemolysin antibodies.



WO 2017/205377 A3

INTERNATIONAL SEARCH REPORT

International application No.

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A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - C07K 16/12, 14/705, 14/31 (2017.01)
 CPC - C07K 16/1271, 14/31, 2317/76; G01N 33/56938

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)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015/091935 A2 (Arsanis Biosciences GmbH) 25 June 2015 (25.06.2015). Especially pg 2 ln 17-18, sheet 1 fig 1; claim 1	1-6, (48-54)/(1-6)
A	WO 2015/031578 A1 (Abbvie, Inc.) 5 March 2015 (05.03.2015). Especially para [0008], SEQ ID NO: 1	1-6, (48-54)/(1-6)
A	US 2014/0294765 A1 (Compugen, Inc.) 2 October 2014 (02.10.2014). Especially SEQ ID NO: 218	1-6, (48-54)/(1-6)
A	US 2008/0025913 A1 (Bowdish et al.) 31 January 2008 (31.01.2008). Especially SEQ ID NO: 28	1-6, (48-54)/(1-6)
A	US 2010/0068199 A1 (Liang et al.) 18 March 2010 (18.03.2010). Especially SEQ ID NO: 21	1-6, (48-54)/(1-6)
A	US 2015/0274814 A1 (Torres et al.) 1 October 2015 (01.10.2015). Especially para [0048], [0052], claim 72	9-14, 17-24, (48-54)/(9-14, 17-24)
A	WO 2015/200522 A2 (Integrated Biotherapeutics Inc.) 30 December 2015 (30.12.2015). Especially SEQ ID NO: 12	9-14, (48-54)/(9-14)
A	US 2013/0251733 A1 (Youd et al.) 26 September 2013 (26.09.2013). Especially SEQ ID NO: 38	17-24, (48-54)/(17-24)



Further documents are listed in the continuation of Box C.



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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/34009

Box No. 1 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, the international search was carried out on the basis of a sequence listing:

- a. ☐ forming part of the international application as filed:
 - ☐ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13ter. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
 - ☒ in the form of an Annex C/ST.25 text file (Rule 13ter. 1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter. 1(b) and Administrative Instructions, Section 713).

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

3. Additional comments:

GenCore SEQ ID NOs: 177, 191, 195, 196, 197, 336, 410

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/34009

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.: 55-66
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:

-----Go to Extra Sheet for continuation-----

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.:
see Extra Sheet
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 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.

INTERNATIONAL SEARCH REPORT

International application No.

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----continuation of Box III (Lack of Unity of Invention)-----

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 1-8, 48-54 (in part), drawn to an antibody or binding portion thereof that binds *Staphylococcus aureus* Leukocidin AB.

The antibody that binds Leukocidin AB will be searched to the extent that the heavy chain variable region comprises CDR-H1 SEQ ID NO: 1, CDR-H2 SEQ ID NO: 21, CDR-H3 SEQ ID NO: 42 (claim 2) [note: VH SEQ ID NO: 177 comprises all three CDR-H sequences] and variable light chain comprises CDR-L1 SEQ ID NO: 63, CDR-L2 SEQ ID NO: 88, CDR-L3 SEQ ID NO: 106 [note: VL SEQ ID NO: 136 comprises all 3 CDR-L sequences]. It is believed that claims 1-6, 48-54 (in part) read on this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 1, 21, 42, 63, 88, 106, 136 and 177. Additional heavy chain and light chain variable region CDRs will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected VH, VL, CDR regions. Applicants must further indicate, if applicable, the claims which read on 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 "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: CDR-H1 SEQ ID NO: 11, CDR-H2 SEQ ID NO: 31, CDR-H3 SEQ ID NO: 53, CDR-L1 SEQ ID NO: 66, CDR-L2 SEQ ID NO: 92, CDR-L3 SEQ ID NO: 117, VH SEQ ID NO: 191 VL SEQ ID NO: 150 (claims 1-8, 48-54 (in part)).

Group II+: Claims 9-16, 25-32, 40-47, 48-54 (in part), drawn to an antibody or binding portion thereof that binds *Staphylococcus aureus* Leukocidin E and/or gamma-hemolysin A (HlgA).

Group II+ will be searched upon payment of additional fee(s). The antibody composition which binds Leukocidin E and/or HlgA may be searched, for example, with the heavy chain variable region comprises CDR-H1 SEQ ID NO: 287, CDR-H2 SEQ ID NO: 292, CDR-H3 SEQ ID NO: 297 (claim 10) [note: VH SEQ ID NO: 336 comprises all three CDR-H sequences] and variable light chain comprises CDR-L1 SEQ ID NO: 303, CDR-L2 SEQ ID NO: 310, CDR-L3 SEQ ID NO: 314 (claim 12) [note: VL SEQ ID NO: 324 comprises all 3 CDR-L sequences] for an additional fee and election as such. It is believed that claims 9-14, 48-54 (in part) read on this exemplary invention. Additional bioactive trigger polynucleotide(s) will be searched upon the payment of additional fees. Additional heavy chain and light chain variable region CDRs will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected VH, VL, CDR regions. Applicants must indicate, if applicable, which claims read on this 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 "+" group(s) will result in only the first named invention to be searched/examined. An exemplary election would be: CDR-H1 SEQ ID NO: 513, CDR-H2 SEQ ID NO: 530, CDR-H3 SEQ ID NO: 549, CDR-L1 SEQ ID NO: 569, CDR-L2 SEQ ID NO: 601, CDR-L3 SEQ ID NO: 622, VH SEQ ID NO: 694, VL SEQ ID NO: 655 (claims 40-45, 48-54 (in part)).

Group III+: Claims 17-24, 48-54 (in part), drawn to an antibody or binding portion thereof that binds *Staphylococcus aureus* Leukocidin D.

Group III+ will be searched upon payment of additional fee(s). The antibody composition which binds Leukocidin D may be searched, for example, with the heavy chain variable region comprises CDR-H1 SEQ ID NO: 372, CDR-H2 SEQ ID NO: 375, CDR-H3 SEQ ID NO: 380 (claim 18) [note: VH SEQ ID NO: 406 comprises all three CDR-H sequences] and variable light chain comprises CDR-L1 SEQ ID NO: 384, CDR-L2 SEQ ID NO: 388, CDR-L3 SEQ ID NO: 392 (claim 20) [note: VL SEQ ID NO: 398 comprises all 3 CDR-L sequences] for an additional fee and election as such. It is believed that claims 17-22, 48-54 (in part) read on this exemplary invention. Additional bioactive trigger polynucleotide(s) will be searched upon the payment of additional fees. Additional heavy chain and light chain variable region CDRs will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected VH, VL, CDR regions. Applicants must indicate, if applicable, which claims read on this 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 "+" group(s) will result in only the first named invention to be searched/examined. An exemplary election would be: CDR-H1 SEQ ID NO: 372, CDR-H2 SEQ ID NO: 376, CDR-H3 SEQ ID NO: 381, CDR-L1 SEQ ID NO: 385, CDR-L2 SEQ ID NO: 389, CDR-L3 SEQ ID NO: 393, VH SEQ ID NO: 407, VL SEQ ID NO: 399 (claims 17-22, 48-54 (in part)).

Group IV+: Claims 33-39, 48-54 (in part), drawn to an antibody or binding portion thereof that binds *Staphylococcus aureus* gamma-hemolysin C (HlgC).

Group IV+ will be searched upon payment of additional fee(s). The antibody composition which binds HlgC may be searched, for example, with the heavy chain variable region comprises CDR-H1 SEQ ID NO: 470, CDR-H2 SEQ ID NO: 471, CDR-H3 SEQ ID NO: 472 (claim 33) [note: VH SEQ ID NO: 494 comprises all three CDR-H sequences] and variable light chain comprises CDR-L1 SEQ ID NO: 473, CDR-L2 SEQ ID NO: 478, CDR-L3 SEQ ID NO: 483 (claim 35) [note: VL SEQ ID NO: 488 comprises all 3 CDR-L sequences] for an additional fee and election as such. It is believed that claims 33-37, 48-54 (in part) read on this exemplary invention. Additional bioactive trigger polynucleotide(s) will be searched upon the payment of additional fees. Additional heavy chain and light chain variable region CDRs will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected VH, VL, CDR regions. Applicants must indicate, if applicable, which claims read on this 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 "+" group(s) will result in only the first named invention to be searched/examined. An exemplary election would be: CDR-H1 SEQ ID NO: 470, CDR-H2 SEQ ID NO: 471, CDR-H3 SEQ ID NO: 472, CDR-L1 SEQ ID NO: 474, CDR-L2 SEQ ID NO: 479, CDR-L3 SEQ ID NO: 484, VH SEQ ID NO: 494, VL SEQ ID NO: 489 (claims 33-39, 48-54 (in part)).

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The inventions listed as Groups I+, II+, III+, 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:

Special Technical Features:

Group I+ inventions have the special technical feature of an antibody that binds to *S. aureus* Leukocidin AB, not required by Groups II+, III+, IV+.

Group II+ inventions have the special technical feature of an antibody that binds to *S. aureus* Leukocidin E and/or HlgA, not required by Groups I+, III+, IV+.

Group III+ inventions have the special technical feature of an antibody that binds to *S. aureus* Leukocidin D, not required by Groups I+, II+, IV+.

Group IV+ inventions have the special technical feature of an antibody that binds to *S. aureus* HlgC, not required by Groups I+, II+, III+.

Among the inventions listed as Groups I+, II+, III+, IV+ are the specific sequences representing CDR regions for specific antibodies recited therein. The inventions do not share a special technical feature, because no significant structural similarities can readily be ascertained among sequences.

Common Technical Feature:

1. Group I+ inventions share the common technical feature of an antibody that competes for leukocidin AB binding with a monoclonal antibody comprising a heavy chain variable region comprising defined CDR-H1, CDR-H2, CDR-H3 sequences and light chain variable region comprising defined CDR-L1, CDR-L2, CDR-L3 sequences.

2. Group II+ inventions share the common technical feature of an antibody that competes for leukocidin E and/or HlgA binding with a monoclonal antibody comprising a heavy chain variable region comprising defined CDR-H1, CDR-H2, CDR-H3 sequences and light chain variable region comprising defined CDR-L1, CDR-L2, CDR-L3 sequences.

3. Group III+ inventions share the common technical feature of an antibody that competes for leukocidin D binding with a monoclonal antibody comprising a heavy chain variable region comprising defined CDR-H1, CDR-H2, CDR-H3 sequences and light chain variable region comprising defined CDR-L1, CDR-L2, CDR-L3 sequences.

4. Group IV+ inventions share the common technical feature of an antibody that competes for HlgC binding with a monoclonal antibody comprising a heavy chain variable region comprising defined CDR-H1, CDR-H2, CDR-H3 sequences and light chain variable region comprising defined CDR-L1, CDR-L2, CDR-L3 sequences.

5. Groups I+, II+, III+, IV+ share the common technical feature of a monoclonal antibody directed against a *Staphylococcus aureus* antigen comprising a heavy chain variable region comprising defined CDR-H1, CDR-H2, CDR-H3 sequences and light chain variable region comprising defined CDR-L1, CDR-L2, CDR-L3 sequences.

However, said common technical features do not represent a contribution over the prior art and is obvious over US 2016/0108106 A1 to Arsanis Biosciences GmbH (hereinafter "Arsanis") [published 21 April 2016], in view of US20150274814 A1 to Torres et al. (hereinafter "Torres").

As to common technical features #1 and #5, Arsanis teaches monoclonal antibodies directed against leukocidin AB (i.e. LukAB) (para [0007]; "It is the objective of the present invention to provide for an antibody directed against the *S. aureus* cytotoxin LukGH (also called LukAB) that is cross-reactive among the different variants of this toxin and provides cross neutralizing potency"; para [0026]; "Specifically, the antibody of the invention is a full-length monoclonal antibody or an antibody fragment thereof comprising at least one antibody domain incorporating the binding site, preferably an antibody selected from the group consisting of murine, chimeric, humanized or human antibodies, heavy-chain antibodies, Fab, Fd, scFv and single-domain antibodies like VH, VHH or VL, preferably a human IgG1 antibody"; para [0138]; Some forms of humanized antibodies preserve all CDR sequences (for example a humanized mouse antibody which contains all six CDRs from the mouse antibody).

As to common technical feature #2, Torres teaches monoclonal antibodies directed against leukocidin E and/or HlgA (para [0048]; "In some embodiments, the anti-LukE and/or anti-LukD antibodies neutralize (e.g., substantially eliminate) LukE and LukD activity, respectively....In the present invention, the anti-LukE antibodies may have some degree of cross-reactivity with other *Staphylococcus* leukocidin S-subunits such as....HlgA"; para [0050]; "Three regions, called hyper-variable or complementarity-determining regions (CDRs), are found in each of VL and VH, which are supported by less variable regions called framework variable regions. The inventive antibodies include IgG monoclonal antibodies as well as antibody fragments or engineered forms. These are, for example, Fv fragments, or proteins wherein the CDRs and/or variable domains of the exemplified antibodies are engineered as single-chain antigen-binding proteins").

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INTERNATIONAL SEARCH REPORT

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As to common technical feature #3, Torres teaches monoclonal antibodies directed against leukocidin D (i.e. LukD) (claim 72; "72. A composition comprising: an antibody component containing all Staphylococcus aureus-specific Leukocidin D (LukD) antibodies and fragments thereof in said composition, wherein said LukD antibodies and fragments thereof are only neutralizing LukD antibodies and fragments thereof, and a pharmaceutically acceptable carrier component"; para [0050]; "Three regions, called hyper-variable or complementarity-determining regions (CDRs), are found in each of VL and VH, which are supported by less variable regions called framework variable regions. The inventive antibodies include IgG monoclonal antibodies as well as antibody fragments or engineered forms. These are, for example, Fv fragments, or proteins wherein the CDRs and/or variable domains of the exemplified antibodies are engineered as single-chain antigen-binding proteins").

As to common technical feature #4, Torres teaches monoclonal antibodies directed against HlgC (para [0048]; In the present invention, the anti-LukE antibodies may have some degree of cross-reactivity with other Staphylococcus leukocidin S-subunits such as...HlgC"; para [0050]; "Three regions, called hyper-variable or complementarity-determining regions (CDRs), are found in each of VL and VH, which are supported by less variable regions called framework variable regions. The inventive antibodies include IgG monoclonal antibodies as well as antibody fragments or engineered forms. These are, for example, Fv fragments, or proteins wherein the CDRs and/or variable domains of the exemplified antibodies are engineered as single-chain antigen-binding proteins").

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups.

Therefore, Groups I+, II+, III+ IV+ inventions lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning item 4: Claims 55-66 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).

Note concerning claim 24: Claim 24 is directed to an antibody or binding portion thereof that competes for leukocidin E binding with a monoclonal antibody. However, the specific CDR-H1, CDR-H2 and CDR-H3 SEQ ID NOs provided correspond exactly to the specific CDR-H1, CDR-H2 and CDR-H3 SEQ ID NOs directed to binding to leukocidin D in independent claim 17. For this reason, claim 24 is restructured as follows: "24. An antibody or binding portion thereof that competes for leukocidin D binding with a monoclonal antibody, wherein said monoclonal antibody is selected from ? "

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continuation of Box III-3:

Claims 1-6, (48-54)(in part), limited to:

leukocidin AB monoclonal antibody comprising SEQ ID NOs: 1, 21, 42, 63, 88, 106, 177, 136;
leukocidin AB monoclonal antibody comprising SEQ ID NOs: 11, 31, 53, 66, 92, 117, 191, 150;
leukocidin AB monoclonal antibody comprising SEQ ID NOs: 14, 34, 56, 77, 99, 121, 195, 154;
leukocidin AB monoclonal antibody comprising SEQ ID NOs: 14, 35, 56, 77, 99, 121, 196, 155;
leukocidin AB monoclonal antibody comprising SEQ ID NOs: 15, 36, 57, 78, 100, 122, 197, 156;

Claims 9-14, 48-54 (in part), limited to:

leukocidin E monoclonal antibody comprising SEQ ID NOs: 287, 292, 297, 303, 310, 314, 336, 324;

Claims 17-24, (48-54)(in part) limited to:

leukocidin D monoclonal antibody comprising SEQ ID NOs: 373, 378, 382, 387, 390, 395, 410, 402