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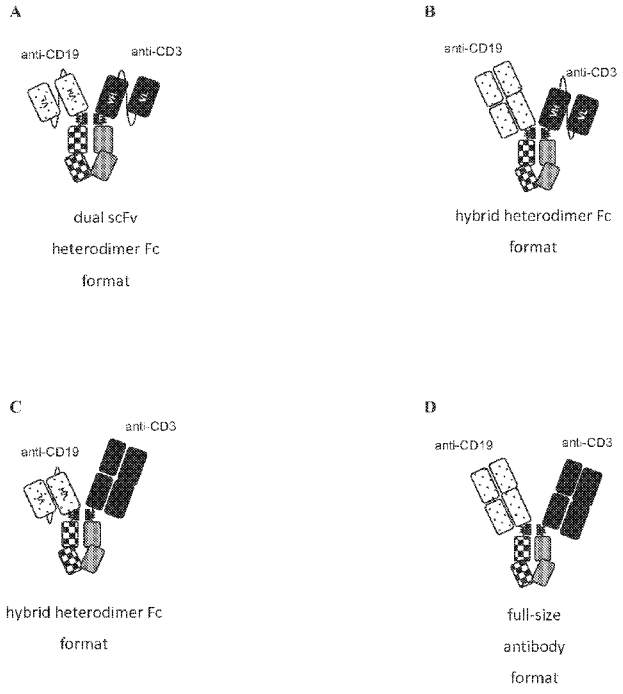
[Continued on next page]

(54) Title: BISPECIFIC CD3 AND CD19 ANTIGEN BINDING CONTRUCTS

Figure 1



(57) Abstract: Bispecific antigen binding contructs are described that bind to CD3 and CD19 or CD20 antigens.

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TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *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))*

(88) Date of publication of the international search report:
12 March 2015

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/46436

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12P 21/08 (2014.01)
CPC - C07K 2319/00, 2317/24
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): C12P 21/08 (2014.01)
CPC: C07K 2319/00, 2317/24; USPC: 530/387.3, 387.1, 386, 380, 350

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)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; Dialog ProQuest; Entrez Pubmed; 'bispecific antibody,' 'heterodimeric Fc,' 'CH3 domain,' 'variant 6754,' 'CD19,' 'CD3,' 'CD20,' 'OKT3,' 'HD37,' 'monovalent specificity,' construct

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2012/0149876 A1 (VON KREUDENSTEIN, TS, et al.) June 14, 2012; abstract; figure 14; paragraphs [0003], [0021], [0023], [0025], [0027], [0074]-[0076], [0079], [0080], [0083], [0088], [0153], [0188], [0196], [0202], [0205], [0207], [0208], [0228]	1-8, 31
Y	COCHLOVIUS, B et al. Treatment Of Human B Cell Lymphoma Xenografts With A CD3 x CD19 Diabody And T Cells. Journal of Immunology. 2000, Vol. 165; pages 888-895; abstract; page 888, second column, second paragraph; page 889, first column, first to second paragraph; page 889, first column, fourth paragraphs; page 890, first paragraph, fourth paragraph; page 893, first column, first paragraph; figure 4.	1-8, 31
A	STANGLMAIER, M et al. Bi20 (FBTA05), A Novel Trifunctional Bispecific Antibody (anti-CD20 3 anti-CD3), Mediates Efficient Killing Of B-cell Lymphoma Cells Even With Very Low CD20 Expression Levels. International Journal of Cancer. 2008, Vol. 123; pages 1181-1189; abstract.	1-8, 31
A	US 2013/0078249 A1 (AST, O et al.) March 28, 2013; abstract	1-8, 31

☐ 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 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
17 December 2014 (17.12.2014)	02 JAN 2015

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

International application No.
PCT/US14/46436

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, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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.: 9-30, 32-35
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:

-***-Please See Supplemental Page-***-

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.:

Groups I+: Claims 1, 2 (in-part), 3 (in-part), 4 (in-part), 5, 6, 7 (in-part), 8 (in-part), 31 (in-part), an isolated bispecific antigen binding polypeptide construct consisting of variant 6754

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.

-***-Continued from Box No. III: Observations Where Unity of Invention Is Lacking:

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 I+: Claims 1-8, 31 and 36 are directed toward a bispecific antigen binding construct comprising a first antigen-binding polypeptide construct which monovalently and specifically binds a CD19 antigen or a CD20 antigen; a second antigen-binding polypeptide construct which monovalently and specifically binds a CD3 antigen; and a heterodimeric Fc comprising first and second Fc polypeptides each comprising a modified CH3 domain, wherein each modified CH3 domain comprises asymmetric amino acid modifications that promote the formation of a heterodimeric Fc and the dimerized CH3 domains having a melting temperature (Tm) of about 68 degrees C or higher; and an isolated polynucleotide encoding said bispecific antigen binding construct.

The bispecific antigen binding construct will be searched to extent that the construct encompasses variant 6754. It is believed that Claims 1, 2 (in-part), 3 (in-part), 4 (in-part), 5, 6, 7 (in-part), 8 (in-part) and 31 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass an isolated bispecific antigen binding polypeptide construct consisting of variant 6754. Additional antigen binding construct(s) will searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected antigen binding construct(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 "+" group(s) will result in only the first claimed invention to be searched/examined. An Exemplary Election would be: an isolated bispecific antigen binding polypeptide construct consisting of variant 6751.

Groups I+ share the technical features including: an isolated bispecific antigen binding construct comprising a first antigen-binding polypeptide construct which monovalently and specifically binds a CD19 antigen or a CD20 antigen; a second antigen-binding polypeptide construct which monovalently and specifically binds a CD3 antigen; a heterodimeric Fc comprising first and second Fc polypeptides each comprising a modified CH3 domain, wherein each modified CH3 domain comprises asymmetric amino acid modifications that promote the formation of a heterodimeric Fc and the dimerized CH3 domains having a melting temperature (Tm) of about 68 degrees C or higher, wherein the first Fc polypeptide is linked to the first antigen-binding polypeptide construct, with or without a first linker, and the second monomeric Fc polypeptide is linked to the second antigen-binding polypeptide construct with or without a second linker; and wherein the first antigen binding polypeptide construct is a Fab and the second antigen binding polypeptide construct is an scFv or the first antigen binding polypeptide construct is an scFv and the second antigen binding polypeptide construct is a Fab; an isolated polynucleotide or set of isolated polynucleotides encoding at least one polypeptide of variant 6754, 6751, 1853, 10151, 6475, 6749, 10152, 10153, 6476, 5850, 5851, 5852 or 6325; and an isolated bispecific antigen binding construct consisting of V1813 or V1812 or V1823.

However, these shared technical features are previously disclosed by US 2012/0149876 A1 to Von Kreudenstein, et al. (hereinafter 'Von Kreudenstein') and further in view of the publication entitled 'Bi20 (FBTA05), A Novel Trifunctional Bispecific Antibody (anti-CD20 X anti-CD3), Mediates Efficient Killing Of B-Cell Lymphoma Cells Even With Very Low CD20 Expression Levels' by Stanglmaier, et al. (hereinafter 'Stanglmaier') and the publication entitled 'Treatment Of Human B Cell Lymphoma Xenografts With A CD3 X CD19 Diabody And T Cells' by Cochlovius, et al. (hereinafter 'Cochlovius').

Von Kreudenstein discloses an isolated bispecific antigen binding construct (a bispecific antibody; paragraphs [0003], [0025]) comprising a first antigen-binding polypeptide construct (comprising an Fab or single chain antibody in a bispecific antibody; paragraphs [0003], [0188]) which monovalently and specifically binds a first antigen (fab or single-chain antibody; paragraphs [0003], [0188]); a second antigen-binding polypeptide construct which monovalently and specifically binds a second antigen (a second single-chain antibody or Fab in a bispecific antibody; paragraphs [0003], [0188]); a heterodimeric Fc (a heterodimeric Fc; paragraph [0003]) comprising first and second Fc polypeptides (comprising a heterodimer Fc region; paragraphs [0013], [0080]) each comprising a modified CH3 domain (paragraph [0080]), wherein each modified CH3 domain comprises asymmetric amino acid modifications that promote the formation of a heterodimeric Fc (paragraph [0080]) and the dimerized CH3 domains having a melting temperature (Tm) of about 68 degrees C or higher (the dimerized CH3 domains having a melting temperature (Tm) of about 70 degrees C or higher; paragraph [0079]), wherein the first Fc polypeptide is linked to the first antigen-binding polypeptide construct (wherein the Fc heterodimer is fused to a variable light or heavy chain domain; figure 14, paragraph [0188]), with or without a first linker (with a hinge region; figure 14), and the second monomeric Fc polypeptide is linked to the second antigen-binding polypeptide construct (wherein the Fc heterodimer is fused to a variable light or heavy chain domain; figure 14, paragraph [0188]) with or without a second linker (with a hinge region; figure 14); and wherein the first antigen binding polypeptide construct is a Fab (paragraphs [0188]) and the second antigen binding polypeptide construct is an scFv (and the second antigen binding polypeptide is a single chain antibody; paragraphs [0188], [0196]) or the first antigen binding polypeptide construct is an scFv (the first antigen binding polypeptide is a single chain antibody; paragraphs [0188], [0196])) and the second antigen binding polypeptide construct is a Fab (the second antigen binding protein is an Fab; paragraph [0188]); and an isolated polynucleotide or set of isolated polynucleotides encoding at least one polypeptide (a nucleic acid encoding a heteromultimer; paragraph [0027]); wherein the bispecific antigen may bind targets including CD19, CD20 and CD3 (wherein the bispecific antigen may bind targets including CD19, CD20 and CD3; paragraph [0207]), and wherein the modifications include T350V (T350V; Claims 1, 91, and 94), L351Y, F405A, Y407V, T366L, K392L, and T394W (L351Y, F405A, Y407V, T366L, K392L, and T394W; paragraph [0020]) to produce heterodimers with increased stability (to produce heterodimers with increased stability; paragraph [0011]).

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Previous Supplemental Page:

Von Kreudenstein does not disclose a first antigen-binding polypeptide construct which monovalently and specifically binds a CD19 antigen or a CD20 antigen; a second antigen-binding polypeptide construct which monovalently and specifically binds a CD3 antigen; an isolated polynucleotide or set of isolated polynucleotides encoding at least one polypeptide of variant 6754, 6751, 1853, 10151, 6475, 6749, 10152, 10153, 6476, 5850, 5851, 5852 or 6325; and an isolated bispecific antigen binding construct consisting of V1813 or V1812 or V1823.

Stanglmaier discloses a bispecific antigen-binding construct (a bispecific antibody; abstract) comprising a first antigen-binding polypeptide construct which monovalently and specifically binds a CD20 antigen (comprising a CD20-specific monoclonal IgG2 antibody; page 1181, column 2, paragraph 5) and a second antigen-binding polypeptide construct which monovalently and specifically binds a CD3 antigen (and an anti-CD3 monoclonal antibody; page 1182, column 1, paragraph 1), wherein the molecule is useful for the treatment of B-cell lymphoma (abstract).

Cochlovius discloses a bispecific antigen-binding construct (a bispecific antibody; abstract) comprising a first antigen-binding polypeptide construct which monovalently and specifically binds a CD19 antigen (comprising ScFv fragment from HD37 (comprising a first antigen-binding polypeptide construct which monovalently and specifically binds a CD19 antigen; page 890, column 1, paragraph 4), and a second antigen-binding polypeptide construct which monovalently and specifically binds a CD3 antigen (and an OKT3 ScFv (a second antigen-binding polypeptide construct which monovalently and specifically binds a CD3 antigen); page 890, column 1, paragraph 4), wherein the CD-3 binding antigen comprises an OKT3 scFv (page 890, column 1, paragraph 4), and nucleic acids encoding said polypeptides (optimized expression vector encoding the bispecific construct; page 890, column 1, paragraph 4); wherein the bispecific antigen binding construct is useful for the treatment of B-cell leukemias and lymphomas (abstract).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the prevoius disclosure of Von Kreudenstein, for producing variants including the specific combination of mutations required to produce any of variants 6754, 6751, 1853, 10151, 6475, 6749, 10152, 10153, 6476, 5850, 5851, 5852, 6325, V1813, V1812 or V1823, including a bispecific construct comprising said Fc mutations in chains including an anti-CD3 OKT3 scFv and anti-CD19 HD37 Fab, provided the previous disclosure of Cochlovius, for producing a bispecific construct, such as a variant of 6754 with increased stability, useful for the treatment of B-cell lymphomas. Furthermore, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Von Kreudenstein, for producing Fc variants, including the specific combination of Fc mutations required to produce any of variants V1813, V1812 or V1823, or a nucleic acid encoding at least one polypeptide of the variants, which binds to the same targets, provided the previous disclosure of Stanglmaier. Moreover, it would have been obvious to a person of ordinary skill in the art at, the time the invention, to have modified the prevoius disclosure of Von Kreudenstain, for implementing the use of a known therapeutic antibody, such as anti-CD3 and anti-CD20 antibodies or binding proteins, as previously disclosed by Stanglmaier, with utilizing known therapeutic antibodies, particularly Foralumab, and Fatumumab (anti-CD3 and anti-CD20 antibodies or binding proteins Foralumab, and Fatumumab; paragraph [00248], Table 1 of the instant PCT application), for producing therapeutic bispecific antibodies such as V1813 (Table 1 of the instant PCT appolication), with increased stability, as previously disclosed by Von Kreudenstein, and having usefulness in the treatment of B-cell lymphomas, as previously disclosed by Stanglmaier, for achieving more rapid clearance of the antibodies for therapeutic use, due to their similarity with accepted therapeutics.

Since none of the special technical features of the Groups I+ 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 Von Kreudenstein, Stanglmaier and Cochlovius references, unity of invention is lacking.