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Published:
— *with international search report (Art. 21(3))*
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06 June 2019 (06.06.2019)

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(54) Title: DOMINANT NEGATIVE LIGAND CHIMERIC ANTIGEN RECEPTOR SYSTEMS

(57) Abstract: The invention provides modified T-cell receptors referred to herein as "dominant negative ligand-chimeric antigen receptors" (DNL-CARs). The present invention also provides T-cells expressing DNL-CARs such T cells also referred to herein as "DNL- CAR-expressing T cells" or "DNL-CAR T cells. Also provided are "tagged-DNL/CAR-T systems" that direct CAR-T cells to tumor cells previously complexed to the DNL-Tag fusion. Also provided are tagged-DNL-antigen fusion proteins wherein the antigen portion of the fusion proteins recruits the patient's own immune system to neutralize cells tagged with the tagged DNL portion of the fusion protein.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/59198

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/32, A61K 35/17 (2019.01)

CPC - C07K 2319/03, C07K 14/7051, C07K 2319/74, C12N 2510/00, C12N 5/0636, C07K 14/705

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.
Y	WO 2017/167217 A I (INNOVATIVE CELLULAR THERAPEUTICS CO., LTD) 5 October 2017 (05.10.2017) Claim 1; page 5, In 27-30; page 11, In 41-42; page 18, In 31-40, page 19, In 29-31; page 20, In 28-36	1-15
Y	US 2009/0318350 A1 (PIENKOS et al.) 24 December 2009 (24.12.2009) Abstract; para [0012]; (para [0043]	1-15
Y	US 2009/0311783 A1 (PIENKOS et al.) 17 December 2009 (17.12.2009) para [0023]; para [0036]	5-6
Y	US 2013/0231286 A1 (CHEN) 5 September 2013 (05.09.2013) Claim 1; Claim 3	7-8
Y	US 2007/0243192 A1 (WICHA et al.) 18 October 2007 (18.10.2007) Claim 1; para [0007]	9-10

☐ 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

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2019	24 APR 2019

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. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/59198

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:
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 searched, the appropriate additional search fees must be paid.

--Please see 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.:
1-15

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.

---Continuation of Box No. III Observations where unity of invention is lacking---

Group I: Claims 1-15, directed to a dominant-negative ligand chimeric antigen receptor (DNL-CAR) wherein the extracellular domain comprises a dominant-negative ligand (DNL), and use of the DNL-CAR.

Group II: Claims 16-21 and 27, directed to a system comprising (a) an isolated tagged-DNL, and (b) a chimeric antigen receptor (CAR) comprising an ScFV extracellular domain that binds the tag on the tagged-DNL.

Group III: Claims 22-26, directed to a method of reducing or eliminating a tumor, comprising (a) contacting the tumor with a tagged-DNL, and (b) contacting the tagged-DNL with a T cell comprising a chimeric antigen receptor (CAR) comprising a ScFV extracellular domain that binds the tag.

Group IV: Claim 28-37, directed to fusion protein comprising a tagged-DNL fused to an antigen that promotes an immune response in a patient.

The inventions listed as Groups I through 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

Groups II - IV do not require a DNL-CAR, comprising a DNL fused to a chimeric antigen receptor [see instant application Fig. 6], as required by group I.

Groups I and III-IV do not require a system for treating cancer comprising (a) an isolated (soluble) tagged DNL and (b) an isolated CAR comprising an ScFV extracellular domain that binds the tag, as required by group II.

Groups I, II and IV do not require a method of reducing the size, growth, or re-growth of a tumor with a system for treating cancer comprising (a) an isolated (soluble) tagged DNL and (b) an isolated CAR comprising an ScFV extracellular domain that binds the tag, as required by group III.

Groups I - III do not require a fusion protein comprising a Tagged DNL fused to a non-lethal immunogenic antigen, as required by group IV.

Common Technical Features

The common technical feature shared by Groups I-IV, is a DNL, or dominant-negative ligand moiety [e.g. Pan-HER antagonist, claim 3].However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by US 2009/0318350 A1 to Pienkos et al (hereinafter 'Pienkos'). Pienkos discloses a dominant negative HER ligand (Abstract-"The HER ligand polypeptide variants of the invention possess Pan-HER antagonistic properties and can inhibit at least one HER-mediated biological activity of one or more HER subtypes, such as inhibition of the receptor's kinase activation activity and subsequently, cell proliferation."; para [0012]-"One aspect of the invention relates to a human epidermal receptor (HER) ligand variant designed with EGF as the starting druggable ligand which is then modified ... wherein at least one amino acid corresponding to G18, G39, R41 or L47 of human wild-type epidermal growth factor (EGF) is substituted with a different amino acid."; para [0043]-"In one embodiment of the invention, the HER ligand variants or Pan-HER antagonists act as dominant negative ligands (DNLs)").

A feature shared by Groups II and III is a system comprising (a) a tagged DNL and (b) a CAR-svFv that binds the tag. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is made obvious by WO 2014/100615 Al to Purdue Research Foundation (hereinafter 'Purdue') in view of Pienkos. Purdue discloses (a) a tagged-tumor receptor ligand covalently bonded to a tag (para [0022]-"In a third embodiment, the invention is directed to a two component cancer therapeutic comprising: (a) a small conjugate molecule (SCM) comprising a targeted moiety conjugated to a tumor receptor ligand"; para [0023]-"Targeted moieties that may be used in the SCMs include... (FITC)..."), and (b) a chimeric antigen receptor (CAR) comprising an ScFV extracellular domain; wherein the ScFV of the CAR targets and binds the tag on the tagged-tumor receptor ligand (para [0022]-"(b) chimeric antigen receptor (CAR)-expressing cytotoxic lymphocytes, wherein the CAR is a fusion protein comprising a recognition region, a co-stimulation domain and an activation signaling domain, and wherein the CAR has binding specificity for the targeted moiety."; para [0027]-"the recognition region of the CAR is a single chain fragment variable (scFv) region of an anti-FITC antibody"). Purdue does not teach that the tumor receptor ligand is a dominant-negative ligand (DNL), however, Pienkos discloses a dominant negative HER ligand (Abstract "The HER ligand polypeptide variants of the invention possess Pan-HER antagonistic properties"; para [0012] "a human epidermal receptor (HER) ligand variant designed with EGF as the starting druggable ligand ... wherein at least one amino acid ... of human wild-type epidermal growth factor (EGF) is substituted"; para [0043]-"In one embodiment of the invention, the HER ligand variants or Pan-HER antagonists act as dominant negative ligands (DNLs)"). It would have been obvious to one of ordinary skill in the art to have used the dominant negative HER ligand of Pienkos as a tumor receptor ligand in the system of Purdue, as said ligand has known tumor inhibitory properties which would have activated CAR cytotoxic activity, and would have allowed treatment of HER2 expressing tumors.

********** See Next Extra Sheet to continue **********

---Continuation of Box No. III Observations where unity of invention is lacking---

A feature shared by Groups I and III is reducing the size of a tumor by contacting the tumor with a T-cell comprising a CAR that acts (elicits a T-cell response) through a DNL binding to a tumor cell. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is made obvious by Purdue in view of Pienkos. Purdue teaches a chimeric antigen receptor (CAR) comprising an ScFV extracellular domain; wherein the ScFV of the CAR targets and binds a tag on a tagged-tumor receptor ligand (para [0022] "a two component cancer therapeutic comprising: (a) a small conjugate molecule (SCM) comprising a targeted moiety conjugated to a tumor receptor ligand, ... (b) chimeric antigen receptor (CAR)-expressing cytotoxic lymphocytes, wherein the CAR is a fusion protein comprising a recognition region, a co-stimulation domain and an activation signaling domain, and wherein the CAR has binding specificity for the targeted moiety."; para [0088] "The cells used in the CAR system ... are cytotoxic lymphocytes ... Upon activation ... these cytotoxic lymphocytes triggers the destruction of target tumor cells"). Purdue does not recite that the tumor receptor ligand is a dominant-negative ligand (DNL), however, Pienkos teaches a dominant negative HER ligand (Abstract "The HER ligand polypeptide variants of the invention possess Pan-HER antagonistic properties and can inhibit at least one HER-mediated biological activity of one or more HER subtypes, such as inhibition of the receptor's kinase activation activity and subsequently, cell proliferation."; para [0012] "One aspect of the invention relates to a human epidermal receptor (HER) ligand variant designed with EGF as the starting druggable ligand which is then modified to a T1E or WVS background and wherein at least one amino acid corresponding to G18, G39, R41 or L47 of human wild-type epidermal growth factor (EGF) is substituted with a different amino acid."; para [0043] "In one embodiment of the invention, the HER ligand variants or Pan-HER antagonists act as dominant negative ligands (DNLs)"). It would have been obvious to one of ordinary skill in the art to have used the dominant negative HER ligand of Pienkos as a tumor receptor ligand in the system of Purdue, as said ligand has known tumor inhibitory properties which would have synergised with CAR activity, and would have allowed treatment of HER2 expressing tumors.

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

Groups I through IV therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.