

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

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Date of mailing
(day/month/year)

22 OCT 2020

Applicant's or agent's file reference
118003-10520

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US2020/040642

International filing date (day/month/year)

02 July 2020

Priority date (day/month/year)

03 July 2019

International Patent Classification (IPC) or both national classification and IPC

IPC(8) - A61K 38/00; A61K 39/00; A61K 39/395; C07K 7/06; C07K 16/18; C12N 15/13 (2020.01)

CPC - A61K 38/00; A61K 39/00; A61K 2039/505; C07K 7/06; C07K 14/47; C07K 16/00; C07K 2317/56;
C07K 2319/00 (2020.08)Applicant **REGENERON PHARMACEUTICALS, INC.**

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Date of completion of this opinion 13 October 2020	Authorized officer Blaine R. Copenhaver PCT Helpdesk: 571-272-4300 Telephone No. 571-272-4300
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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

- ☒ the international application in the language in which it was filed.
- ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(b)).

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established 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 13*ter*.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 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ 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.

5. Additional comments:

SEQ ID NOs: 2, 10, 22, 30, 42, 50, 62, 70, 82, 90, 102, 110, 112, 122, 130, 142, 150, 162, 170, 180, 186, 195, 196, 203, 211, 219, 230, 238, 250, 258, 269, 271, 273, 274, 276, 277, 280, and 291 were searched.

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 16-57, 62-75

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 16-57, 62-75 are so unclear that no meaningful opinion could be formed (*specify*):

Claims 16-57 and 62-75 are multiple dependent claims not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 16-57, 62-75

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*. I(a) or (b).

☐ See Supplemental Box for further details.

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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	8-15, 58-61	YES
	Claims	1-7	NO
Inventive step (IS)	Claims	8-15, 58-61	YES
	Claims	1-7	NO
Industrial applicability (IA)	Claims	1-15, 58-61	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-7 lack novelty under PCT Article 33(2) as being anticipated by Irving et al. (hereinafter Irving).

Regarding claim 1, Irving discloses an antigen-binding protein that binds specifically to an HLA-A2:New York esophageal squamous cell carcinoma 1 peptide (NY-ESO-1 peptide) complex at least one amino acid corresponding to an amino acid selected from the group consisting of M160, W161, and Q164 of SEQ ID NO: 271 (Pg. 23068, left-hand column, we generated a panel of HLA-A*0201/NY-ESO-1157-165-specific T cell receptors (TCR); Pg. 23071, right-hand column, first paragraph, we tested the binding of soluble TCR by ELISA with plate-immobilized A2/NY-ESO157-165 complexes containing Ala substituted NY-ESO157-165 peptides. All of the TCR, including the extreme supraphysiologic affinity TCR wtc51m, exhibited similar binding patterns (Fig. 1G) with peptide residues M160, W161, I162, and Q164 being critical contact residues; Pg. 23072, Fig. 1G ... binding to the WT NY-ESO157-165 peptide-MHC complex is set to 1 and the relative change in binding to the Ala replacements calculated and shown in log10 scale on the y axis. NY-ESO157-165 peptide residues M160, W161, I162, and Q164 are marked with gray arrows as their replacement with Ala abolishes TCR binding, wherein Fig. 1G of Irving shows a panel of soluble TCRs shows decreased TCR binding ratio at M160A, W161A, I162A, and Q164A).

Regarding Claim 2, Irving discloses the antigen-binding protein of claim 1, wherein said isolated antigen-binding protein specifically binds to a conformational epitope of the HLA-A2 presented NY-ESO-1 polypeptide (Pg. 23068, left-hand column, we generated a panel of HLA-A*0201/NY-ESO-1157-165-specific T cell receptors (TCR); Pg. 23071, right-hand column, first paragraph, we tested the binding of soluble TCR by ELISA with plate-immobilized A2/NY-ESO157-165 complexes containing Ala substituted NY-ESO157-165 peptides. All of the TCR, including the extreme supraphysiologic affinity TCR wtc51m, exhibited similar binding patterns (Fig. 1G) with peptide residues M160, W161, I162, and Q164 being critical contact residues; Pg. 23072, Fig. 1G ... binding to the WT NY-ESO157-165 peptide-MHC complex is set to 1 and the relative change in binding to the Ala replacements calculated and shown in log10 scale on the y axis. NY-ESO157-165 peptide residues M160, W161, I162, and Q164 are marked with gray arrows as their replacement with Ala abolishes TCR binding, wherein Fig. 1G of Irving shows a panel of soluble TCRs shows decreased TCR binding ratio at M160A, W161A, I162A, and Q164A).

Regarding claim 3, Irving discloses the antigen-binding protein of claim 1, wherein said at least one amino acid is at least two amino acids (Pg. 23068, left-hand column, we generated a panel of HLA-A*0201/NY-ESO-1157-165-specific T cell receptors (TCR); Pg. 23071, right-hand column, first paragraph, we tested the binding of soluble TCR by ELISA with plate-immobilized A2/NY-ESO157-165 complexes containing Ala substituted NY-ESO157-165 peptides. All of the TCR, including the extreme supraphysiologic affinity TCR wtc51m, exhibited similar binding patterns (Fig. 1G) with peptide residues M160, W161, I162, and Q164 being critical contact residues; Pg. 23072, Fig. 1G ... binding to the WT NY-ESO157-165 peptide-MHC complex is set to 1 and the relative change in binding to the Ala replacements calculated and shown in log10 scale on the y axis. NY-ESO157-165 peptide residues M160, W161, I162, and Q164 are marked with gray arrows as their replacement with Ala abolishes TCR binding, wherein Fig. 1G of Irving shows a panel of soluble TCRs shows decreased TCR binding ratio at M160A, W161A, I162A, and Q164A).

Regarding claim 4, Irving discloses the antigen-binding protein of claim 3, wherein said at least one amino acid is three amino acids (Pg. 23068, left-hand column, we generated a panel of HLA-A*0201/NY-ESO-1157-165-specific T cell receptors (TCR); Pg. 23071, right-hand column, first paragraph, we tested the binding of soluble TCR by ELISA with plate-immobilized A2/NY-ESO157-165 complexes containing Ala substituted NY-ESO157-165 peptides. All of the TCR, including the extreme supraphysiologic affinity TCR wtc51m, exhibited similar binding patterns (Fig. 1G) with peptide residues M160, W161, I162, and Q164 being critical contact residues; Pg. 23072, Fig. 1G ... binding to the WT NY-ESO157-165 peptide-MHC complex is set to 1 and the relative change in binding to the Ala replacements calculated and shown in log10 scale on the y axis. NY-ESO157-165 peptide residues M160, W161, I162, and Q164 are marked with gray arrows as their replacement with Ala abolishes TCR binding, wherein Fig. 1G of Irving shows a panel of soluble TCRs shows decreased TCR binding ratio at M160A, W161A, I162A, and Q164A).

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 19 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claim 19 is indefinite for the following reason(s):

With regards to claim 19, the claim recites dependency from itself (claim 19), which is improper. Herein, claim 19 is best interpreted as depending from claim 18.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Regarding claim 5, Irving discloses an antigen-binding protein that binds specifically to an HLA-A2:NY-ESO-1 157-165 peptide complex, wherein said isolated antigen-binding protein interacts with amino acids M160, W161, and Q164 of said NY-ESO-1 157-165 peptide (Pg. 23068, left-hand column, we generated a panel of HLA-A*0201/NY-ESO-1157-165-specific T cell receptors (TCR); Pg. 23071, right-hand column, first paragraph, we tested the binding of soluble TCR by ELISA with plate-immobilized A2/NY-ESO157-165 complexes containing Ala substituted NY-ESO157-165 peptides. All of the TCR, including the extreme supraphysiologic affinity TCR wt51m, exhibited similar binding patterns (Fig. 1G) with peptide residues M160, W161, I162, and Q164 being critical contact residues; Pg. 23072, Fig. 1G ... binding to the WT NY-ESO157-165 peptide-MHC complex is set to 1 and the relative change in binding to the Ala replacements calculated and shown in log10 scale on the y axis. NY-ESO157-165 peptide residues M160, W161, I162, and Q164 are marked with gray arrows as their replacement with Ala abolishes TCR binding, wherein Fig. 1G of Irving shows a panel of soluble TCRs shows decreased TCR binding ratio at M160A, W161A, I162A, and Q164A). Further, in regards to the limitation relating to "as determined by X-ray crystallography at a resolution of 4.0 Å or higher," a recitation of the process of determining which amino acids of the NY-ESO-1 157-165 peptide the instant antigen-binding protein interacts with does not structurally limit the claim. The claimed antigen-binding protein and the antigen-binding protein of Irving are indistinguishable, even if the amino acid contacts of the antigen-binding protein of Irving were determined by a different process.

Regarding claim 6, Irving discloses the antigen-binding protein of claim 5. Further, in regards to the limitations relating to "as determined by X-ray crystallography at a resolution of 4.0 Å or higher" and "wherein said resolution is 3.5 Å or higher," a recitation of the process of determining which amino acids of the NY-ESO-1 157-165 peptide the instant antigen-binding protein interacts with does not structurally limit the claim. The claimed antigen-binding protein and the antigen-binding protein of Irving are indistinguishable, even if the amino acid contacts of the antigen-binding protein of Irving were determined by a different process.

Regarding claim 7, Irving discloses the antigen-binding protein of claim 6. Further, in regards to the limitations relating to "as determined by X-ray crystallography at a resolution of 4.0 Å or higher" and "wherein said resolution is 3.3 Å or higher," a recitation of the process of determining which amino acids of the NY-ESO-1 157-165 peptide the instant antigen-binding protein interacts with does not structurally limit the claim. The claimed antigen-binding protein and the antigen-binding protein of Irving are indistinguishable, even if the amino acid contacts of the antigen-binding protein of Irving were determined by a different process.

Claims 8-15 and 58-61 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest:

Regarding claim 8, the prior art of record, individually or in combination, does not teach or fairly suggest an antigen-binding protein that specifically binds to an HLA-A2:NY-ESO-1 157- 165 peptide complex but does not specifically bind to one or more off-target peptides selected from the group consisting of BCL9L 1351-1359 (SEQ ID NO: 273), GRID1 7-15 (SEQ ID NO: 274), ZDHHC1 376-384 (SEQ ID NO: 276), ITCH 807-815 (SEQ ID NO: 277), and URB1 1853- 1861 (SEQ ID NO: 280), wherein the ratio of i) said antigen-binding protein binding to cells pulsed with said one or more off-target peptides to ii) unpulsed cells is less than about nine.

The following prior art is made of record to support and further define the reasons why claims 8 meets the criteria set out in PCT Article 33 (2)-(3):

(i) "An MHC-restricted antibody-based chimeric antigen receptor requires TCR-like affinity to maintain antigen specificity," to Maus et al. (hereafter Maus) teaches an antigen-binding protein that binds specifically to an HLA-A2 presented NY-ESO-1 polypeptide (Pg. 1, left-hand column, last paragraph-Pg. 2, right-hand column, third paragraph, a high-affinity Fab restricted to HLA-A2/NY-ESO-1157-165 has been generated (3M4E5) and affinity-matured by shifting the binding affinity from the MHC alpha-helix to the peptide (T1) ... The T1 antibody displayed high specificity for HLA-A2/NY- ESO-1157-165 by BIAcore and enzyme-linked immunosorbent assay (ELISA) assays. The T1 Fab structure was also determined to bind the NY-ESO-1157-165/HLAA*0201 complex in a very similar fashion to a native TCR specific for the same MHC/peptide antigen), but Maus fails to teach but does not specifically bind to one or more off-target peptides selected from the group consisting of BCL9L 1351-1359 (SEQ ID NO: 273), GRID1 7-15 (SEQ ID NO: 274), ZDHHC1 376-384 (SEQ ID NO: 276), ITCH 807-815 (SEQ ID NO: 277), and URB1 1853-1861 (SEQ ID NO: 280), wherein the ratio of i) said antigen-binding protein binding to cells pulsed with said one or more off-target peptides to ii) unpulsed cells is less than about nine.

(ii) "In-silico discovery of cancer-specific peptide-HLA complexes for targeted therapy" to Dhanik et al. (hereafter Dhanik) teaches an HLA-A2:NY-ESO-1 157-165 peptide and GRID1 7-15 (SEQ ID NO: 274) (Pg. 10, left-hand column, second paragraph, The cancer testis antigen 1 A (CTAG1A, also known as CTAG1B/NY-ESO-1) peptide SLLMWITQC in complex with HLA-A*02:01, wherein SLLMWITQC of Dhanik comprises a peptide of 100% identity to instant SEQ ID NO: 269; Table 7 includes GRID1 7-15 (WLLPWICQC), wherein WLLPWICQC of Dhanik comprises a peptide of 100% identity to instant SEQ ID NO: 274; see Para. [0078] of this instant application, a NY-ESO-1 peptide includes amino acids 157-165 of SEQ ID NO: 271 (SLLMWITQC (SEQ ID NO:269)).

None of the prior art taken alone or in combination fairly teach or suggest an antigen-binding protein that specifically binds to an HLA-A2: NY-ESO-1 157-165 peptide complex but does not specifically bind to one or more off-target peptides selected from the group consisting of BCL9L 1351-1359 (SEQ ID NO: 273), GRID1 7-15 (SEQ ID NO: 274), ZDHHC1 376-384 (SEQ ID NO: 276), ITCH 807-815 (SEQ ID NO: 277), and URB1 1853-1861 (SEQ ID NO: 280), wherein the ratio of i) said antigen-binding protein binding to cells pulsed with said one or more off-target peptides to ii) unpulsed cells is less than about nine.

Claims 9-15 depend from claim 8, and therefore meet the criteria set out in PCT Article 33(2)-(3) for at least the same reasons as claim 8.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Regarding claim 58, the prior art of record, individually or in combination, does not teach or fairly suggest an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular binding domain that specifically binds to a conformational epitope of an HLA-A2 presented New York esophageal squamous cell carcinoma 1 peptide (NY-ESO-1 peptide), a transmembrane domain, and an intracellular signaling domain.

The following prior art is made of record to support and further define the reasons why claim 58 meet the criteria set out in PCT Article 33 (2)-(3):

(i) "An MHC-restricted antibody-based chimeric antigen receptor requires TCR-like affinity to maintain antigen specificity," to Maus et al. (hereafter Maus) teaches an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular binding domain that specifically binds to an HLA-A2 presented New York esophageal squamous cell carcinoma 1 peptide (Pg. 1, Abstract, a T cell receptor-like CAR based on an antibody that recognizes HLA-A*0201 presenting a peptide epitope derived from the cancer-testis antigen NY-ESO-1; Pg. 2, left-hand column, second paragraph, we have developed a novel TCR-like antibody into a CAR based on the scFv of a high-affinity antibody (T1) that recognizes HLA-A*0201 in combination with a peptide epitope derived from NY-ESO-1; Pg. 2, left-hand column, third paragraph, The T1 antibody displayed high specificity for HLA-A2/NY- ESO-1157-165 ... We constructed a second-generation CAR based on the scFv of the T1 Fab. The scFv of T1 was cloned into the backbone of the CD28-CD3ζ bi-cistronic γ-retroviral vector ... (Figure 1a); Pg. 3, Figure 1. T1/28z CAR construct. (a) ... single-chain variable fragment of the T1 (HLA-A2/NYESO1-specific) antibody fused to human CD28 and human CD3zeta), but Maus fails to teach a conformational epitope of an HLA-A2 presented NY-ESO-1 peptide.

(ii) US 2016/0081314 A1 to Regeneron Pharmaceuticals, Inc. (hereafter Regeneron Pharmaceuticals) teaches an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular binding domain that specifically binds to an HLA-A2 presented NY-ESO-1 peptide (Example 1 ... Chimeric Antigen Receptors, Para. [0227] Sequences encoding VH and VL domains of antibodies recognizing peptide-MHC complexes were incorporated into synthetically produced 1.9 kb nucleotide sequences, where the immunoglobulin Vk and VH domain sequences were placed upstream of TCRA C and TCRB C sequences, respectively (FIG. 3) ... describe an antibody recognizing NY-ESO-1 peptide (SLLMWITQVNY, SEQ ID NO: 1) complexed with HLA-A2; Para. [0230] CARs comprising VL and VH of NY-ESO-1).

None of the prior art taken alone or in combination fairly teach or suggest a conformational epitope of an HLA-A2 presented NY-ESO-1 peptide.

Claims 59-61 depend from claim 58, and therefore meet the criteria set out in PCT Article 33(2)-(3) for at least the same reasons as claim 58.

Claims 1-15 and 58-61 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.