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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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[Continued on next page]

(54) Title: REPLICATION COMPETENT ATTENUATED VACCINIA VIRUSES WITH DELETION OF THYMIDINE KINASE WITH AND WITHOUT THE EXPRESSION OF HUMAN FLT3L OR GM-CSF FOR CANCER IMMUNOTHERAPY

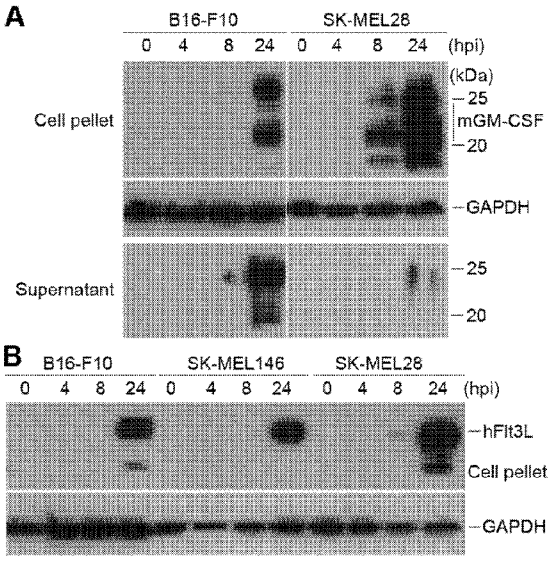

Figure 4

(57) Abstract: The present invention relates generally to the fields of oncology, virology and immunotherapy. More particularly, it concerns the use of poxviruses, specifically the replication competent attenuated vaccinia virus with deletion of thymidine kinase (VC-TK$^-$) with and without the expression of human Flt3L or GM-CSF as oncolytic and immunotherapy. The foregoing poxviruses can also be used in combination with immune checkpoint blocking agents. The foregoing poxviruses can also be inactivated via Heat or UV-treatment and the inactivated virus can be used as immunotherapy either alone or in combination with immune checkpoint blocking agents.

WO 2017/147553 A3

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, 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).

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))*

— *with sequence listing part of description (Rule 5.2(a))*

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5 October 2017

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/19548

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 38/19, 48/00; A61P 35/00 (2017.01)
CPC - A61K 9/0019, 35/768, 38/19, 38/191, 38/193, 38/208, 38/2013, 45/06, 48/00, 48/0066, 48/0075; A61N 5/10

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	EP 2 136 633 B1 (SILLAJEN BIOTHERAPEUTICS INC.) October 28, 2015; paragraphs [0009], [0012], [0013], [0035], [0036], [0059]; Claims 1, 11, 12	1-5/2-4, 7-9, 10/7-8, 11-12, 14/11-12, 16-22, 23/21-22, 24/21-22, 25/24/21-22, 26-32, 33/29, 33/31-32, 34/33/29, 34/33/31-32
A	Brandt, T et al. The N-Terminal Domain Of The Vaccinia Virus E3L-Protein Is Required For Neurovirulence. Virology, 2005, Vol. 333, No. 2, pp 263-270; abstract, page 264, first column, third paragraph. DOI: 10.1128/JVI.75.2.850-856.2001.	1-5/2-4, 7-9, 10/7-8, 11-12, 14/11-12, 16-22, 23/21-22, 24/21-22, 25/24/21-22, 26-32, 33/29, 33/31-32, 34/33/29, 34/33/31-32
A	US 2008/0181870 A1 (LOWENSTEIN, P et al.) July 31, 2008; abstract	1-5/2-4, 7-9, 10/7-8, 11-12, 14/11-12, 16-22, 23/21-22, 24/21-22, 25/24/21-22, 26-32, 33/29, 33/31-32, 34/33/29, 34/33/31-32
A	WO 2015/084897 A2 (MIRIMMUNE, LLC) June 11, 2015; paragraphs [0030], [0047]	11-12, 14/11, 29-33, 33/29, 33/31-32, 34/33/29, 34/33/31-32

☐ 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
21 June 2017 (21.06.2017)	08 AUG 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/19548

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:

   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).

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:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/19548

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.: 6, 13, 15
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.:
1-5/2-4, 7-9, 10/7-8, 11-12, 14/11-12, 16-22, 23/21-22, 24/21-22, 25/24/21-22, 26-32, 33/29, 33/31-32, 34/33/29, 34/33/31-32;
E3LD83N-TKhFIt3L

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
Information on patent family members

International application No.
PCT/US17/19548

-***-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 1-5, 7-14, 16-34 and E3Ldelta83N-TK^- -hFlt3L are directed toward an isolated and purified active substance; methods of treating a subject with one or more tumors therewith; and compositions comprising the active substance.

The substance, methods and compositions will be searched to the extent they encompass E3Ldelta83N-TK^- -hFlt3L (first exemplary active substance). Applicant is invited to elect additional active substance(s) to be searched. Additional active substances will be searched upon the payment of additional fees. It is believed that claims 1-5, 7-14, 16-19, 20 (in-part), 21 (in-part), 22 (in-part), 23 (in-part), 24 (in-part), 25 (in-part), 26 (in-part), 27 (in-part), 28 (in-part), 29 (in-part), 30 (in-part), 31 (in-part), 32 (in-part), 33 (in-part) and 34 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass E3Ldelta83N-TK^- -hFlt3L (active substance). 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 active substance encompassing E3Ldelta83N-TK^- (first exemplary elected active substance).

Groups I+ share the technical features including: an isolated and purified active substance comprising E3Ldelta83N-TK^- in replicative or inactivated form suitable for use as an immunotherapeutic agent against a malignant solid tumor; a composition comprising an effective amount for treating a patient afflicted with a solid malignant tumor an active ingredient comprising E3Ldelta83N-TK^- in replicative or inactivated form, or both, and a pharmaceutically acceptable excipient; a method for treating a solid malignant tumor in a subject comprising delivering to tumor cells of the subject an amount of replication competent or inactivated E3Ldelta83N-TK^- virus effective to induce the immune system of the subject to mount an immune response against the tumor and conjointly administering or having administered to the subject a second amount of an immune checkpoint blocking agent effective to block immune suppressive mechanisms within the tumor elicited by tumor cells, stromal cells, or tumor infiltrating immune cells; and a method for treating a solid malignant tumor in a subject comprising delivering to tumor cells of the subject an amount of E3Ldelta83N-TK^- virus or viral constructs, including replicative and inactivated versions thereof; effective to accomplish one or more of the following (regardless of order): induce the immune system of the subject to mount an immune response against the tumor; reduce the size of the tumor; eradicate the tumor; inhibit growth of the tumor; inhibit metastasis of the tumor; and reduce or eradicate metastatic tumor.

However, these shared Technical features are previously disclosed by EP 2 136 633 B1 to SillaJen Biotherapeutics Inc. (hereinafter 'SillaJen') in view of the article 'The N-terminal domain of the vaccinia virus E3L-protein is required for neurovirulence, but not induction of a protective immune response' by Brandt et al. (hereinafter 'Brandt') and US 2015/0250837 A1 to Morningside Technology Ventures Ltd. (hereinafter 'Morningside').

SillaJen discloses an isolated and purified (isolated and purified; paragraph [0059]) active substance (vaccinia virus (active substance); paragraphs [0009], [0059]) comprising TK^- virus in replicative form, comprising a further modification in an E3L gene (comprising TK^- virus in replicative form, comprising a further modification in an E3L gene; paragraph [0009], Claims 1, 11, 12) suitable for use as an immunotherapeutic agent against a malignant solid tumor (suitable for producing anti-viral response for the treatment of a solid tumor (suitable for use as an immunotherapeutic agent against a malignant solid tumor); paragraphs [0035], [0036]); a composition comprising an effective amount (a composition comprising an effective amount; paragraphs [0013], [0136]) for treating a patient afflicted with a solid malignant tumor an active ingredient comprising TK^- vaccinia virus in replicative form (for treating a patient afflicted with a solid malignant tumor an active ingredient comprising TK^- vaccinia virus in replicative form; paragraph [0009]), further comprising a modification in an E3L gene (further comprising a modification in an E3L gene; Claims 1, 11, 12); and a pharmaceutically acceptable excipient (and a pharmaceutically acceptable excipient; paragraph [0136]); and a method for treating a solid malignant tumor in a subject (a method for treating a solid malignant tumor in a subject; paragraph [0009]) comprising delivering to tumor cells of the subject (comprising injecting into the tumor (comprising delivering to tumor cells of the subject); paragraph [0009]) an amount of replication competent TK^- virus further comprising a modification in an E3L gene (an amount of replication competent TK^- virus further comprising a modification in an E3L gene; paragraphs [0009], [0013], Claims 1, 11, 12); effective to induce the immune system of the subject to mount an immune response against the tumor (effective to produce immune-based oncolysis (effective to induce the immune system of the subject to mount an immune response against the tumor); paragraphs [0009], [0013], [0036]) and conjointly administering or having administered to the subject a second amount of an immunotherapeutic agent (conjointly administering or having administered to the subject a second amount of an immunotherapeutic agent; paragraph [0012]).

SillaJen does not disclose E3Ldelta83N-TK^-; an immune checkpoint blocking agent effective to block immune suppressive mechanisms within the tumor elicited by tumor cells, stromal cells, or tumor infiltrating immune cells.

Brandt discloses a recombinant vaccinia virus containing a deletion of the N-terminal domain of the E3L protein (E3Ldelta83N)(a recombinant vaccinia virus containing a deletion of the N-terminal domain of the E3L protein (E3Ldelta83N); abstract, page 264, first column, third paragraph), wherein the viral strain is attenuated by 100-fold, but still enables the production of an immune response (wherein the viral strain is attenuated by 100-fold, but still enables the production of an immune response; page 264, first column, fourth paragraph).

Morningside discloses oncolytic viruses for the treatment of cancer (oncolytic viruses for the treatment of cancer; abstract), wherein the viruses encode a gene for a PD-1 binding agent, such as an antibody fragment (wherein the viruses encode a gene for a PD-1 binding agent, such as an antibody fragment; abstract) to induce cancer killing immune response (to induce cancer killing immune response; abstract); wherein the construct comprises tumor-cell specific promoters (wherein the construct comprises tumor-cell specific promoters; paragraph [0062]).

-***-Continued on Next Supplemental Page-***-

-***-Continued from Previous Supplemental Page:-***-

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of SillaJen to have included an EL3delta83N modification in order to attenuate the virus, as disclosed by Brandt, reducing its toxicity to a host, while retaining the potential of the virus to produce an immune response. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of SillaJen to have provided concurrent immunotherapy, as disclosed by SillaJen, including the production of an anti-PD-1 antibody by the virus specifically in host tumor cells, as disclosed by Morningside, in order to avoid potential negative side effects of long-term production of the immune modulator by non-tumor cells infected by the vector, or to provide the antibody, itself, concurrently, to enable clearance of the antibody by the subject, minimizing the duration of the effects in time to avoid undesirable autoimmune effects

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 SillaJen, Brandt and Morningside references, unity of invention is lacking.