(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 April 2024 (18.04.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/077601 A1

(51) International Patent Classification:
A61K 39/07 (2006.01) *A61K 38/17* (2006.01)
A61K 38/03 (2006.01) *C07K 14/47* (2006.01)

(21) International Application Number:
PCT/CN2022/125413

(22) International Filing Date:
14 October 2022 (14.10.2022)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicants: **GUANGDONG TCRCURE BIOPHARMA TECHNOLOGY CO., LTD.** [CN/CN]; Building 12, No.6, Nanjiang Second Road, Zhujiang Street, Nansha District, Guangzhou, Guangdong 511462 (CN). **CHONGQING TCRCURE SCIENCE AND TECHNOLOGY CO., LTD.** [CN/CN]; 10th Floor, Building 5, Tianan Digital City, 101 Cuibai Road, Chunhui Road, Dadukou District, Chongqing 400084 (CN).

(72) Inventors: **ZHANG, Lifeng**; Building 12, No.6, Nanjiang Second Road, Zhujiang Street, Nansha District, Guangzhou, Guangdong 511462 (CN). **WU, Haiyang**; 10th Floor, Building 5, Tianan Digital City, 101 Cuibai Road, Chunhui Road, Dadukou District, Chongqing 400084 (CN). **XU, Wei**; 10th Floor, Building 5, Tianan Digital City, 101 Cuibai Road, Chunhui Road, Dadukou District, Chongqing 400084 (CN).

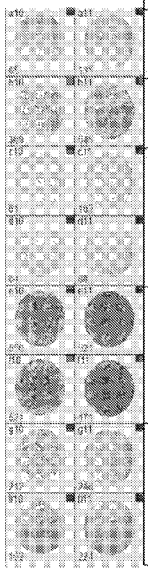
(74) Agent: **BEIJING QIKUN INTELLECTUAL PROPERTY AGENCY LTD.**; Huang Kejun Building B-1616, Xing Fu Building, No.3 Dong San Huan Bei Road, Chaoyang District, Beijing 100027 (CN).

(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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, 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,

(54) Title: PEPTIDE VACCINES AGAINST GLIOMA AND USES THEREOF

(57) Abstract: A peptide vaccine pharmaceutical composition and a method of use for stimulating immune responses against the glioma H3K27M mutation are provided. Having a length of at least 12 amino acid residues, the peptide vaccine is capable of stimulating CD4 T cell response, and optionally CD8 T cell response as well, after administration.



Group	EN-17	EN-23
PSG1	0.50%	0.45%
PSG2_KQ	**2.84%**	**1.82%**
PSG2_RM	0.47%	0.34%
	0.49%	0.29%
PHA	4.28%	1.74%
	4.01%	1.57%
NCG	0.53%	0.35%
	0.48%	0.32%

FIG. 1

WO 2024/077601 A1

[Continued on next page]

LU, LV, MC, ME, 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))*

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

PEPTIDE VACCINES AGAINST GLIOMA AND USES THEREOF

BACKGROUND

[0001] Diffuse intrinsic pontine glioma (DIPG) is a rare and dismal brain tumor that primarily affects children, with most diagnoses occurring between 4 and 7 years of age. Approximately 200-300 children are diagnosed with DIPG per year in the United States. The medium survival of DIPG patients is 9-12 months after diagnosis. The location of the tumor, pons, contains nerves sustaining basic life functions. Therefore, to surgically remove the tumor is not an option. Currently radiotherapy is the only responsive treatment for DIPG. No improvement in patient survival has been achieved for more than four decades.

[0002] The point mutation of replacing the lysine 27 (K27) on the tail of histone H3 with a methionine residue (M), or H3K27M, has been identified as the driver mutation of DIPG (Schwartzentruber J, et al., 2012; Wu G et al., 2012). It is estimated that 60-70% DIPG tumors carry heterozygous mutations of H3K27M. All H3K27M occurs among two histone H3 variants (H3.1 and H3.3), with the H3.3 mutation being found to occur more frequently (~70%) (Argersinger DP, et al. 2021; Zhang X et al., 2019). It is also noteworthy that in addition to DIPG, the H3K27M mutations have also been identified to be associated with other pediatric brain tumors (e.g., pediatric high-grade glioma), and with several other adult cancers, including glioma, acute myeloid leukaemia (AML), and melanoma, etc. (Lowe BR, et al. 2019)

[0003] Previously, a peptide vaccine against H3K27M has been developed to treat DIPG (WO2016179326A1). The peptide vaccine substantially contains a 10 amino acid (10-AA) long peptide, (R/A)MSAP(S/A)TGGV (SEQ ID NO:), which is an epitope restricted to HLA-A*02, and thus the peptide vaccine is designed to specifically trigger CD8 T cell responses or cytotoxic T cell responses. However, another important T cell responses, i.e., $CD4^+$ T cell responses or the T-helper cell mediated immune responses, which have profound impacts on the antitumor immunity, are purposefully excluded in the vaccine design in this above art, because the peptide is as short as 10 amino acids which is only able to trigger HLA class I restricted responses. It has been shown that the $CD8^+$ T cell epitopes bound by MHC class I range from 8 to 11 residues (Rosa DS, et al. 2010; Hemmer B et al., 2000), whereas the $CD4^+$ T lymphocyte usually recognize peptides of

12-16 amino acids (Rosa DS, et al. 2010; Hemmer B et al., 2000) or 13-17 amino acids (Chicz RM, et al. 1992; Sercarz EE, et al. 2003).

SUMMARY

5

[0004] In a first aspect, a pharmaceutical composition is provided in the present disclosure, which is substantially a peptide vaccine composition. The pharmaceutical composition comprises a peptide (i.e. peptide vaccine), which has a length of at least 12 amino acid residues and comprises a 4-AA peptide segment RMSA (SEQ ID NO: 26). The pharmaceutical composition is, after administration to a subject according to a therapeutically effective regimen thereof, capable of stimulating CD4 T cell response against histone 3 (H3) K27M mutation (H3K27M) in the subject. Thus in contrast with the 10-AA peptide vaccine disclosed in WO2016179326A1, which can only stimulate the CD8 T cell responses against the H3.3K27M mutation, the peptide vaccine provided in this present disclosure can stimulate the CD4 T cell response against H3K27M mutation.

10

15

[0005] Herein, the 4-AA peptide segment RMSA (SEQ ID NO: 26) in the peptide vaccine substantially corresponds to the "K27M" mutant form (as cited in the literature) of the 4-AA region RKSA (SEQ ID NO: 30), which corresponds to position 27-30 of both the human histone 3.1 (i.e., H3.1) variant (SEQ ID NO: 22) and the human histone 3.3 (i.e., H3.3) variant (SEQ ID NO: 24). Thus the peptide vaccine provided herein is able to stimulate the CD4 T cell response against both the H3.1 K27M mutation and the H3.3 K27M mutation in the subject, depending on the sequence of the peptide vaccine.

20

[0006] According to some embodiments, the pharmaceutical composition is further capable of stimulating CD8 T cell response against the H3K27M mutation in the subject after its administration. In other words, some embodiments of the peptide vaccine can stimulate both CD4 T cell response and CD8 T cell response after administration.

25

[0007] According to some embodiments of the pharmaceutical composition, the peptide comprises a 10-AA segment of RMSAP(S/A)TGGV (SEQ ID NO: 25). Herein, depending on the different mutant H3 genotypes of the target tumour tissues in the subject, the peptide vaccine may comprise a 10-AA segment of RMSAPSTGGV (SEQ ID NO: 5) to thereby be able to stimulate the CD4, and optionally CD8, T cell responses against the H3.3K27M mutation in the subject, or comprise a 10-AA segment of RMSAPATGGV (SEQ ID NO: 29) to thereby be able to stimulate the CD4, and optionally CD8, T cell responses against the H3.1K27M mutation in the subject.

30

35

[0008] Further according to some embodiments of the pharmaceutical composition, the

peptide comprises a 18-AA segment of KQLATKAARMSAP(S/A)TGGV (SEQ ID NO: 1). Depending on the different mutant H3 genotypes of the target tumour tissues in the subject, the peptide vaccine may comprise an 18-AA segment of KQLATKAARMSAPSTGGV (SEQ ID NO: 2) to thereby be able to stimulate the CD4, and optionally CD8, T cell responses against the H3.3K27M mutation in the subject, or comprise an 18-AA segment of KQLATKAARMSAPATGGV (SEQ ID NO: 27) to thereby be able to stimulate the CD4, and optionally CD8, T cell responses against the H3.1K27M mutation in the subject.

[0009] Further according to one specific embodiment, the peptide in the pharmaceutical composition consists of the 18-AA KQLATKAARMSAPSTGGV (SEQ ID NO: 2). As illustrated in great detail by the Examples set forth below, this 18-AA peptide vaccine, when formulated in a pharmaceutical composition comprising the poly ICLC adjuvant and administered to the patients, both the CD4 and CD8 T cell responses against the H3.3K27M mutation can be stimulated. will be provided in the Examples set forth below.

[0010] According to another specific embodiment, the peptide in the pharmaceutical composition consists of the 18-AA KQLATKAARMSAPATGGV (SEQ ID NO: 27), which is designed to stimulate similar T cell responses against the H3.1K27M mutation.

[0011] In any of the embodiments as set forth above, the pharmaceutical composition can further comprise an adjuvant. According to some embodiments, the adjuvant comprises a toll-like receptor (TLR) agonist, which can optionally include ligands for any of TLR3, TLR4, TLR7/TLR8, and TLR9. Optionally, a TLR3 ligand is used as an adjuvant for the pharmaceutical composition comprising the peptide vaccine, such as poly I:C (polyriboinosinic: polyribocytidylic acid). Further optionally, certain derivatives of poly I:C with improved stability, safety and/or adjuvanticity, such as Poly ICLC and Poly I:C12U. Optionally, other TLR ligands can also be used, and non-limiting examples can include monophosphoryl lipid A (MPL, as TLR4 ligand) or its derivatives, imiquimod and resiquimod (both as TLR7/8 ligand), cpG ODN (TLR9 ligand). More examples and relevant information for such adjuvant can reference to US patent application No.: US20110038888A1, Toussi DN and Massari P, 2014, whose disclosure is incorporated by reference in its entirety. According to some other embodiments, the adjuvant comprises an exogenous substance other than the TLR agonist, which can include Bacillus Calmette-Guerin (BCG) vaccine, but can also include a substance with diverse nature and material property.

[0012] Herein optionally, poly ICLC is used as the adjuvant, and in the pharmaceutical

composition, poly ICLC and the peptide can have a ratio of approximately 1:0.5-1:5 (e.g., 1:1, 1:2, 1:3, 1:4) by weight.

[0013] Further optionally, the pharmaceutical composition can be configured to have a dosage form that comprises approximately 0.5 mg poly ICLC and approximately 0.5-2 mg of the peptide.

[0014] Further optionally, the pharmaceutical composition can be configured to be in a formulation that is suitable for subcutaneous injection to the subject.

[0015] In a second aspect, a method of using the pharmaceutical composition provided in the first aspect to stimulate an immune response in a subject in need thereof is further provided.

[0016] As used herein, a "subject" is referred to as a human individual having a cancer, and the cancer is characterized to have a K27M mutation in histone H3 in cancer tissues/cells. Non-limiting examples of a subject can include a human individual with glioma, DIPG, acute myeloid leukaemia (AML), melanoma (Lowe BR, et al. 2019), but other examples of the subject can also include other cancers as long as the cancer tissues/cells contain H3K27M. It is also to be noted that although currently K27M mutation has been identified only in two variants of histone H3, i.e., H3.1 and H3.3, the pharmaceutical composition and method of uses thereof as disclosed herein shall also be interpreted to cover other H3 variants (e.g., H3.2, SEQ ID NO: 23) or other H3 mutations, as long as the variant or mutant H3 contains K27M.

[0017] Herein, the method substantially comprises a step of: administering, on a therapeutically effective regimen, the pharmaceutical composition according to any one of the embodiments as described above in the first aspect to the subject, thereby inducing the immune response in the subject. Herein, the immune response comprises CD4 T cell response against the H3K27M mutation, and optionally, the immune response further comprises CD8 T cell response against the H3K27M mutation.

[0018] According to some embodiments of the method, prior to the administering step, the method further comprises a step of: determining the mutated variant type of H3 in the subject.

[0019] Herein, if the mutated variant type of H3 is determined to be H3.3, in the administering step, the peptide in the pharmaceutic composition comprises RMSAPSTGGV (SEQ ID NO: 5). Further optionally, the peptide in the pharmaceutic composition comprises KQLATKAARMSAPSTGGV (SEQ ID NO: 2). Further optionally, the peptide has a length of 18 AA, and consists of

KQLATKAARMSAPSTGGV (SEQ ID NO: 2).

[0020] Herein, if the mutated variant type of H3 is determined to be H3.1, in the administering step, the peptide in the pharmaceutic composition comprises RMSAPATGGV (SEQ ID NO: 29). Further optionally, the peptide in the pharmaceutic composition comprises KQLATKAARMSAPATGGV (SEQ ID NO: 27). Further optionally, the peptide has a length of 18 AA, and consists of KQLATKAARMSAPATGGV (SEQ ID NO: 27).

[0021] In any of the embodiments of the method described above, the cancer can comprise at least one of glioma, acute myeloid leukaemia (AML), or melanoma. Optionally, the cancer can comprise glioma, and further optionally, the cancer comprises DIPG.

[0022] In any of the embodiments of the method described above, the subject carries HLA-A*02 allele.

[0023] In any of the embodiments of the method described above, the subject carries at least one of an HLA-DRB1*07:01 allele or an HLA-DRB1*01:01 allele.

[0024] In any of the embodiments of the method described above, the therapeutically effective regimen comprises administering the pharmaceutical composition to the subject through subcutaneous injection.

[0025] In a third aspect, the present disclosure further provides a T-cell that expresses a T-cell receptor (TCR) or a functional fragment thereof.

[0026] Herein the TCR or its functional fragment is capable of binding to a peptide/MHC II complex, wherein the peptide in the peptide/MHC II complex has a length of at least 12 amino acid residues and comprises RMSA (SEQ ID NO: 26).

[0027] According to some embodiments of the T-cell, the TCR or its functional fragment is further capable of binding to a complex formed between the peptide and MHC I.

[0028] Herein optionally, the peptide comprises RMSAP(S/A)TGGV (SEQ ID NO: 25). Further optionally, the peptide comprises KQLATKAARMSAP(S/A)TGGV (SEQ ID NO: 1).

[0029] Herein according to some embodiments of the T cell, the peptide comprises KQLATKAARMSAPSTGGV (SEQ ID NO: 2), and further optionally, the peptide has a length of 18 AA, and consists of KQLATKAARMSAPSTGGV (SEQ ID NO: 2). Herein optionally, the TCR or the fragment thereof is incapable of bind to a complex between a second peptide and MHC II complex, and the second peptide comprises an amino acid sequence of KQLATKAAR**K**SAPSTGGV (SEQ ID NO: 6).

[0030] Herein according to some other embodiments of the T cell, the peptide comprises

KQLATKAARMSAPATGGV (SEQ ID NO: 27), and further optionally, the peptide has a length of 18 AA, and consists of KQLATKAARMSAPATGGV (SEQ ID NO: 27). Herein optionally, the TCR or the fragment thereof is incapable of bind to a complex between a second peptide and MHC II complex, and the second peptide comprises an amino acid sequence of KQLATKAARKSAPATGGV (SEQ ID NO: 28).

[0031] According to some embodiments of the T-cell, the TCR is heterologous to the T-cell. Here optionally, the TCR is transduced or transfected into the T-cell via a vector, such as a retroviral vector.

[0032] All the sequences referenced in here, and elsewhere in the application as well, are listed in Table 1.

Table 1. Sequences referenced in the application

Sequences	Length	SEQ ID NOS	NOTE
KQLATKAARMSAP(S/A)TGGV	18	SEQ ID NO: 1	H3.1&H3.3 (K27M)
KQLATKAARMSAPSTGGV	18	SEQ ID NO: 2	H3.3 (K27M); "KQ-RMS"
KQLATKAARKSAPSTGGV	18	SEQ ID NO: 6	H3.3 (w/o K27M); "KQ-RKS"
KQLATKAARMSAPATGGV	18	SEQ ID NO: 27	H3.1 (K27M)
KQLATKAARKSAPATGGV	18	SEQ ID NO: 28	H3.1 (w/o K27M)
KQLATKAARMSAPATGGV	18	SEQ ID NO: 3	H3.1 (K27M)
(R/A)MSAP(S/A)TGGV	10	SEQ ID NO: 4	H3.1&H3.3 (K27M)
RMSAP(S/A)TGGV	10	SEQ ID NO: 25	
RMSAPSTGGV	10	SEQ ID NO: 5	H3.3 (K27M); "RMS"
RKSAPSTGGV	10	SEQ ID NO: 7	H3.3 (w/o K27M); "RKS"
RMSAPATGGV	10	SEQ ID NO: 29	H3.1 (K27M)
RMSA	4	SEQ ID NO: 26	mutant 4-AA core region in H3.1 and H3.3
RKSA	4	SEQ ID NO: 30	wildtype 4-AA core region in H3.1 and H3.3
KQLATKAARMSA	12	SEQ ID NO: 8	
KQLATKAARMSAP	13	SEQ ID NO: 9	
KQLATKAARMSAPS	14	SEQ ID NO: 10	
KQLATKAARMSAPST	15	SEQ ID NO: 11	
KQLATKAARMSAPSTG	16	SEQ ID NO: 12	
KQLATKAARMSAPSTGG	17	SEQ ID NO: 13	
QLATKAARMSAP	12	SEQ ID NO: 14	
ATKAARMSAPST	12	SEQ ID NO: 15	
KQLATKAARMSA	12	SEQ ID NO: 16	
TKAARMSAPSTG	12	SEQ ID NO: 17	
ATKAARMSAPSTG	13	SEQ ID NO: 18	
LATKAARMSAPST	13	SEQ ID NO: 19	
TKAARMSAPSTGG	13	SEQ ID NO: 20	
AARMSAPSTGGV	12	SEQ ID NO: 21	
MARTKQTARKSTGGKAPRKQL ATKAAR**K**SAPATGGVKKPHRY RPGTVALREIRRYQKSTELLIRK LPFQRLVREIAQDFKTDLRFQS	136	SEQ ID NO: 22	H3.1 (wildtype); NP_003522.1; The underlined and bold-font "K" represents the residue for the H3.1K27M mutation

SAVMALQEACEAYLVGLFEDT NLCAIHAKRVTIMPKDIQLARR IRGERA			
MARTKQTARKSTGGKAPRKQL ATKAARKSAPATGGVKKPHRY RPGTVALREIRRYQKSTELLIRK LPFQRLVREIAQDFKTDLRFQS SAVMALQEASEAYLVGLFEDT NLCAIHAKRVTIMPKDIQLARR IRGERA	136	SEQ ID NO: 23	H3.2 (wildtype); NP_066403.2;
MARTKQTARKSTGGKAPRKQL ATKAAR**K**SAPSTGGVKKPHRY RPGTVALREIRRYQKSTELLIRK LPFQRLVREIAQDFKTDLRFQS AAIGALQEASEAYLVGLFEDTN LCAIHAKRVTIMPKDIQLARRIR GERA	136	SEQ ID NO: 24	H3.3 (wildtype); NP_005315.1; The underlined and bold-font "K" represents the residue for the H3.3K27M mutation.

DEFINITIONS

[0033] As used herein, the terms “about” or “approximately” when used in conjunction with a number refers to any number within 1, 5 or 10% of the referenced number.

[0034] As used herein, the terms “neoplasia,” “hyperplasia,” and “tumor” are often commonly referred to as “cancer,” which is a general name for more than 100 diseases that are characterized by uncontrolled, abnormal growth of cells. As used herein, a “tumor” also includes a normal, benign, or malignant mass of tissue.

[0035] As used herein, the term “peptide” refers to a polymer of amino acids linked by amide bonds (or peptide bonds) as is known to those of skill in the art. A peptide can be a polymer of 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids linked by covalent amide bonds. In some embodiments, the peptide is a polymer of 6 to 8, 8 to 10, 10 to 15, 10 to 20, 10 to 25, 10 to 30, 10 to 40, 10 to 50, or 25 to 25 amino acids linked by covalent amide bonds. In certain embodiments, the peptide is a polymer of 50 to 65, 50 to 75, 50 to 85, 50 to 95, 50 to 100, 75 to 100 amino acids linked by covalent amide bonds. As used herein, the term can refer to a single peptide chain linked by covalent amide bonds. The term can also refer to multiple peptide chains associated by non-covalent interactions such as ionic contacts, hydrogen bonds, Van der Waals contacts and hydrophobic contacts. Those of skill in the art will recognize that the term includes peptides that have been modified, for example by post-translational processing such as signal peptide cleavage, disulfide bond formation, glycosylation (e.g.,

N-linked glycosylation), protease cleavage and lipid modification (e.g. S-palmitoylation).

[0036] As used herein, the term “conservative substitution” refers to replacement of an amino acid of one class with another amino acid of the same class. In particular embodiments, a conservative substitution does not alter the structure or function, or both, of a peptide. Classes of amino acids for the purposes of conservative substitution include hydrophobic (Met, Ala, Val, Leu, Ile), neutral hydrophilic (Cys, Ser, Thr), acidic (Asp, Glu), basic (Asn, Gln, His, Lys, Arg), conformation disrupters (Gly, Pro) and aromatic (Trp, Tyr, Phe).

[0037] As used herein, the term "immune response" means a reaction occurring within a human subject that is purported for fighting cancers. An immune response may include the innate immune response and the adaptive immune response; while the former includes immune cells such as neutrophils, macrophages, and monocytes, and cytokines and complement, the latter includes immune cells such as dendritic cells, T cells, B cells, and antibodies to stimulate antigen-specific immune reactions.

[0038] The term “T-cell response” is referred to as one type of adaptive immune response mediated by T cells, which typically includes the specific proliferation and activation of effector functions induced by a peptide *in vitro* or *in vivo* against the specific antigens. There are two major subtypes of T cells: $CD8^+$ T cells (i.e. killer T cells, cytotoxic T cells, or effector T cells) and $CD4^+$ T cells (i.e. helper T cells), and thus the T cell response include the CD4 T cell response and the CD8 T cell response.

[0039] The term "CD8 T cell response", "$CD8^+$ T cell response" or alike, means one type of the T cell response that is mediated by $CD8^+$ T cells or $CD8^+$ lymphocytes. The $CD8^+$ T cells typically serve to destroy virus-infected cells and tumor cells, and typically recognize their targets by binding to short peptide (8-11 AA in length) associated with MHC class I molecules present on the surface of all nucleated cells. The $CD8^+$ T cells also produce several key cytokines including TNFα, IL-2 and IFNγ, which can influence the effector functions of other cells, in particular macrophages and NK cells.

[0040] The term "CD4 T cell response", "$CD4^+$ T cell response" or alike, means another type of the T cell response that is mediated by $CD4^+$ T cells or $CD4^+$ lymphocytes. The $CD4^+$ T cells typically assist other lymphocytes, including maturation of B cells and activation of cytotoxic T cells and macrophages. When the $CD4^+$ T cells are presented with longer peptide antigens (12-16 AA in length) by MHC class II molecules, which are expressed on the surface of antigen-presenting cells (APCs), the $CD4^+$ T cells become activated such that they divide rapidly and secrete cytokines (e.g. IFNγ, IL-2, etc.) that

regulate or assist the immune response. Herein, the term "antigen-presenting cells" refers to dendritic cells (DC), monocytes/macrophages, B lymphocytes or other cell type(s) expressing the necessary MHC/co-stimulatory molecules, which effectively allow for T cell recognition of the presented peptide.

[0041] The term "MHC" refers to "major histocompatibility antigen". In humans, the MHC genes are known as HLA ("human leukocyte antigen") genes. Although there is no consistently followed convention, some literature uses HLA to refer to HLA protein molecules, and MHC to refer to the genes encoding the HLA proteins. As such the terms "MHC" and "HLA" are equivalents when used herein. The HLA system in man has its equivalent in the mouse, i.e., the H2 system. The most intensely-studied HLA genes are the nine so-called classical MHC genes: HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, and HLA-DRB1. In humans, the MHC is divided into three regions: Class I, II, and III. The A, B, and C genes belong to MHC class I, whereas the six D genes belong to class II. MHC class I molecules are made of a single polymorphic chain containing 3 domains (alpha 1, 2 and 3), which associates with beta 2 microglobulin at cell surface. Class II molecules are made of 2 polymorphic chains, each containing 2 chains (alpha and beta).

[0042] Class I MHC molecules are expressed on virtually all nucleated cells. Peptide fragments presented in the context of class I MHC molecules are recognized by CD8 T lymphocytes (cytotoxic T lymphocytes or CTLs). $CD8^+$ T cells or lymphocytes frequently mature into cytotoxic effectors which can lyse cells bearing the stimulating antigen. Class II MHC molecules are expressed primarily on activated lymphocytes and antigen-presenting cells. $CD4^+$ T lymphocytes (helper T lymphocytes or HTLs) are activated with recognition of a unique peptide fragment presented by a class II MHC molecule, usually found on an antigen presenting cell like a macrophage or dendritic cell. $CD4^+$ T lymphocytes proliferate and secrete cytokines that either support an antibody-mediated response through the production of IL-4 and IL-10 or support a cell-mediated response through the production of IL-2 and IFNγ.

[0043] Functional HLAs are characterized by a deep binding groove to which endogenous as well as foreign, potentially antigenic peptides bind. The groove is further characterized by a well-defined shape and physicochemical properties. HLA class I binding sites are closed, in that the peptide termini are pinned down into the ends of the groove. They are also involved in a network of hydrogen bonds with conserved HLA residues. In view of these restraints, the length of bound peptides is limited to 8-10 residues. In contrast to

HLA class I binding sites, class II sites are open at both ends. This allows peptides to extend from the actual region of binding, thereby “hanging out” at both ends. Class II HLAs can therefore bind peptide ligands of variable length, usually more than 12 amino acid residues.

5 **[0044]** As used herein, the term "adjuvant" is referred to as an exogenous substance that, when simultaneous administered to a subject with a particular vaccine (e.g. the peptide vaccine disclosed herein) can enhance the immune responses in the subject to the vaccine, and is also called "immune adjuvant". There is no limitation to the adjuvant that can be used simultaneously with the peptide vaccine as disclosed herein, which can include 10 exogenous substances that have a wide variety of nature and origin, such as mineral salts, oil and water-based emulsions, polymers, microparticles, liposomes, saponins, microbial products and cytokines. There is also no limitation to the mechanisms of action of the adjuvant in the present disclosure, which can involve non-specific effects (i.e., antigen depot at the immunization site) to specific activation of immune cells leading to improved 15 host innate and adaptive responses.

[0045] As used herein, the term "poly I:C" is referred to as polyinosinic:polycytidylic acid, and can also be abbreviated as poly(I:C) or alike, which is typically used in the form of a sodium salt to simulate immune response. The term "poly ICLC", or poly I:CLC or alike, is referred to as a mixture of poly I:C with stabilizers carboxymethylcellulose and 20 polylysine.

[0046] As used herein, the term “therapeutically effective regimen” refers to a regimen for dosing, timing, frequency, manner, and duration of the administration of the pharmaceutical composition comprising the peptide vaccine in a subject in need thereof for the treatment and/or management of his or her cancer that is characterized by having 25 the H3K27M mutation, and the dosing, timing, frequency, and duration of the administration are configured in a level that is sufficient to stimulate the immune responses (e.g. CD4, and optionally CD8, T cell responses) against the H3K27M mutation in the subject.

[0047] Unless specifically stated or obvious from context, as used herein, the term “or” is 30 understood to be inclusive.

[0048] Unless specifically stated or obvious from context, as used herein, the terms “a”, “an”, and “the” are understood to be singular or plural.

[0049] Unless specifically stated or obvious from context, as used herein, the term "approximately", “about”, "around", or alike, is understood as within a range of normal

tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless obvious from context, all numerical values provided herein can be understood to be modified by the term.

5 **[0050]** The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

[0051] The term "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word 10 "substantially" may be omitted from the definition of the invention.

[0052] All references, patents, or patent applications as referred to herein are specifically incorporated by reference in their entireties.

BRIEF DESCRIPTION OF THE DRAWINGS

15

[0053] FIG. 1 shows the IFN-γ ELISpot results. Briefly, the peripheral blood mononuclear cells (PBMC) samples from two DIPG patients: EN-17 and EN-23 were cultured with antigen stimulation. After culture expansion and antigen stimulation, 20,000 cells from each sample were collected into each well for an ELISpot assay to detect IFN-γ secreting 20 cells. PSG1: peptide stimulated group 1. The cells were stimulated with the corresponding peptide only once and expanded for 2-3 weeks. PSG2: peptide stimulated group 2. The cells were stimulated with the corresponding peptide at the beginning of the culture expansion, and then re-stimulated with the antigen peptide before the ELISpot assay. PHA: phytohaemagglutinin, a positive control antigen for ELISpot. NCG: negative 25 control group (no peptide stimulation). KQ: The long peptide (18 aa) carrying the point mutation. RM: The short peptide (10 aa) carrying the point mutation.

[0054] FIG. 2 shows the EN-17 C27 TCR validation results. Briefly, EN-17 C27 is a candidate TCR cloned from CD4 T cells. A selected PBMC sample was transduced with a retroviral vector carrying the candidate TCR. Transduced PBMC cells were then 30 subjected to different peptide stimulations and the T cell responses were examined using FACS. The CD4 T cells and CD8 T cells were gated and studied separately. All the FACS results are shown in the same format, with the T cell activation markers shown along the X axis and SSC (i.e. side scatter, which is related to the granularity of cells, and thus is an optical measurement for each individual cells in the FACS analysis) shown along the Y

axis. **(A)** CD4 T cell responses. **(B)** CD8 T cell responses.

[0055] FIG. 3 shows the EN-10 C01 and C04 TCR validation results. Briefly, EN-10 C01 and C04 are two candidate TCRs cloned from CD8 T cells. The selected PBMC samples were transduced with the corresponding retroviral vector carrying the candidate TCR. Transduced PBMC cells were then subjected to different peptide stimulations and the T cell responses were examined using FACS. Only CD8 T cell responses are shown. CD4 T cell responses are weak (Data not shown). All the FACS results are shown in the same format, with the T cell activation markers shown along the X axis and SSC shown along the Y axis. **(A)** EN-10 C01. **(B)** EN-10 C04.

DETAILED DESCRIPTION

[0056] The present disclosure provides a peptide vaccine that can be used to stimulate the CD4 T cell response, and optionally the CD8 T cell response as well, against the H3K27M mutation in subjects carrying the somatic mutation who have been administered with a pharmaceutical composition comprising the peptide vaccine. Due to the capability to stimulate significant T cell responses against this clinically crucial mutation that is strongly associated with human glioma, and especially the pediatric and adult DIPG, this peptide vaccine has the potential for treating the disease.

[0057] In a first aspect, a pharmaceutical composition is provided, which comprises a peptide (i.e., peptide vaccine) having a length of at least 12 amino acid residues (e.g., 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, etc.) and comprises a 4-AA peptide segment RMSA (SEQ ID NO: 26). The pharmaceutical composition is capable, after administration to a subject according to a therapeutically effective regimen thereof, of stimulating CD4 T cell response against the H3K27M mutation in the subject. Further optionally and preferably, the pharmaceutical composition is further capable of stimulating the CD8 T cell responses.

[0058] In other words, according some embodiments, the pharmaceutical composition comprising the peptide vaccine can stimulate only the CD4 T cell responses, but not the CD8 T cell responses, against the H3K27M mutation; whereas according some other embodiments, the pharmaceutical composition comprising the peptide vaccine can stimulate both the CD4 T cell responses and the CD8 cell responses, against the H3K27M mutation.

[0059] Thus in contrast with the 10-AA peptide vaccine disclosed in WO2016179326A1, which can only stimulate the CD8 T cell responses against the H3.3K27M mutation, the

peptide vaccine in the pharmaceutical composition provided in this present disclosure can distinguishingly stimulate the CD4 T cell response against H3K27M mutation.

[0060] Herein the H3 can be either H3.1 or H3.3, whose wildtype sequences are listed in SEQ ID NO: 22 and SEQ ID NO: 24 respectively. The subject carrying the H3.1K27M mutation or the H3.3K27M mutation may benefit from the peptide vaccine, who may have at least one of glioma (e.g., DIPG), acute myeloid leukaemia (AML), and melanoma, etc.

[0061] According to some embodiments, the peptide comprises a 10-AA segment of RMSAP(S/A)TGGV (SEQ ID NO: 25). Herein, depending on the different mutant H3 genotypes of the target tumour tissues in the subject, the peptide vaccine may comprise a 10-AA segment of RMSAPSTGGV (SEQ ID NO: 5) to thereby be able to stimulate the CD4, and optionally CD8, T cell responses against the H3.3K27M mutation in the subject, or alternatively may comprise a 10-AA segment of RMSAPATGGV (SEQ ID NO: 29) to thereby be able to stimulate the CD4, and optionally CD8, T cell responses against the H3.1K27M mutation in the subject, or alternatively may comprise both of the above two peptides, one of which comprise the 10-AA segment of SEQ ID NO: 25, and another of which comprise the 10-AA segment of SEQ ID NO: 29.

[0062] Further according to some embodiments of the pharmaceutical composition, the peptide comprises a 18-AA segment of KQLATKAARMSAP(S/A)TGGV (SEQ ID NO: 1). Depending on the different mutant H3 genotypes of the target tumour tissues in the subject, the peptide vaccine may comprise an 18-AA segment of KQLATKAARMSAPSTGGV (SEQ ID NO: 2) to thereby be able to stimulate the CD4, and optionally CD8, T cell responses against the H3.3K27M mutation in the subject, or comprise an 18-AA segment of KQLATKAARMSAPATGGV (SEQ ID NO: 27) to thereby be able to stimulate the CD4, and optionally CD8, T cell responses against the H3.1K27M mutation in the subject, or alternatively comprise both.

[0063] Further according to one specific embodiment, the peptide in the pharmaceutical composition consists of the 18-AA sequence KQLATKAARMSAPSTGGV (SEQ ID NO: 2) that specifically targets H3.3K27M, which will be covered in more detail in Example 1 below for experimental data. According to another specific embodiment, the peptide in the pharmaceutical composition consists of the 18-AA sequence KQLATKAARMSAPATGGV (SEQ ID NO: 27) that specifically targets H3.1K27M.

[0064] It is noted that depending on different needs, the pharmaceutical composition may comprise one single peptide as described above, or may comprise a combination of

multiple peptides as described above.

[0065] The pharmaceutical composition may be formulated in a dosage form that allows the administration thereof into a subject in need. As such, the pharmaceutical composition may comprise, in addition to the peptide vaccine, an adjuvant, which may comprise, for example, poly ICLC. The pharmaceutical composition may be formulated to allow administration via subcutaneous injection, but may also be formulated to allow other administration manners as known by the skilled artisan in the field.

[0066] In a second aspect, a method of using the pharmaceutical composition as described above in the first aspect is further provided. Such method may be directed to using the pharmaceutical composition for stimulating an immune response, especially the T cell responses, in a subject in need thereof, who may have a cancer (e.g., DIPG, glioma, AML, or melanoma, etc.) and may carry the H3K27M mutation.

[0067] Herein, the method substantially comprises a step of: administering, on a therapeutically effective regimen, the pharmaceutical composition according to any one of the embodiments as described above in the first aspect to the subject, thereby inducing the immune response in the subject. Herein, the immune response comprises CD4 T cell response against the H3K27M mutation, and optionally, the immune response further comprises CD8 T cell response against the H3K27M mutation.

[0068] The peptide vaccine in the pharmaceutical composition to be administered to the subject in the method disclosed herein may comprise the 4-AA RMSA (SEQ ID NO: 26), or further optionally may comprise the 10-AA RMSAP(S/A)TGGV (SEQ ID NO: 25), or further optionally may comprise the 18-AA KQLATKAARMSAP(S/A)TGGV (SEQ ID NO: 1), i.e. one of the 18-AA KQLATKAARMSAP<u>S</u>TGGV (SEQ ID NO: 2) or the 18-AA KQLATKAARMSAP<u>A</u>TGGV (SEQ ID NO: 27).

[0069] In the method, the subject may carry the HLA-A*02 allele to thus allow the CD8 T cell response to be stimulated in addition to the CD4 T cell response. However, it is possible that for other subject who may carry an HLA allele other than HLA-A*02, CD8 T cell responses may also be stimulated.

[0070] In the method, the subject may carry at least one of an HLA-DRB1*07:01 allele or an HLA-DRB1*01:01 allele for definitive CD4 T cell response, but may also carry other alleles to also have CD4 T cell response.

[0071] In the method, the administration of the pharmaceutical composition comprising the peptide vaccine may be through subcutaneous injection, but may be through other administration routes.

[0072] In a third aspect, the present disclosure further provides a T-cell that expresses a T-cell receptor (TCR) or a functional fragment thereof. Herein the TCR or its functional fragment is capable of binding to a peptide/MHC II complex, wherein the peptide in the peptide/MHC II complex has a length of at least 12 amino acid residues and comprises the 4-AA segment RMSA (SEQ ID NO: 26), or further optionally comprises the 10-AA segment RMSAP(S/A)TGGV (SEQ ID NO: 25), or further optionally comprises the 18-AA KQLATKAARMSAP(S/A)TGGV (SEQ ID NO: 1). According to some embodiments of the T-cell, the TCR or its functional fragment is further capable of binding to a complex formed between the peptide and MHC I.

[0073] Herein according to one specific embodiment of the T cell, the peptide comprises KQLATKAARMSAPSTGGV (SEQ ID NO: 2), and further optionally, the peptide has a length of 18 AA, and consists of KQLATKAARMSAPSTGGV (SEQ ID NO: 2). Herein optionally, the TCR or the fragment thereof is incapable of bind to a complex between a second peptide and MHC II complex, and the second peptide comprises an amino acid sequence of KQLATKAARKSAPSTGGV (SEQ ID NO: 6). As such, the TCR has a specific binding to the K27M mutant peptide, but not to the wildtype peptide, of the H3.3 variant, conferring capability for specifically stimulating the CD4 T cell responses against the H3.3K27M mutation, but not the wildtype H3.3.

[0074] According to another specific embodiment of the T cell, the peptide comprises KQLATKAARMSAPATGGV (SEQ ID NO: 27), and further optionally, the peptide has a length of 18 AA, and consists of KQLATKAARMSAPATGGV (SEQ ID NO: 27). Herein optionally, the TCR or the fragment thereof is incapable of bind to a complex between a second peptide and MHC II complex, and the second peptide comprises an amino acid sequence of KQLATKAARKSAPATGGV (SEQ ID NO: 28). As such, the TCR has a specific binding to the K27M mutant form, but not to the wildtype form, of H3.1, conferring capability for specifically stimulating the CD4 T cell responses against the H3.1K27M mutation, but not the wildtype H3.1.

[0075] According to some embodiments of the T-cell, the TCR is heterologous to the T-cell. Here optionally, the TCR is transduced or transfected into the T-cell via a vector, such as a retroviral vector.

[0076] In the following, one specific example (Example 1) is provided for the testing of one specific embodiment of the peptide vaccine (i.e., the 18-AA KQLATKAARMSAPSTGGV (SEQ ID NO: 2)) in terms of its capabilities to induce or stimulate immune responses, especially the CD4 T cell responses and/or CD8 T cell

responses against the H3.3K27M mutation, after its administration into DIPG patients.

[0077] EXAMPLE 1

[0078] 1.1. Peptide vaccine design:

[0079] In order to design the peptide vaccine, a computational prediction on HLA binding and antigen presentation was first performed based upon Immune Epitope Database (IEDB, www.iedb.org). Specifically, the MHC-II binding predictions module (IEDB recommended 2.22) was used for the prediction. '12-18' was used as the 'Select length(s)' to let algorithm to predict all epitopes containing the H3K27M mutation with the length from 12AA to 18AA. The 'IEDB recommended 2.22' used a Consensus approach, combining NN-align, SMM-align, CombLib and Sturniolo if any corresponding predictor is available for the HLA allele, otherwise NetMHCIIpan is used. After the prediction, the least predicting score (percentile rank listed in the above tables) was recorded to represent the specificity of a certain epitope binding to the corresponding HLA allele. The smaller the score, the more specific the epitope binds to the HLA allele. The prediction result is summarized in Table 2.

Table 2. Computational Prediction on Class II HLA binding and antigen presentation

Allele	peptide	percentile rank
HLA-DRB1*07:01	KQLATKAA**RMSA** (SEQ ID NO: 8)	9.2
HLA-DRB1*07:01	KQLATKAA**RMSA**P (SEQ ID NO: 9)	12
HLA-DRB1*07:01	KQLATKAA**RMSA**PS (SEQ ID NO: 10)	13
HLA-DRB1*07:01	KQLATKAA**RMSA**PST (SEQ ID NO: 11)	15
HLA-DRB1*07:01	KQLATKAA**RMSA**PSTG (SEQ ID NO: 12)	17
HLA-DRB1*07:01	KQLATKAA**RMSA**PSTGG (SEQ ID NO: 13)	18
HLA-DRB1*07:01	QLATKAA**RMSA**P (SEQ ID NO: 14)	18
HLA-DRB1*01:01	ATKAA**RMSA**PST (SEQ ID NO: 15)	14
HLA-DRB1*01:01	KQLATKAA**RMSA** (SEQ ID NO: 16)	14
HLA-DRB1*01:01	TKAA**RMSA**PSTG (SEQ ID NO: 17)	14
HLA-DRB1*01:01	ATKAA**RMSA**PSTG (SEQ ID NO: 18)	17
HLA-DRB1*01:01	LATKAA**RMSA**PST (SEQ ID NO: 19)	17
HLA-DRB1*01:01	TKAA**RMSA**PSTGG (SEQ ID NO: 20)	17
HLA-DRB1*01:01	AA**RMSA**PSTGGV (SEQ ID NO: 21)	17

Note: the common 4-AA core segment in each sequence is in bold and underlined fonts.

[0080] As the prediction results show, various epitopes containing the point mutation are predicted to have high affinity with class II HLA molecules, and all of the epitopes have the core 4-AA segment (R**M**SA, SEQ ID NO: 26) that harbors the K27M mutation (i.e. the M residue on the 2nd position of the 4-AA segment). It is noted that some of the selected class II HLA molecules are high-frequency molecules in human population, such as HLA-DRB1*01:01 and HLA-DRB1*07:01.

[0081] Based on these above prediction results, a series of candidate peptide vaccines are designed. In addition to the above listed high-rank candidate peptides (i.e. percentile rank <=15, including SEQ ID NOS: 8-11 and 15-17), a longer peptide vaccine candidate designed to have 18 amino acids (i.e. KQLATKAAR**M**SAPSTGGV (SEQ ID NO: 2), with the point mutation residue M located at the 10th position from the N-terminus of the peptide vaccine) was also selected. A series of experiments were subsequently carried out using this 18-AA peptide vaccine candidate.

[0082] 1.2. Experimental tests on the peptide vaccine

[0083] 1.2.1 Patient Recruitment:

[0084] Newly-diagnosed DIPG patients aged 5 and above were selected. After signing the consent, patients with HLA-A*02 subtype and with H3.3K27M genotyping results from the tumor biopsy were given the vaccine treatment.

[0085] 1.2.2 Vaccine Administration:

[0086] The peptide vaccine is first prepared in an appropriate pharmaceutical composition, with three dosage forms (dose #1, #2 and #3) designed (see Table 3), which all showed good safety profiles. The peptide vaccine with the dosage formulation indicated as "dose #3" is finally used for the experiments with patients.

Table 3. Vaccine Recipe and Dose-finding

	Peptide	**Poly ICLC**	**Solution**
dose #1	0.5mg	0.5mg	dissolved in normal saline, total volume 1ml
dose #2	1mg	0.5mg	
dose #3	2mg	0.5mg	

[0087] Briefly, the peptide vaccine is administered (in the dosage formulation dose #3 in Table 2) through subcutaneous injection two weeks after conformal radiotherapy is completed. The day of the first vaccine injection is defined as Day 1(D1). The injections will be administered on D3, D15, D29, D57, D85, and thereafter, one injection every 8

weeks until disease progression. Periphery blood samples were collected from different time points and used for pharmacokinetic (PK) and pharmacodynamic (PD) studies and for TCR cloning work. Blood samples used for TCR cloning were collected from patients after the fifth and sixth vaccine injections.

[0088] All PBMC samples used in TCR cloning were collected from patients received vaccination dose #3. The PBMC sample used for TCR cloning from patient EN17 was collected after the patient received his/her sixth vaccine injection. The PBMC sample used for TCR cloning from patient EN10 was collected after the patient received his/her sixth vaccine injection. The PBMC sample used for TCR cloning from patient EN23 was a combined PBMC sample, from the patient's PBMC samples after the patient received his/her third and fourth vaccine injections.

[0089] 1.2.3 CD4 and CD8 T cell responses detected in the periphery blood samples of vaccinated patients

[0090] Periphery blood samples from vaccinated DIPG patients were collected and Peripheral Blood Mononuclear Cells (PBMCs) were collected by Ficoll Gradient. PBMC samples were stimulated with corresponding peptides for 2-3 weeks after which cells were seeded into IFN-γ detecting ELISpot plates and restimulated with the corresponding peptides. An enhancement of IFN-γ signal was considered as the presence of antigen-specific T cells (FIG. 1). The sample was then single cell RNA sequenced by 10× Genomics. Candidate TCRs were picked based on the transcription profile, introduced into primary human T cells and validated by pulsing the corresponding peptide (FIG. 2 and FIG. 3). In this technical disclosure, we use the data to illustrate that our vaccination approach indeed provoked strong tumor antigen specific immune responses.

[0091] The following terms and/or abbreviations used in this disclosure, including the drawings and the description, are summarized in Table 4.

Table 4. Description of the terms and abbreviations used in Example 1

Terms/Abbreviations	Description
EN-10; EN-17; EN-23	These are the identifiers of three different vaccinated DIPG patients. The identifiers are also used to label PB samples
KQ-RMS or KQ	The long peptide (18 AA) carrying the point mutation (KQLATKAARMSAPSTGGV, SEQ ID NO: 2)
KQ-RKS	The long peptide (18 aa) without the point mutation (KQLATKAARKSAPSTGGV, SEQ ID NO: 6)
RMS or RM	The short peptide (10 aa) carrying the point mutation (RMSAPSTGGV, SEQ ID NO: 5)
RKS	The short peptide (10 aa) without the point mutation (RKSAPSTGGV, SEQ ID NO: 7)

Note: underlined amino acid residues represent the key letters in each term/abbreviation.

[0092] In greater detail, we used specific culture conditions to culture the PBMC samples. The antigen peptide and suitable antigen presenting cells were included in the culture. By doing so, we seek to specifically stimulate the expansion of the antigen-specific T cells. After 2-3 weeks, the cells were seeded into IFN-γ detecting ELISpot plates and restimulated with the corresponding peptides. Upon activation, T cells release IFN-γ in response to antigen stimulation. IFN-γ secretion could be detected by ELISpot. One spot can be interpreted as the detection of one IFN-γ secreting cell. FIG. 1 shows an IFN-γ ELISpot result. The results show that PBMC samples (EN-17; EN-23) responded to the stimulation of the long peptide (KQ), but not to the stimulation of the short peptide (RM). Therefore, T cell responses were detected specifically to the stimulation of the long peptide. Most likely, these are CD4 T cell responses.

[0093] 1.2.4 TCR cloning and experimental procedures:

[0094] The following is a brief description of the TCR cloning experimental procedure. Briefly, PB samples were collected from vaccinated patients at different time points. PBMCs were isolated from the blood samples. Isolated PBMCs were subjected to culture conditions optimised for T cell expansion and antigen stimulation. Autologous B or DC cells culturally expanded separately were used as antigen-presenting cells (APCs) to present the long peptide antigen (MHC class II antigen). Meanwhile, B cells and DCs are also suitable to present the short peptide (MHC class I antigen). Patient T cells were expanded and stimulated by the peptide antigen for a few weeks. To monitor the T cell status along the time course, a small fraction of the cultured T cells were collected at different time points. The collected cells were subjected to a 2nd round of antigen stimulation and the T cell responses were measured by ELIspot and FACS. Samples shown significant T cell activation signals were subjected to single-cell transcriptome sequencing. Candidate TCRs were selected from the single-cell transcriptome data analysis and individually subjected to the downstream functional validation. For the functional validation of each candidate TCR, the TCR was introduced to a selected PBMC sample with suitable HLA subtypes through viral transduction using a retroviral vector. The T cells ($CD4^+$ and $CD8^+$ T cells) from the transduced PBMC sample express the candidate TCR as a foreign transgene. The transduced PBMC sample was expanded and subjected to antigen stimulation. FACS analysis was used to exam the T cell responses. In FACS analysis, $CD4^+$ T cells and $CD8^+$ T cells were gated separately to specifically exam each type's immune responses. More details are provided in the

following.

[0095] First, suitable samples with an enhancement of IFN-γ signal (EN-17 and EN-23 PSG2_KQ) post restimulation were selected and subjected to the downstream single-cell RNA-Seq analysis. More specifically, PBMC samples (post vaccination) were initially stimulated and expanded for 2-3 weeks. Then examined with ELISpot. Samples with enhanced IFN-γ post restimulation, in this ELISpot experiment, EN-17 and EN-23 PSG2-KQ samples were then single cell RNA-sequenced.

[0096] After the single-cell data was analyzed, candidate TCRs were selected based on the transcription profile and subjected to functional validation experiments. More specifically, candidate TCRs were retrovirally (viral backbone pMP71) introduced into primary human T cells. Corresponding peptides were pulsed into TCR transduced T cells and flow cytometry analyses were used to monitor the responses. As an example, FIG. 2 shows the functional validation results of EN-17 C27, a candidate TCR cloned from the CD4 T compartment of EN-17 PBMC sample. The CD4 T cells and CD8 T cells were gated and studied separately. CD134 (OX40) is an activation marker for CD4 T cells. Specifically, the CD4 T cells upregulate the cell-surface expression of CD134 in response to antigen stimulation. CD107a, CD137, and CD69 are three other T cell activation markers. Both CD4 and CD8 T cells upregulate the expression of CD107a and CD137 and CD69 in response to antigen stimulation.

[0097] FIG. 2 shows that EN-17 C27 (a TCR cloned from CD4 T cells) is validated as an H3K27M antigen specific TCR. It does NOT respond to the stimulation of the short peptides (RMS or RKS, Fig. 2A). It specifically responded to the stimulation of the long peptide carrying the point mutation (KQ-RMS), but not to the long peptide without the point mutation (KQ-RKS) (Fig. 2A).

[0098] EN-17 C27 is a CD4 TCR being introduced into PBMC cells as a foreign TCR. Both CD4 and CD8 T cells can be transduced by the retroviral vector. When EN-17 C27 is introduced into a CD8 T cell, it still interacts with its cognate antigen (KQ-RMS presented by class II HLA). However, in this case, the interaction can NOT be reinforced by the CD4 molecule as the host cell is a CD8 T cell. Moreover, the CD8 molecule is helpless to the TCR and antigen interaction. This is a good test on the candidate TCR. A good candidate TCR, with high binding-affinity to its antigen can trigger T cell responses independent of CD4 or CD8. The data show that EN-17 C27 can trigger CD8 T cell responses independent of CD4 (FIG. 2B).

[0099] These data show that EN-17 C27 is an H3K27M antigen specific CD4 TCR with high antigen-binding affinity. By now, we have cloned 7 antigen-specific CD4 TCRs from the vaccinated patient samples (6 from patient EN-17 and 1 from patient EN-23). Meanwhile, we have cloned only 2 CD8 TCRs. The cloned CD8 TCRs are functionally weak compared to the CD4 TCRs (FIG. 3).

[0100] FIG. 3 shows the functional validation results of the two CD8 TCRs cloned. Both TCRs were cloned from a single patient sample EN-10. Both TCRs can only trigger weak responses in CD4 T cells (data not shown). Therefore, they have low binding-affinity to the antigen. EN-10 C01 cannot distinguish the peptide antigen with or without the point mutation (FIG. 3A), but EN-10 C04 is specific to the mutated peptide (FIG. 3B). Interestingly, C01 showed a better response to the long peptide than the short peptide (FIG. 3). Since CD8 TCRs can only recognize antigen peptide with 8-12 aa. It is possible that the originally designed short peptide vaccine (RMS) is not the best antigen peptide design to trigger C01 responses. It is possible that a sub-region of the long peptide (KQ-RMS), different from “RMS”, serves as the cognate antigen for EN-10 C01. It shows that the long peptide design not only includes CD4 T cells into the immune responses, but also serves a better antigen to trigger CD8 responses than the prior peptide design.

[0101] 1.2.5 Conclusion:

[0102] In consistent with our vaccine design, both CD8 and CD4 T cell responses were detected in the peripheral blood of vaccinated patients. Importantly, stronger immune responses were detected in the CD4 T compartment than in CD8 T cells. These data shows that the peptide vaccine can stimulate anticancer immunity in DIPG patients. Moreover, CD4 T cell mediated immune responses triggered by the long peptide vaccine may play a more significant role in cancer treatment than CD8 T cell responses.

References

Schwartzentruber J, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* 2012 Jan 29;482(7384):226-31.

Wu G, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet* 2012 Jan 29;44(3):251-3.

Argersinger DP, et al. New Developments in the Pathogenesis, Therapeutic Targeting, and Treatment of H3K27M-Mutant Diffuse Midline Glioma. *Cancers* 2021, 13(21), 5280.

Zhang X et al., Oncohistone Mutations in Diffuse Intrinsic Pontine Glioma. *Trends Cancer* 2019 Dec;5(12):799-808.

Lowe BR, et al. Histone H3 Mutations: An Updated View of Their Role in Chromatin Deregulation and Cancer. *Cancers*. 2019 May; 11(5): 660.

Rosa DS, et al. $CD4^+$ T Cell Epitope Discovery and Rational Vaccine Design. *Archivum Immunologiae et Therapiae Experimentalis* volume 58, pages 121–130 (2010)

Hemmer B et al., Minimal peptide length requirements for $CD4^+$ T cell clones—implications for molecular mimicry and T cell survival. *International Immunology*, 2000 March; 12(3): 375-383.

Chicz RM, et al. Predominant naturally-processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. Nature, 1992, vol. 358 (764-768).

Sercarz EE, et al. MHC-guided processing: binding of large antigen fragments. Nat. Rev. Immunol, 2003, vol. 3 (621-629)

Toussi DN and Massari P, Immune Adjuvant Effect of Molecularly-defined Toll-Like Receptor Ligands. Vaccines 2014 Jun; 2(2): 323-353.

CLAIMS

1. A pharmaceutical composition, comprising a peptide, wherein the peptide has a length of at least 12 amino acid residues and comprises RMSA (SEQ ID NO: 26), wherein the pharmaceutical composition, after administration to a subject on a therapeutically effective regimen, is capable of stimulating CD4 T cell response against histone 3 (H3) K27M mutation (H3K27M) in the subject.

2. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition, after administration to the subject on a therapeutically effective regimen, is further capable of stimulating CD8 T cell response against the H3K27M mutation in the subject.

3. The pharmaceutical composition of claim 1 or claim 2, wherein the peptide comprises RMSAP(S/A)TGGV (SEQ ID NO: 25).

4. The pharmaceutical composition of claim 3, wherein the peptide comprises an amino acid sequence of KQLATKAARMSAP(S/A)TGGV (SEQ ID NO: 1).

5. The pharmaceutical composition of claim 4, wherein H3 in the subject has a variant type of H3.3, wherein the peptide has an amino acid sequence of KQLATKAARMSAPSTGGV (SEQ ID NO: 2).

6. The pharmaceutical composition of claim 4, wherein H3 in the subject has a variant type of H3.1, wherein the peptide has an amino acid sequence of KQLATKAARMSAPATGGV (SEQ ID NO: 27).

7. The pharmaceutical composition of any one of claims 1-6, further comprising an adjuvant.

8. The pharmaceutical composition of claim 7, wherein the adjuvant comprises a toll-like receptor agonist.

9. The pharmaceutical composition of claim 8, wherein the toll-like receptor agonist is poly I:C.

10. The pharmaceutical composition of claim 9, wherein the toll-like receptor agonist is poly ICLC.

11. The pharmaceutical composition of claim 10, wherein the poly ICLC and the peptide have a ratio of approximately 1:0.5-1:5 by weight.

12. The pharmaceutical composition of claim11, wherein the poly ICLC and the peptide have a ratio of approximately 1:4 by weight.

13. The pharmaceutical composition of any one of claims 1-12, wherein a dosage form of the pharmaceutical composition comprises approximately 0.5 mg poly ICLC and approximately 0.5-2 mg of the peptide.

14. The pharmaceutical composition of claim 13, wherein the dosage form of the pharmaceutical composition comprises approximately 0.5 mg poly ICLC and approximately 2 mg of the peptide.

15. The pharmaceutical composition of any one of claims 1-14, wherein the pharmaceutical composition is configured as a formulation for subcutaneous injection to the subject.

16. A method of stimulating an immune response in a subject having a cancer, wherein the cancer is characterized to have a K27M mutation in histone H3, the method comprising a step of:

administering, on a therapeutically effective regimen, the pharmaceutical composition according to any one of claims 1-10 to the subject, thereby inducing the immune response in the subject.

17. The method of claim 16, wherein the immune response comprises CD4 T cell response against the H3K27M mutation.

18. The method of claim 17, wherein the immune response further comprises CD8 T cell response against the H3K27M mutation.

19. The method of any one of claims 16-18, further comprising, prior to the administering step, a step of determining the mutated variant type of H3 in the subject.

20. The method of claim 19, wherein the mutated variant type of H3 is determined to be H3.3, wherein:

in the administering step, the peptide in the pharmaceutic composition comprises RMSAPSTGGV (SEQ ID NO: 5).

21. The method of claim 20, wherein the peptide in the pharmaceutic composition comprises KQLATKAARMSAPSTGGV (SEQ ID NO: 2).

22. The method of claim 21, wherein the peptide in the pharmaceutic composition consists of KQLATKAARMSAPSTGGV (SEQ ID NO: 2).

23. The method of claim 19, wherein the mutated variant type of H3 is determined to be H3.1, wherein:

in the administering step, the peptide in the pharmaceutic composition comprises RMSAPATGGV (SEQ ID NO: 29).

24. The method of claim 23, wherein the peptide in the pharmaceutic composition comprises KQLATKAARMSAPATGGV (SEQ ID NO: 27).

25. The method of claim 24, wherein the peptide in the pharmaceutic composition consists of KQLATKAARMSAPATGGV (SEQ ID NO: 27).

26. The method of any one of claims 16-25, wherein the cancer comprises at least one of glioma, acute myeloid leukaemia (AML), or melanoma.

27. The method of claim 26, wherein the cancer comprises glioma.

28. The method of claim 27, wherein the cancer comprises DIPG.

29. The method of any one of claims 16-28, wherein the subject carries HLA-A*02 allele.

30. The method of any one of claims 16-28, wherein the subject carries at least one of an HLA-DRB1*07:01 allele or an HLA-DRB1*01:01 allele.

31. The method of any one of claims 16-29, wherein the therapeutically effective regimen comprises administering the pharmaceutical composition to the subject through subcutaneous injection.

32. A T-cell expressing a T-cell receptor (TCR) or a fragment thereof, wherein the TCR is capable of binding to a peptide/MHC II complex, wherein the peptide in the peptide/MHC II complex has a length of at least 12 amino acid residues and comprises RMSA (SEQ ID NO: 26).

33. The T-cell of claim 32, wherein the TCR or the fragment thereof is further capable of binding to a complex formed between the peptide and MHC I.

34. The T-cell of claim 32 or claim 33, wherein the peptide comprises RMSAP(S/A)TGGV (SEQ ID NO: 25).

35. The T-cell of claim 34, wherein the peptide comprises an amino acid sequence of KQLATKAARMSAP(S/A)TGGV (SEQ ID NO: 1).

36. The T-cell of claim 35, wherein the peptide comprises an amino acid sequence of KQLATKAARMSAPSTGGV (SEQ ID NO: 2).

37. The T-cell of claim 36, wherein the peptide consists of an amino acid sequence of KQLATKAARMSAPSTGGV (SEQ ID NO: 2).

38. The T-cell of claim 36 or claim 37, wherein the TCR or the fragment thereof is incapable of bind to a complex between a second peptide and MHC II complex, wherein the second peptide comprises an amino acid sequence of KQLATKAARKSAPSTGGV (SEQ ID NO: 6).

39. The T-cell of claim 35, wherein the peptide comprises an amino acid sequence of KQLATKAARMSAPATGGV (SEQ ID NO: 27).

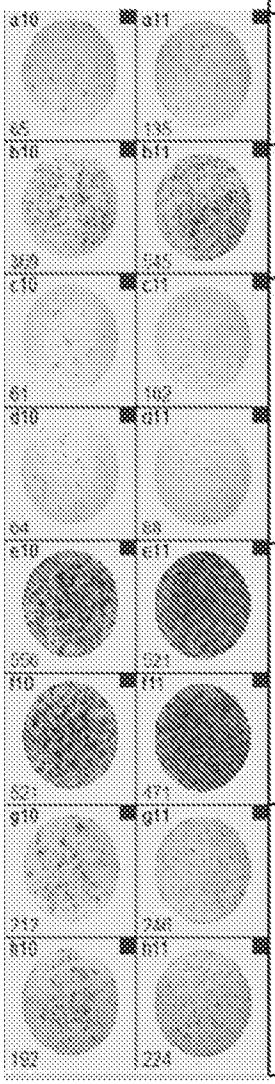
40. The T-cell of claim 39, wherein the peptide consists of an amino acid sequence of KQLATKAARMSAPATGGV (SEQ ID NO: 27).

41. The T-cell of claim 39 or claim 40, wherein the TCR or the fragment thereof is incapable of bind to a complex between a second peptide and MHC II complex, wherein the second peptide comprises an amino acid sequence of KQLATKAARKSAPATGGV (SEQ ID NO: 28).

42. The T-cell of any one of claims 32-41, wherein the TCR is heterologous to the T-cell.

43. The T-cell of claim 42, wherein the TCR is transduced or transfected into the T-cell via a vector.

44. The T-cell of claim 43, wherein the vector is a retroviral vector.



Group	EN-17	EN-23
PSG1	0.50%	0.45%
PSG2_KQ	**2.84%**	**1.82%**
PSG2_RM	0.47%	0.34%
	0.49%	0.29%
PHA	4.28%	1.74%
	4.01%	1.57%
NCG	0.53%	0.35%
	0.48%	0.32%

FIG. 1

FIG. 2A

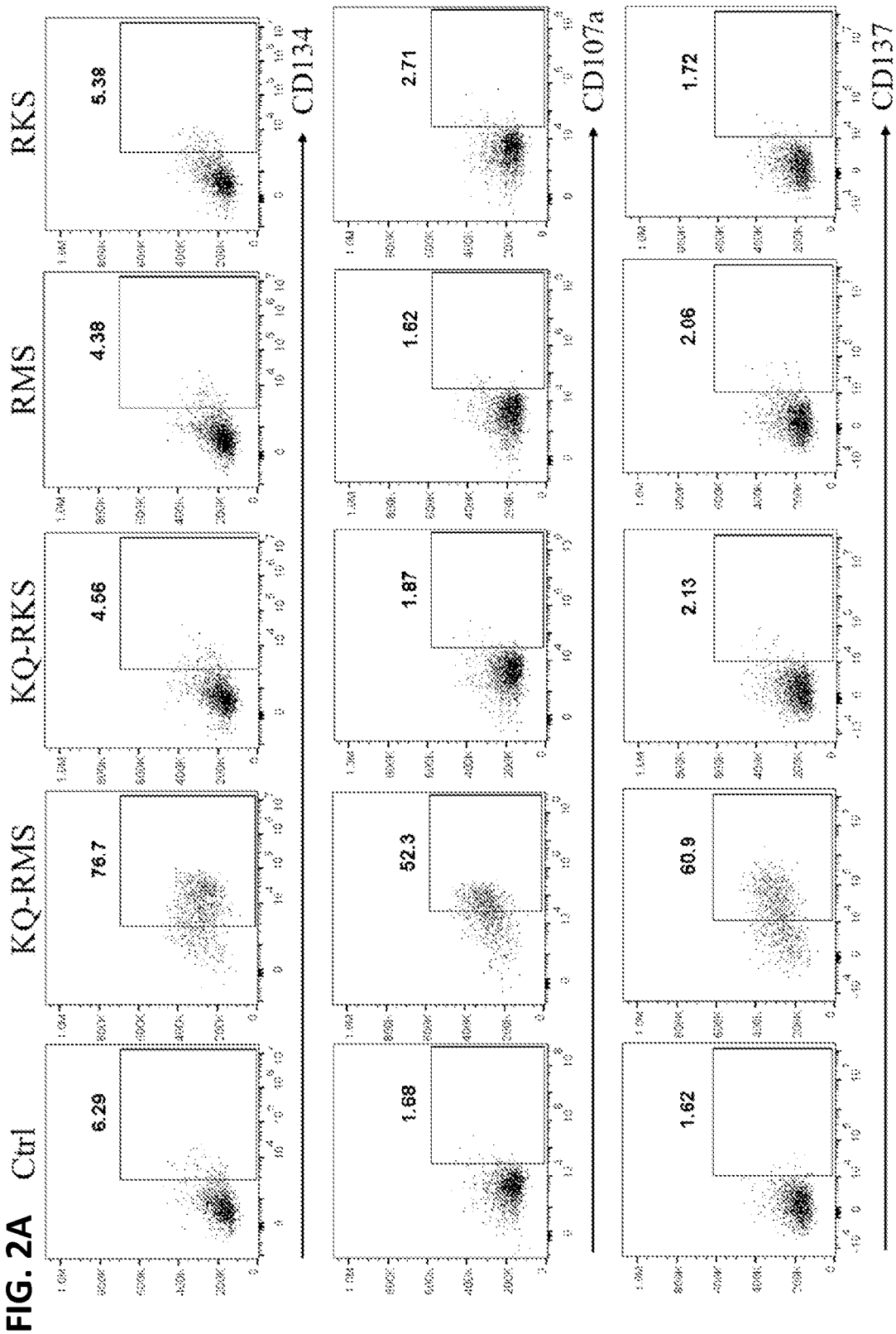
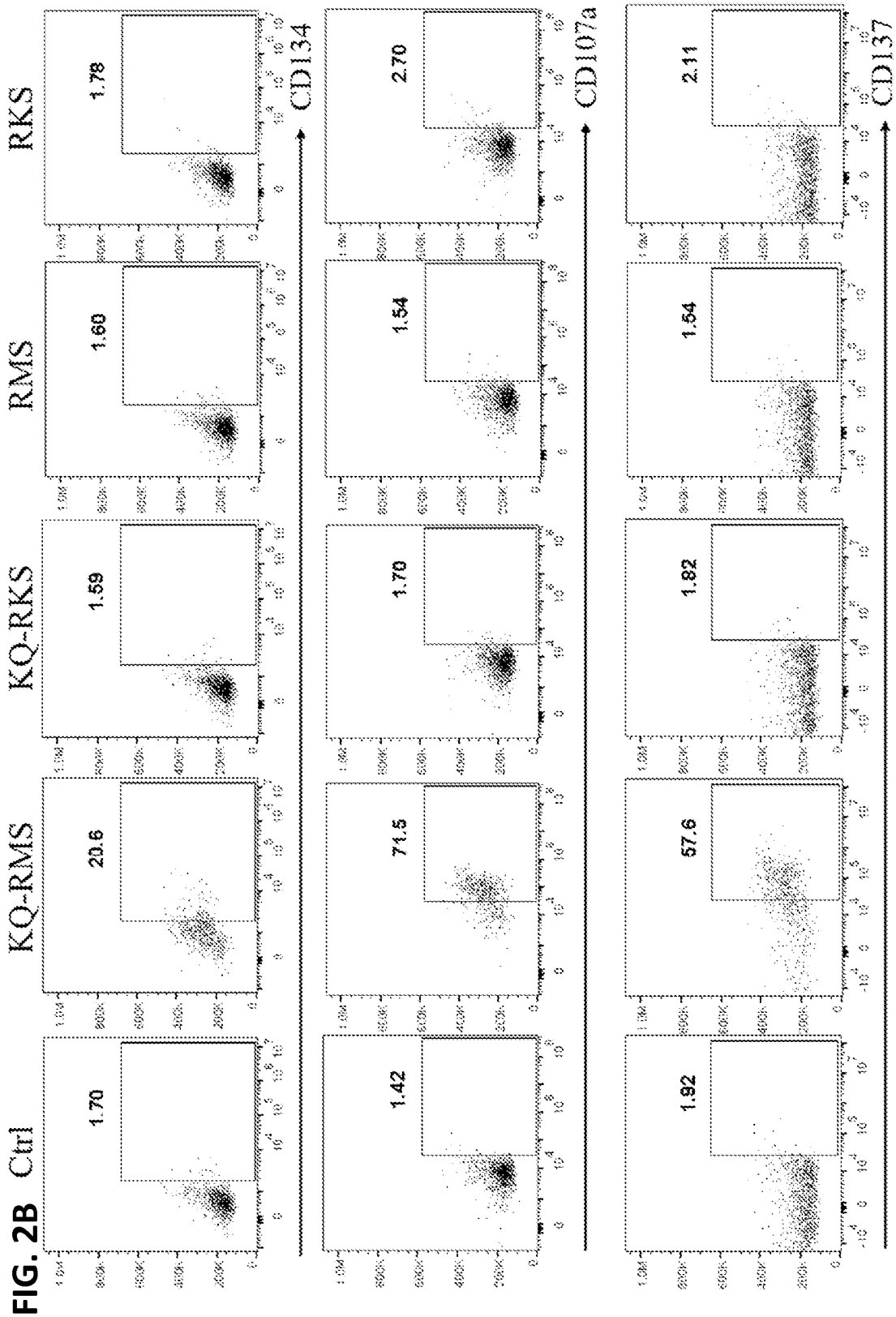

Ctrl
KQ-RMS
KQ-RKS
RMS
RKS
6.29
76.7
4.66
4.38
5.38
CD134
1.68
52.3
1.87
1.62
2.71
CD107a
1.62
60.9
2.13
2.06
1.72
CD137

FIG. 2B


Ctrl
KQ-RMS
KQ-RKS
RMS
RKS
1.70
20.6
1.59
1.60
1.78
1.42
71.5
1.70
1.54
2.70
1.92
57.6
1.82
1.54
2.11
CD134
CD107a
CD137

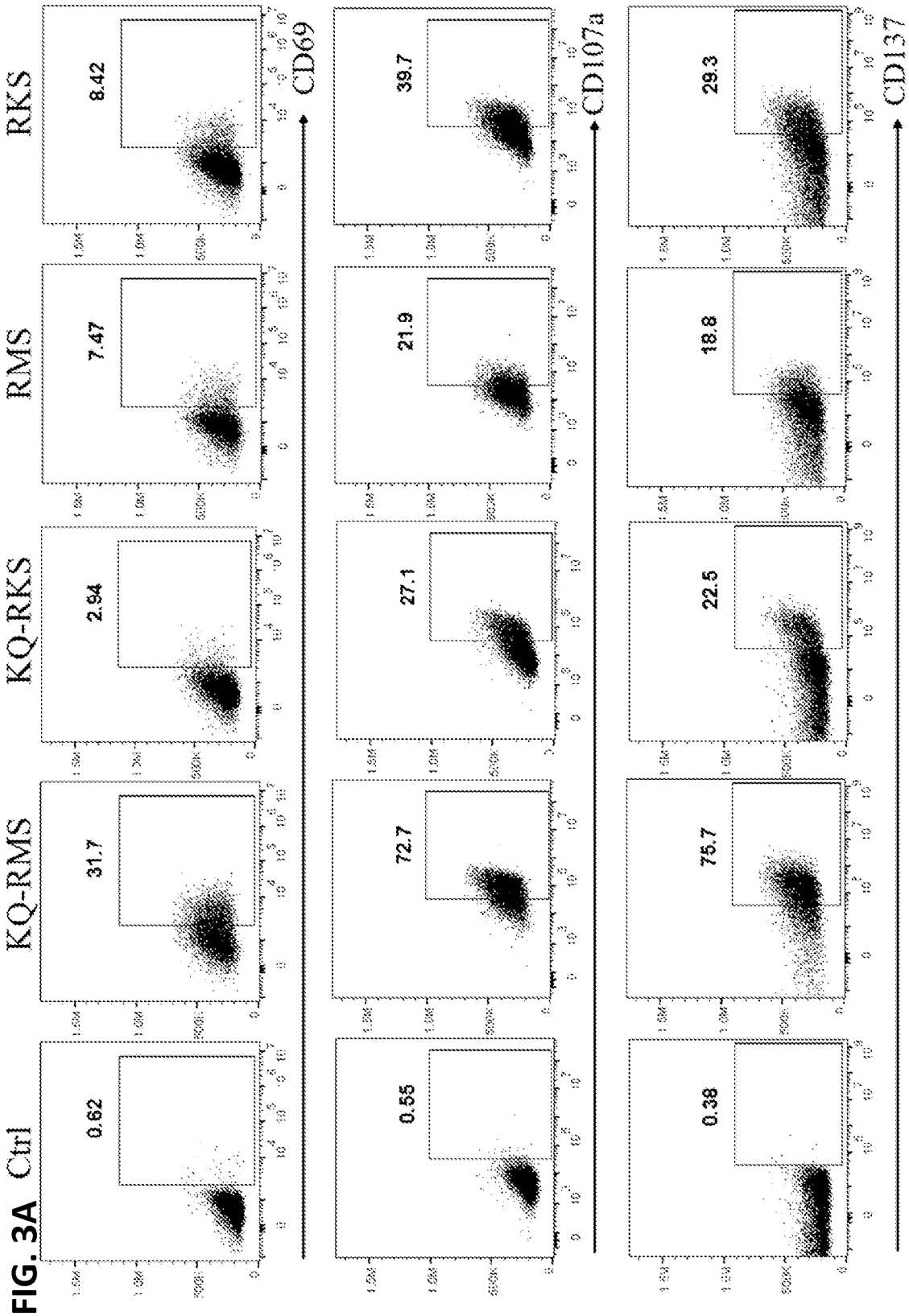
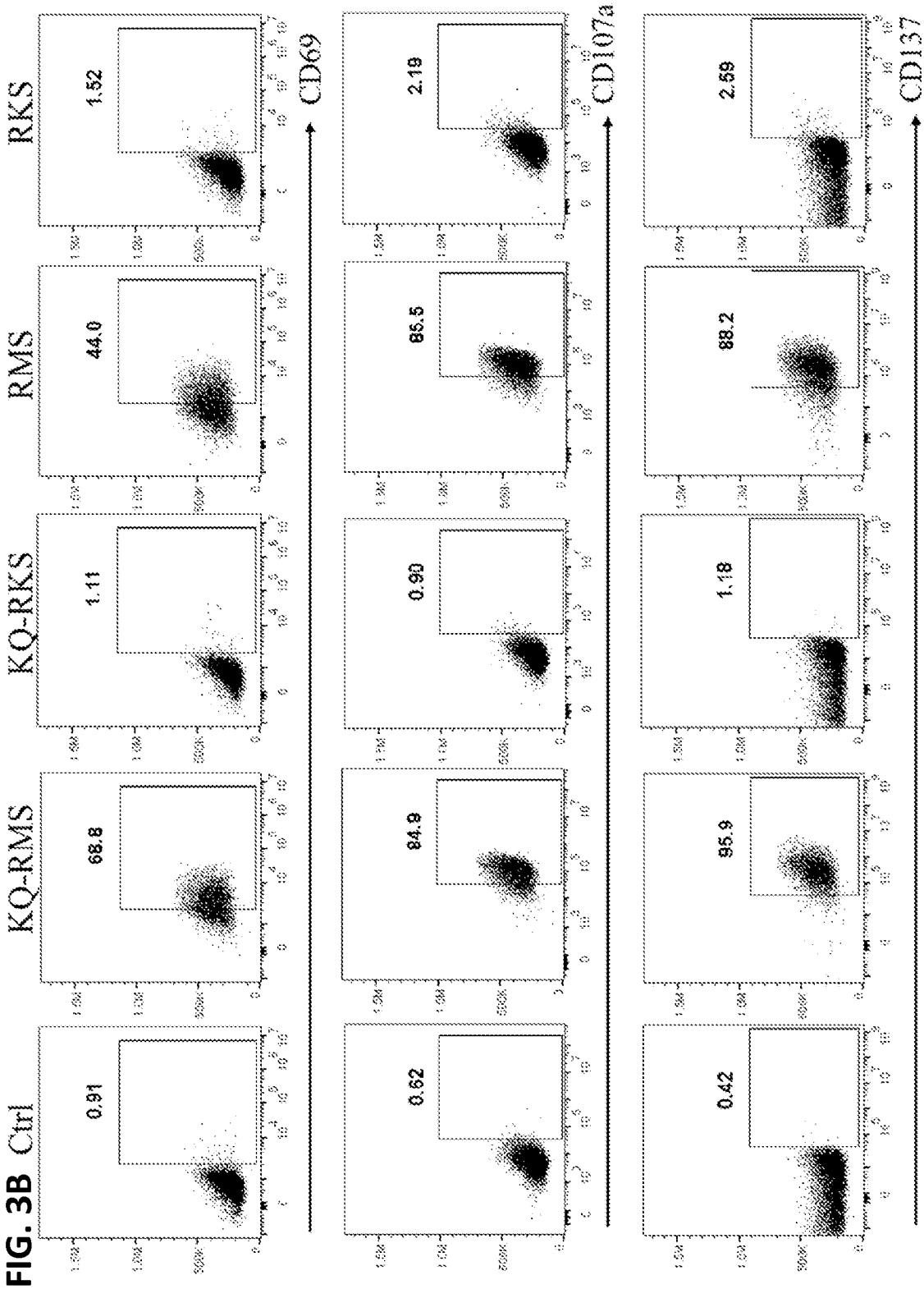

Ctrl
KQ-RMS
KQ-RKS
RMS
RKS
0.62
31.7
2.54
7.47
8.42
CD69
0.55
72.7
27.1
21.9
39.7
CD107a
0.38
75.7
22.5
18.8
29.3
CD137

FIG. 3A

FIG. 3B


Ctrl
KQ-RMS
KQ-RKS
RMS
RKS
0.91
68.8
1.11
44.0
1.52
CD69
0.62
84.9
0.90
85.5
2.19
CD107a
0.42
95.9
1.18
88.2
2.59
CD137

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/125413

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/07(2006.01)i;A61K 38/03(2006.01)i;A61K 38/17(2006.01)i;C07K 14/47(2006.01)i

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: A61K C07K

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)

CNABS, DWPI, USTXT, CNTXT, VEN, CNKI, NCBI, EBI, bing, baidu, patentics, wanfang, chaoxing, duxiu, S GUANGDONG TCRCURE BIOPHARMA TECHNOLOGY CO., LTD., CHONGQING TCRCURE SCIENCE AND TECHNOLOGY CO., LTD. , ZHANG Lifeng, WU Haiyang, XU Wei, RMSA, H3K27M, stimulating CD4 T cell response against histone 3 (H3) K27M mutation, stimulating CD8 T cell response against the H3K27M mutation, H3.3 CTL peptides, CD4, CD8, HLA, HLA-A*02 allele, HLA-DRB1*07: 01 allele, HLA-DRB1*01: 01 allele, T-cell expressing a T-cell receptor, TCR, RMSAP(S/A)TGGV, RMSAPSTGGV, RMSAPATGGV, KQLATKAARMSAP(S/A)TGGV, KQLATKAARMSAPSTGGV, KQLATKAARMSAPATGGV, KQLATKAARMSAPS TGGV, KQLATKAARMSAPATGGV, cancer, tumor, glioma, acute myeloid leukaemia, AML, melanoma, DIPG, adjuvant, toll-like receptor agonist, poly I: C, poly ICLC, SEQ ID NOs:1-29.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016179326 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 10 November 2016 (2016-11-10) claims 1-43, description paragraphs 0003-0012, 0037-0043, example 1	1-3, 7-20, 23, 26-34, 42-44
Y	WO 2016179326 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 10 November 2016 (2016-11-10) claims 1-43, description paragraphs 0003-0012, 0037-0043, example 1	4-19, 21-22, 24-31, 35-44
Y	US 2019338035 A1 (EUREKA THERAPEUTICS,INC.) 07 November 2019 (2019-11-07) claims 1-5, description SEQ ID NO:1	4-19, 21-22, 24-31, 35-44
X	US 10441644 B2 (REGENTS OF THE UNIVERSITY OF CALIFORNIA) 15 October 2019 (2019-10-15) claims 1-4, description columns 1-4, examples 1-2	1-3, 7-20, 23, 26-34, 42-44

✓ 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

"D" document cited by the applicant in the international application

"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
14 March 2023	**10 May 2023**
Name and mailing address of the ISA/CN **CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China**	Authorized officer **ZHU,Ning**
Facsimile No. **(86-10)62019451**	Telephone No. **(+86) 010-53961956**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 102460173 A (加利福尼亚大学董事会) 16 May 2012 (2012-05-16) 全文	1-44
A	CN 105440119 A (伊玛提克斯生物技术有限公司) 30 March 2016 (2016-03-30) 全文	1-44
A	CN 108779149 A (伊玛提克斯生物技术有限公司) 09 November 2018 (2018-11-09) 全文	1-44
A	CN 112920257 A (伊玛提克斯生物技术有限公司) 08 June 2021 (2021-06-08) 全文	1-44
A	CN 114040921 A (伊玛提克斯生物技术有限公司) 11 February 2022 (2022-02-11) 全文	1-44
A	TW 201920252 A (德商英麦提克生物技术股份有限公司（德国）) 01 June 2019 (2019-06-01) 全文	1-44
A	CN 1218404 A (西特尔公司) 02 June 1999 (1999-06-02) 全文	1-44
A	CN 111647062 A (伊玛提克斯生物技术有限公司) 11 September 2020 (2020-09-11) 全文	1-44
A	CN 112399853 A (伊玛提克斯生物技术有限公司) 23 February 2021 (2021-02-23) 全文	1-44
A	CN 102459314 A (肿瘤疗法科学股份有限公司) 16 May 2012 (2012-05-16) 全文	1-44
A	TW 202126676 A (德商英麦提克生物技术股份有限公司（德国）) 16 July 2021 (2021-07-16) 全文	1-44
Y	CHHEDA, Z.S.等. "Novel and shared neoantigen derived from histone 3 variant H3.3K27M mutation for glioma T cell therapy." *J. Exp. Med.*, Vol. 215, No. 1, 31 December 2018 (2018-12-31), 第141-157页	26, 29-31, 44

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/125413

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.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/125413

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.: **16-31**
because they relate to subject matter not required to be searched by this Authority, namely:

The subject matter of claims 16-31 relates to a method of treating a disease in a subject in need thereof by therapy method, and therefore does not warrant an international search according to the criteria set out in Rule 39.1(iv). However, the search has been carried out and based on the use of effective amount of the fusion protein or the pharmaceutical composition for the manufacturing of a medicament.

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).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/125413

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2016179326	A1	10 November 2016	None			
US	2019338035	A1	07 November 2019	TW	201831517	A	01 September 2018
				EP	3568468	A1	20 November 2019
				EP	3568468	A4	30 December 2020
				WO	2018132597	A1	19 July 2018
				US	11274157	B2	15 March 2022
US	10441644	B2	15 October 2019	US	2020101148	A1	02 April 2020
				US	11185577	B2	30 November 2021
				US	2020009236	A1	09 January 2020
				US	10849965	B2	01 December 2020
				US	2017281742	A1	05 October 2017
				US	2021038704	A1	11 February 2021
CN	102460173	A	16 May 2012	CA	2758933	A1	21 October 2010
				JP	2012524278	A	11 October 2012
				AU	2010236415	A1	01 December 2011
				BRPI	1013546	A2	09 April 2019
				US	2012094949	A1	19 April 2012
				EP	2419737	A2	22 February 2012
				EP	2419737	A4	13 February 2013
				WO	2010120942	A2	21 October 2010
				WO	2010120942	A3	24 February 2011
CN	105440119	A	30 March 2016	AT	461214	T	15 April 2010
				JP	2010502173	A	28 January 2010
				JP	5627180	B2	19 November 2014
				CA	2621389	A1	15 March 2007
				CA	2621389	C	19 July 2016
				AU	2010236029	A1	18 November 2010
				AU	2010236029	B2	28 June 2012
				KR	20130019033	A	25 February 2013
				KR	101386827	B1	17 April 2014
				DE	602005020046	D1	29 April 2010
				AU	2006289290	A1	15 March 2007
				AU	2006289290	B2	25 November 2010
				KR	20130019034	A	25 February 2013
				KR	101386790	B1	21 April 2014
				US	2016115212	A1	28 April 2016
				US	10618945	B2	14 April 2020
				SI	2135878	T1	30 March 2012
				PT	1806358	E	28 May 2010
				SI	1806359	T1	30 June 2010
				ES	2341802	T3	28 June 2010
				HK	1220709	A1	12 May 2017
				US	2008206218	A1	28 August 2008
				US	7833970	B2	16 November 2010
				KR	20080052643	A	11 June 2008
				KR	101386355	B1	17 April 2014
				EP	1806358	A2	11 July 2007
				EP	1806358	A3	03 October 2007
				EP	1806358	B1	17 March 2010

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/125413

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
		PL	1806359	T3	31 August 2010
		DK	1806359	T3	14 June 2010
		ES	2373907	T3	10 February 2012
		EP	2138509	A1	30 December 2009
		NZ	566104	A	29 April 2011
		EP	1760088	A1	07 March 2007
		EP	1760088	B1	05 March 2008
		CY	1107973	T1	04 September 2013
		CY	1112186	T1	09 December 2015
		SI	1760088	T1	30 June 2008
		EP	2135878	A1	23 December 2009
		EP	2135878	B1	09 November 2011
		SI	1922335	T1	30 April 2010
		EA	200800677	A1	29 August 2008
		EA	013876	B1	30 August 2010
		SI	1806358	T1	30 June 2010
		US	2010040590	A1	18 February 2010
		US	10196432	B2	05 February 2019
		NZ	588813	A	30 June 2011
		CY	1110187	T1	14 January 2015
		AT	461215	T	15 April 2010
		DE	602005005196	D1	17 April 2008
		DE	602005005196	T2	26 June 2008
		UA	98295	C2	10 May 2012
		WO	2007028574	A2	15 March 2007
		WO	2007028574	A3	24 May 2007
		WO	2007028574	A9	02 May 2008
		ES	2337399	T3	23 April 2010
		BRPI	0615462	A2	17 May 2011
		DE	602005020047	D1	29 April 2010
		US	2008206216	A1	28 August 2008
		AT	451388	T	15 December 2009
		CY	1109849	T1	10 September 2014
		EP	1922335	A2	21 May 2008
		EP	1922335	B1	09 December 2009
		PT	1806359	E	25 May 2010
		ES	2302546	T3	16 July 2008
		PL	2135878	T3	29 June 2012
		PT	1760088	E	14 May 2008
		AT	388164	T	15 March 2008
		DK	2135878	T3	27 February 2012
		ES	2341295	T3	17 June 2010
		NO	20081683	L	04 April 2008
		NO	340870	B1	03 July 2017
		US	2008206217	A1	28 August 2008
		US	7833969	B2	16 November 2010
		EP	1806359	A1	11 July 2007
		EP	1806359	B1	17 March 2010
		DK	1806358	T3	28 June 2010
		HRP	20100128	T1	30 April 2010

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/125413

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
				CY	1110051	T1	14 January 2015
				PT	1922335	E	03 March 2010
				CA	2929252	A1	15 March 2007
				PL	1806358	T3	31 August 2010
				US	2008207520	A1	28 August 2008
				US	7807642	B2	05 October 2010
				DK	1922335	T3	19 April 2010
				PL	1922335	T3	31 May 2010
				HRP	20110981	T1	29 February 2012
				PL	1760088	T3	31 October 2008
				AT	532795	T	15 November 2011
				DK	1760088	T3	09 June 2008
				PT	2135878	E	05 January 2012
				DE	602006011030	D1	21 January 2010
CN	108779149	A	09 November 2018	CR	20180491	A	09 January 2019
				JP	2019515650	A	13 June 2019
				KR	20230004914	A	06 January 2023
				IL	261785	A	31 October 2018
				PH	12018501865	A1	15 July 2019
				US	2020085930	A1	19 March 2020
				AU	2017235364	A1	25 October 2018
				AU	2017235364	B2	06 January 2022
				CO	2018010009	A2	28 September 2018
				PE	20181824	A1	29 November 2018
				SG	10202006119	TA	28 August 2020
				US	2017296641	A1	19 October 2017
				KR	20180121530	A	07 November 2018
				EP	3430027	A1	23 January 2019
				GB	201604458	D0	27 April 2016
				CL	2018002591	A1	05 November 2018
				MA	43719	A	28 November 2018
				AU	2022202307	A1	28 April 2022
				AU	2022202307	B2	15 September 2022
				EA	201891701	A1	28 February 2019
				MX	2018011215	A	22 November 2018
				BR	112018017022	A2	05 February 2019
				SG	11201806905	UA	27 September 2018
				MA	43327	A1	31 December 2018
				AU	2022287621	A1	02 February 2023
				CA	3016410	A1	21 September 2017
				WO	2017157972	A1	21 September 2017
CN	112920257	A	08 June 2021	AU	2022202512	A1	12 May 2022
				US	2020179454	A1	11 June 2020
				US	10751369	B2	25 August 2020
				AU	2019225879	A1	09 July 2020
				AU	2019225879	B2	27 January 2022
				US	2020306308	A1	01 October 2020
				US	10869895	B2	22 December 2020
				US	2019255112	A1	22 August 2019
				US	10894064	B2	19 January 2021

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/125413

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
		JP	2022050398	A	30 March 2022
		US	2021137985	A1	13 May 2021
		US	11154576	B2	26 October 2021
		US	2020155605	A1	21 May 2020
		US	10772915	B2	15 September 2020
		US	2021052651	A1	25 February 2021
		US	10993964	B2	04 May 2021
		US	2021213064	A1	15 July 2021
		US	11141437	B2	12 October 2021
		US	2020179453	A1	11 June 2020
		US	10799537	B2	13 October 2020
		TW	201936630	A	16 September 2019
		AU	2022202520	A1	12 May 2022
		MX	2020007808	A	05 October 2020
		US	2020085873	A1	19 March 2020
		US	10695376	B2	30 June 2020
		AU	2022202522	A1	12 May 2022
		US	2021338735	A1	04 November 2021
		US	2020038446	A1	06 February 2020
		US	10695373	B2	30 June 2020
		EA	202091833	A1	25 November 2020
		CL	2020002387	A1	12 February 2021
		US	2020261506	A1	20 August 2020
		US	10792308	B2	06 October 2020
		CL	2021000842	A1	03 September 2021
		US	2020297771	A1	24 September 2020
		US	10869894	B2	22 December 2020
		US	2021060074	A1	04 March 2021
		US	11040069	B2	22 June 2021
		KR	20200116527	A	12 October 2020
		US	2021113619	A1	22 April 2021
		US	11058727	B2	13 July 2021
		US	2019255113	A1	22 August 2019
		US	11077142	B2	03 August 2021
		US	2020306305	A1	01 October 2020
		US	10888583	B2	12 January 2021
		SG	11202006630	RA	29 September 2020
		US	2021060078	A1	04 March 2021
		US	11065279	B2	20 July 2021
		US	2020323909	A1	15 October 2020
		US	10869896	B2	22 December 2020
		TW	202043257	A	01 December 2020
		TWI	776160	B	01 September 2022
		US	2020085874	A1	19 March 2020
		US	10695377	B2	30 June 2020
		US	2020323914	A1	15 October 2020
		US	11020433	B2	01 June 2021
		US	2021338734	A1	04 November 2021
		US	2020323919	A1	15 October 2020
		US	11020434	B2	01 June 2021

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/125413

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
		US	2021244765	A1	12 August 2021
		US	11179418	B2	23 November 2021
		IL	276845	A	29 October 2020
		US	2020268798	A1	27 August 2020
		US	10874695	B2	29 December 2020
		US	2021052654	A1	25 February 2021
		US	11026977	B2	08 June 2021
		MA	51890	A	30 December 2020
		US	2020206268	A1	02 July 2020
		US	10888582	B2	12 January 2021
		US	2020163996	A1	28 May 2020
		US	10709737	B2	14 July 2020
		US	2021145886	A1	20 May 2021
		US	11147838	B2	19 October 2021
		AU	2020204568	A1	30 July 2020
		AU	2020204568	B2	03 February 2022
		US	2020038447	A1	06 February 2020
		US	10695374	B2	30 June 2020
		US	2020093862	A1	26 March 2020
		US	10702553	B2	07 July 2020
		EP	3755708	A1	30 December 2020
		CL	2020002123	A1	16 October 2020
		US	2020323911	A1	15 October 2020
		US	10888585	B2	12 January 2021
		CO	2020009869	A2	21 August 2020
		US	2020222465	A1	16 July 2020
		US	10780124	B2	22 September 2020
		US	2020323915	A1	15 October 2020
		US	10898519	B2	26 January 2021
		US	2020171091	A1	04 June 2020
		US	10813953	B2	27 October 2020
		CA	3086916	A1	29 August 2019
		WO	2019162110	A1	29 August 2019
		US	2020261504	A1	20 August 2020
		US	10780122	B2	22 September 2020
		PE	20210376	A1	02 March 2021
		IL	276987	A	29 October 2020
		BR	112020014272	A2	15 December 2020
		US	2021401892	A1	30 December 2021
		US	11369636	B2	28 June 2022
		US	2020360433	A1	19 November 2020
		US	10905719	B2	02 February 2021
		US	2021330708	A1	28 October 2021
		US	11376281	B2	05 July 2022
		US	2020323906	A1	15 October 2020
		US	11013766	B2	25 May 2021
		US	2021260120	A1	26 August 2021
		US	11197892	B2	14 December 2021
		US	2020197441	A1	25 June 2020
		US	10792307	B2	06 October 2020

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/125413

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
		US	2020323918	A1	15 October 2020
		US	10905720	B2	02 February 2021
		US	2020108097	A1	09 April 2020
		US	10709736	B2	14 July 2020
		MX	2020007767	A	12 October 2020
		SG	10202010171	VA	27 November 2020
		US	2020323913	A1	15 October 2020
		US	10898518	B2	26 January 2021
		US	2020297770	A1	24 September 2020
		US	10869893	B2	22 December 2020
		CL	2021000841	A1	03 September 2021
		US	2020261505	A1	20 August 2020
		US	10780123	B2	22 September 2020
		US	2020268799	A1	27 August 2020
		US	10869890	B2	22 December 2020
		US	2020306311	A1	01 October 2020
		US	10888586	B2	12 January 2021
		US	2020155604	A1	21 May 2020
		US	10709738	B2	14 July 2020
		BR	122020014377	A2	29 December 2020
		CR	20200365	A	18 September 2020
		US	2021052652	A1	25 February 2021
		US	11013768	B2	25 May 2021
		US	2021100845	A1	08 April 2021
		US	11103535	B2	31 August 2021
		US	2021145888	A1	20 May 2021
		US	2019255110	A1	22 August 2019
		US	10993962	B2	04 May 2021
		DE	102018107224	A1	22 August 2019
		US	2020323907	A1	15 October 2020
		US	10869892	B2	22 December 2020
		US	2020268800	A1	27 August 2020
		US	10869891	B2	22 December 2020
		US	2022000922	A1	06 January 2022
		US	11357797	B2	14 June 2022
		US	2021052653	A1	25 February 2021
		US	11013769	B2	25 May 2021
		US	2019255164	A1	22 August 2019
		US	11033583	B2	15 June 2021
		US	2021069251	A1	11 March 2021
		US	11052113	B2	06 July 2021
		EA	202091754	A1	08 February 2021
		US	2021060077	A1	04 March 2021
		US	11052114	B2	06 July 2021
		US	2021145887	A1	20 May 2021
		US	11147839	B2	19 October 2021
		US	2020038448	A1	06 February 2020
		US	10695375	B2	30 June 2020
		PE	20211300	A1	20 July 2021
		JP	2021514178	A	10 June 2021

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/125413

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
				US	2020297772	A1	24 September 2020
				US	10874696	B2	29 December 2020
				PH	12020551295	A1	12 July 2021
				US	2020323910	A1	15 October 2020
				US	10888584	B2	12 January 2021
				US	2020323916	A1	15 October 2020
				US	11033585	B2	15 June 2021
				US	2021137986	A1	13 May 2021
				KR	20200123134	A	28 October 2020
CN	114040921	A	11 February 2022	None			
TW	201920252	A	01 June 2019	TW	202035441	A	01 October 2020
				TWI	750644	B	21 December 2021
				US	2022265795	A1	25 August 2022
				TW	202138386	A	16 October 2021
				US	2022265793	A1	25 August 2022
				US	2022280623	A1	08 September 2022
				IL	258609	A	28 June 2018
				IL	298653	A	01 January 2023
				US	2022354936	A1	10 November 2022
				US	2021290745	A1	23 September 2021
				US	11235042	B2	01 February 2022
				US	2022257736	A1	18 August 2022
				US	2019262444	A1	29 August 2019
				US	10525116	B2	07 January 2020
				AU	2022204032	A1	30 June 2022
				US	2021290744	A1	23 September 2021
				CL	2019002949	A1	31 January 2020
				US	2021283231	A1	16 September 2021
				US	11224644	B2	18 January 2022
				AU	2020264365	A1	03 December 2020
				AU	2020264365	B2	17 March 2022
				AU	2020264365	C1	30 June 2022
				CL	2018001533	A1	13 July 2018
				CL	2019002947	A1	28 February 2020
				CL	2019002945	A1	28 February 2020
				US	2021353728	A1	18 November 2021
				US	2020093906	A1	26 March 2020
				US	11433124	B2	06 September 2022
				CL	2019002943	A1	31 January 2020
				US	2022273783	A1	01 September 2022
				US	2022265794	A1	25 August 2022
				CO	2018004869	A2	22 November 2018
				US	2021275656	A1	09 September 2021
				TWI	712614	B	11 December 2020
				US	2022249635	A1	11 August 2022
				US	2022280622	A1	08 September 2022
				CL	2019002948	A1	28 February 2020
				US	2022323559	A1	13 October 2022
				CL	2019002946	A1	28 February 2020
				US	2021346481	A1	11 November 2021

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/125413

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
				US	11235041	B2	01 February 2022
				CL	2019002944	A1	31 January 2020
				US	2022257738	A1	18 August 2022
				HK	1255541	A1	23 August 2019
				CL	2019002942	A1	21 February 2020
				US	2019269767	A1	05 September 2019
				US	10434159	B2	08 October 2019
				US	2020000902	A1	02 January 2020
				US	10765730	B2	08 September 2020
CN	1218404	A	02 June 1999	JP	2002515868	A	28 May 2002
				JP	4210734	B2	21 January 2009
				US	2002168374	A1	14 November 2002
				BR	9708217	A	27 July 1999
				CA	2248659	A1	25 September 1997
				AU	2336597	A	10 October 1997
				AU	725550	B2	12 October 2000
				EP	0888120	A1	07 January 1999
				EP	0888120	A4	05 January 2000
				EP	0888120	B1	19 November 2008
				WO	9734617	A1	25 September 1997
CN	111647062	A	11 September 2020	WO	2018189148	A1	18 October 2018
				TW	201841934	A	01 December 2018
				US	2021322478	A1	21 October 2021
				US	11542303	B2	03 January 2023
				US	2021213065	A1	15 July 2021
				US	2018291082	A1	11 October 2018
				US	11427614	B2	30 August 2022
				US	2020339656	A1	29 October 2020
				US	2020339657	A1	29 October 2020
				US	2020199194	A1	25 June 2020
				US	11020432	B2	01 June 2021
				US	2020223900	A1	16 July 2020
				US	11007223	B2	18 May 2021
				US	2020190160	A1	18 June 2020
				US	2020339658	A1	29 October 2020
				US	11103534	B2	31 August 2021
CN	112399853	A	23 February 2021	MA	53004	A	26 May 2021
				PH	12020552013	A1	28 June 2021
				WO	2020002063	A1	02 January 2020
				US	2021330772	A1	28 October 2021
				US	11246917	B2	15 February 2022
				PE	20211499	A1	11 August 2021
				US	2022370583	A1	24 November 2022
				US	11576955	B2	14 February 2023
				US	2021138052	A1	13 May 2021
				US	2020000897	A1	02 January 2020
				US	10946081	B2	16 March 2021
				CA	3097698	A1	02 January 2020
				CO	2020013659	A2	10 November 2020
				IL	279843	A	01 March 2021

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/125413

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
				US	2021128708	A1	06 May 2021
				US	2020000895	A1	02 January 2020
				US	10925947	B2	23 February 2021
				EP	3813873	A1	05 May 2021
				US	2020289632	A1	17 September 2020
				US	10828357	B2	10 November 2020
				US	2022362364	A1	17 November 2022
				US	11554164	B2	17 January 2023
				TW	202019462	A	01 June 2020
				US	2020323970	A1	15 October 2020
				US	10869917	B2	22 December 2020
				BR	112020022250	A2	23 February 2021
				SG	11202009659	XA	28 January 2021
				AU	2019294731	A1	29 October 2020
				JP	2021528046	A	21 October 2021
				KR	20210025534	A	09 March 2021
				CL	2020003371	A1	07 May 2021
				US	2021330771	A1	28 October 2021
				US	2021128707	A1	06 May 2021
				US	11058755	B2	13 July 2021
				CR	20200632	A	25 January 2021
CN	102459314	A	16 May 2012	BRPI	1013938	A2	24 September 2019
				EP	2430039	A1	21 March 2012
				EP	2430039	A4	05 December 2012
				WO	2010131452	A1	18 November 2010
				AU	2010248702	A1	17 November 2011
				MX	2011012013	A	23 February 2012
				CA	2761393	A1	18 November 2010
				SG	175998	A1	29 December 2011
				KR	20120029394	A	26 March 2012
				TW	201102081	A	16 January 2011
				US	2012135020	A1	31 May 2012
				US	8530430	B2	10 September 2013
				RU	2011150283	A	20 June 2013
				RU	2531348	C2	20 October 2014
				JP	2012526519	A	01 November 2012
				JP	5786178	B2	30 September 2015
TW	202126676	A	16 July 2021		None		