

SUMMARY OF THE ~~INVENTION~~DISCLOSURE

Described herein, according to some ~~embodiment~~examples, are humanized antibodies that specifically bind human poliovirus receptor (PVR; CD155) and prevent binding of PVR to at least one of ligands, T cell immunoreceptor with Ig and ITIM domains (TIGIT), CD96 and CD226 (DNAM-1). The humanized antibodies of the present ~~invention~~disclosure, selected from a larger collection of antibody clones, have improved properties compared to other known anti-PVR antibodies. These improved properties include but are not limited to reduced immunogenicity potential, improved binding affinity and activity, biophysical properties and improved expression. As PVR binding to CD226 results in down-regulation of surface expression of CD226 on T and NK cells and reduced activity of CD226 to stimulate T and NK cells and tumor cell killing, the antibodies of the present ~~invention~~disclosure can restore the expression and/or activity of CD226 on these cells. Proper expression and functioning of CD226 allows for increased tumor killing by immune cells, especially CD8+ T cells and NK cells.

A large collection of humanized antibodies was produced by combining specific sets of CDR sequences and human framework sequences and introducing specific mutations in these sequences to produce improved antibodies with modified variable regions. The newly designed humanized variable regions preserve the residues critical for the maintenance of the antibody's conformation and binding affinity, while having the lowest incidence of potential T cell epitopes, thus minimizing the risk of adverse immune response towards the antibodies. The antibodies disclosed herein were designed based on factors including homology, T-cell epitopes, key residues, and predicted structures.

Unexpectedly, variants having a combination of specific human frameworks a point mutation of Glutamic acid to Asparagine in the last residue of CDR2 of the light chain variable region (position 56 according to Kabat numbering), show strong affinity to human PVR and improved immune activity.

Several humanized antibody variants according to the ~~invention~~disclosure were found to be particularly suitable for chimeric antigen receptor (CAR) applications due to their lower affinity, which may be useful in targeting the highly expressed PVR tumor cells, without targeting normal tissues.

Advantageously, several humanized antibody variants according to the present

~~invention~~disclosure have improved producibility and are capable of being produced with exceptionally high yield compared to other variants.

It is now disclosed that the humanized antibody described herein show high efficacy in cytotoxic T and NK cell stimulation, and in treatment of cancer in humanized mouse models, including in vivo models of pancreatic cancer and lung cancer.

The present ~~invention~~disclosure thus provides, in some ~~embodiment~~examples, highly-specific, non-immunogenic, humanized antibodies against human PVR having improved affinity, activity and/or reproducibility.

In one aspect, provided herein is a humanized antibody or antigen binding fragment thereof comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 47; and the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 48.

In one aspect, provided herein is a single chain variable fragment (scFv) of a humanized antibody as described herein, comprising an amino acid sequence selected from SEQ ID NO: 56, SEQ ID NO: 57, and an analog thereof having at least 90% sequence similarity to any of said sequences.

In one aspect, provided herein is a nucleic acid encoding at least one chain or region of the humanized antibody or antigen binding fragment thereof as described herein.

In one aspect, provided herein is a cell line comprising the nucleic acid as described herein.

In one aspect, provided herein is a chimeric antigen receptor (CAR) comprising a combination of heavy and light chain variable region sequences of the humanized antibody as described herein.

In one aspect, provided herein is a pharmaceutical composition comprising the humanized antibody or antigen binding fragment as described herein or the CAR as described

herein, and a pharmaceutically acceptable excipient, carrier, or diluent.

In one aspect, provided herein is a use of the humanized antibody or antigen binding fragment thereof as described herein, the CAR as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for increasing surface expression and/or signaling of CD226 on CD8+ T or NK cells.

In one aspect, provided herein is a use of the humanized antibody or antigen binding fragment thereof as described herein, the CAR as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating cancer in an individual.

In one aspect, provided herein is the humanized antibody or antigen binding fragment thereof as described herein, the CAR as described herein, or the pharmaceutical composition as described herein for use in therapy.

The present ~~invention~~disclosure provides, according to one ~~aspect~~example, a humanized antibody that specifically binds human poliovirus receptor (PVR, CD155), or a fragment thereof comprising at least the antigen binding site, wherein the antibody or a fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain comprises a variable region having an amino acid sequence at least about 90% identical to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6; and wherein the light chain comprises a variable region having an amino acid sequence at least about 90% identical to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

According to some ~~embodiment~~examples, the antibody comprises heavy-chain variable region amino-acid sequence comprising the CDR sequences set forth in SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12, and light-chain variable region amino-acid sequence comprising the CDR sequences set forth in SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15.

According to some ~~embodiment~~examples, the humanized antibody or fragment thereof is an IgG monoclonal antibody. According to some ~~embodiment~~examples, the

humanized monoclonal antibody has a heavy chain constant region selected from IgG4 and IgG1.

In certain ~~embodiment~~[examples](#), the humanized antibody or fragment thereof is an IgG4 subclass.

In certain ~~embodiment~~[examples](#), the humanized antibody or antigen binding fragment thereof is an IgG1 subclass.

- 5 According to some ~~embodiment~~[examples](#), the humanized antibody or fragment thereof comprises a human IgG4 constant region having S228P (also named S241P) substitution in the hinge region.

According to some [embodiment examples](#), the humanized antibody or fragment thereof is a monoclonal antibody, Fab, F(ab)₂, a single-domain antibody, or a single chain variable fragment (scFv).

According to some [embodiment examples](#), the humanized antibody or antigen binding
5 fragment thereof comprises a heavy chain variable region comprising the amino acid sequence
QVQLVQSGAE(L/V)KKPGASVK(I/V)SCKATGYTFSNYWIEW(I/V)(K/R)QAPGQGLEW(I/
M)GEIFPGSGRINFNEKFKGR(A/V)TFTADTSI(D/S)T(T/A)YM(Q/E)LS(S/R)L(T/R)SDD(S/T
)AVYYCARTKIYGNSFDYWGGGT(T/L)VTVSS (SEQ ID NO: 47); and a light chain variable
region comprising the amino acid sequence
10 DI(M/Q)MTQSPS(F/S)LSASVGDRVTITC(K/R)ASQDVGTA(V/A)WYQQKPGKAPK(L/S)
LIYWASSRHEGVP(D/S)RF(T/S)GSGSGTDFLTISS
LQ(S/P)EDFA(D/T)YFCQQYSRYPLTFGQGT KLEIK (SEQ ID NO: 48).

According to some [embodiment examples](#), the humanized antibody or fragment thereof comprises a heavy chain variable region comprising:

- 15 i. a set of three CDR sequences comprising the sequences set forth in SEQ ID Nos. 10-12; and
- ii. a set of four heavy chain framework (FR) sequences: (A) FR-H1 selected from the group consisting of SEQ ID NOs: 18, 22, and 26; (B) FR-H2 selected from the group consisting of SEQ ID NOs: 19, 23, and 28; (C) FR-H3 selected from the group consisting of SEQ ID NOs: 20, 24, 27, and 29; and (D) FR-H4 selected from the group consisting of SEQ ID NOs: 21 and 25.
- 20

According to some [embodiment examples](#), the humanized antibody or antigen binding fragment thereof comprising a light chain variable region comprising:

- 25 i. a set of three CDR sequences comprising the sequences set forth in SEQ ID Nos. 13-15; and
- ii. a set of four light chain framework sequences: (A) FR-L1 selected from the group consisting of SEQ ID NOs: 30 and 34; (B) FR-L2 selected from the group consisting of SEQ ID NOs: 31 and 37; (C) FR-L3 selected from the group consisting of SEQ ID NOs: 32, 35, and 36; and (D) FR-L4 is SEQ ID NO: 33.

According to some ~~embodiment~~examples, the humanized antibody or antigen binding fragment thereof comprising a heavy chain variable region and a light chain variable region, the heavy chain variable region comprising:

- i. a set of three CDR sequences comprising the sequences set forth in SEQ ID Nos. 10-12; and
- ii. a set of four heavy chain (HC) framework (FR) sequences: (A) FR-H1 selected from the group consisting of SEQ ID NOs: 18, 22, and 26; (B) FR-H2 selected from the group consisting of SEQ ID NOs: 19, 23, and 28; (C) FR-H3 selected from the group consisting of SEQ ID NOs: 20, 24, 27, and 29; (D) FR-H4 selected from the group consisting of SEQ ID NOs: 21 and 25; and the light chain variable region comprising:
 - i. a set of three CDR sequences comprising the sequences set forth in SEQ ID Nos. 13-15; and
 - ii. a set of four light chain (LC) framework (FR) sequences: (A) FR-L1 selected from the group consisting of SEQ ID NOs: 30 and 34; (B) FR-L2 selected from the group consisting of SEQ ID NOs: 31 and 37; (C) FR-L3 selected from the group consisting of SEQ ID NOs: 32, 35, and 36; and (D) FR-L4 is SEQ ID NO: 33.

According to some ~~embodiment~~examples, the heavy chain variable region of the humanized monoclonal antibody comprises an amino acid sequence at least about 90% identical to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6; and the light chain variable region comprises an amino acid sequence at least about 90% identical to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9. According to some ~~embodiment~~examples, the heavy chain variable region of the humanized monoclonal antibody comprises an amino acid sequence at least about 95% identical to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6; and the light chain variable region comprises an amino acid sequence at least about 95% identical to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9. In certain ~~embodiment~~examples, the heavy chain variable region of the humanized monoclonal antibody comprises an amino acid sequence at least about 97% identical to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, and the light chain variable region

comprises an amino acid sequence at least about 97% identical to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9. In certain ~~embodiment~~[examples](#), the heavy chain variable region of the humanized monoclonal antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

According to some ~~embodiment~~[examples](#), the humanized antibody comprises a combination of a heavy chain variable region and a light chain variable region, wherein the combination is selected from the group consisting of:

- i. a heavy chain variable region sequence set forth in SEQ ID NO: 1 and a light chain variable region sequence set forth in SEQ ID NO: 2;
- ii. a heavy chain variable region sequence set forth in SEQ ID NO: 4 and a light chain variable region sequence set forth in SEQ ID NO: 8;
- 15 iii. a heavy chain variable region sequence set forth in SEQ ID NO: 5 and a light chain variable region sequence set forth in SEQ ID NO: 2;
- iv. a heavy chain variable region sequence set forth in SEQ ID NO: 5 and a light chain variable region sequence set forth in SEQ ID NO: 8;
- v. a heavy chain variable region sequence set forth in SEQ ID NO: 4 and a light chain variable region sequence set forth in SEQ ID NO: 2;
- 20 vi. a heavy chain variable region sequence set forth in SEQ ID NO: 1 and a light chain variable region sequence set forth in SEQ ID NO: 8;
- vii. a heavy chain variable region sequence set forth in SEQ ID NO: 6 and a light chain sequence set forth in SEQ ID NO: 2; and
- 25 viii. a heavy chain variable region sequence set forth in SEQ ID NO: 6 and a light chain variable region sequence set forth in SEQ ID NO: 8.

According to some ~~embodiment~~[examples](#), the heavy chain variable region of the humanized monoclonal antibody comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NO: 1, and the light chain variable region comprises an amino acid sequence at least about 90% identical to that set forth in SEQ ID NO: 2.

According to some ~~embodiment~~[examples](#), the heavy chain variable region of the humanized

monoclonal antibody comprises an amino acid sequence identical to that set forth in SEQ ID NO: 1, and the light chain variable region comprises an amino acid sequence identical to that set forth in SEQ ID NO: 2.

5 According to some ~~embodiment~~examples, the humanized antibody inhibits binding of PVR to at least one of TIGIT, CD96, and CD226.

According to some ~~embodiment~~examples, the antibody inhibits binding of PVR to TIGIT, CD96, and CD226.

10 According to some ~~embodiment~~examples, the humanized antibody is an IgG4 antibody comprising a heavy chain sequences set forth in SEQ ID NO: 49, or a sequence having at least 90% identity. According to some ~~embodiment~~examples, the humanized antibody is an IgG1 comprising a heavy chain sequence set forth in SEQ ID NO: 50 or a sequence having at least 90% identity.

According to some ~~embodiment~~examples, the humanized antibody comprising a light chain sequence set forth in SEQ ID NO: 49.

15 According to some ~~embodiment~~examples, the humanized antibody exhibits improved antibody-dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) compared to other, known antibodies.

Polynucleotide sequences encoding the humanized antibody or antigen binding fragment thereof, are provided according to another ~~aspect~~example of the ~~invention~~disclosure.

20 According to some ~~embodiment~~examples, a polynucleotide sequence encoding the amino acid sequences of a heavy chain variable region, a light chain variable region or both, as described above is provided.

25 According to some ~~embodiment~~examples, a polynucleotide is provided encoding a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

According to some ~~embodiment~~examples, a polynucleotide is provided encoding a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

According to some [embodimentexamples](#), the polynucleotide encodes a humanized antibody or antibody fragment thereof comprising: a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6; and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9. Each combination of heavy and light chain variable regions represents a separate [embodimentexample](#) of the [inventiondisclosure](#).

According to some [embodimentexamples](#), the polynucleotide sequence encoding the humanized antibody heavy chain variable region comprises a sequence selected from the group consisting of SEQ ID NOs: 38-42, or a variant thereof having at least 90% sequence identity. Each possibility represents a separate [embodimentexample](#) of the [inventiondisclosure](#).

According to some [embodimentexamples](#), the polynucleotide sequence encoding the humanized antibody light chain variable region comprises a sequence selected from the group consisting of SEQ ID NOs: 43-46, or a variant thereof having at least 90% sequence identity. Each possibility represents a separate [embodimentexample](#) of the [inventiondisclosure](#).

In a further [aspectexample](#), the present [inventiondisclosure](#) provides a nucleic acid construct comprising a nucleic acid molecule encoding at least one humanized antibody chain or fragment thereof as described herein. According to some [embodimentexample](#)s the nucleic acid construct is a plasmid.

Also described is a cell line comprising the nucleic acids encoding the antibodies of the present [inventiondisclosure](#). The cell line is for expression of the humanized antibody or fragment thereof as described herein. In certain [embodimentexamples](#), the cell line is a mammalian cell line such as a Chinese Hamster Ovary (CHO) cell line.

According to some [embodimentexamples](#), the cell line is a bacterial, plant, murine (e.g., NS0 and Sp2/0), rat (e.g., YB2/0), hamster (e.g., BHK and CHO) or human (e.g., PER.C6).

According to an [aspectexample](#), the present [inventiondisclosure](#) provides a chimeric antigen receptor (CAR) comprising an extracellular portion (binding domain), containing any of the humanized antibodies or fragment thereof as described herein. According to some [embodimentexamples](#), a CAR comprising a combination of heavy and light chain variable region sequences described above,

having unique combination of CDR and framework sequences and improved binding and other properties, is provided.

According to some [embodiment examples](#), the CAR comprises a combination of heavy and light chain variable regions, the heavy chain variable region comprises an amino acid sequence with at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6; and the light chain variable region comprises an amino acid sequence with at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

According to some [embodiment examples](#), the CAR comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

According to some [embodiment examples](#), the CAR comprises a combination of a humanized antibody heavy and light chain variable regions, wherein the combination is selected from the group consisting of:

- i. a heavy chain variable region sequence set forth in SEQ ID NO: 1 and a light chain variable region sequence set forth in SEQ ID NO: 9;
- ii. a heavy chain variable region sequence set forth in SEQ ID NO: 3 and a light chain variable region sequence set forth in SEQ ID NO: 9;
- iii. a heavy chain variable region sequence set forth in SEQ ID NO: 4 and a light chain variable region sequence set forth in SEQ ID NO: 9;
- iv. a heavy chain variable region sequence set forth in SEQ ID NO: 5 and a light chain variable region sequence set forth in SEQ ID NO: 9; and
- v. a heavy chain variable region sequence set forth in SEQ ID NO: 6 and a light chain variable region sequence set forth in SEQ ID NO: 9.

According to some [embodiment examples](#), the CAR comprises a heavy chain variable region sequence selected from the group consisting of SEQ ID NO: 1, 3, 4, 5, and 6, and a light chain variable region sequence set forth in SEQ ID NO: 9, a transmembrane domain, and an

intracellular T cell signaling domain.

A single chain variable region (scFv) comprising the heavy chain and light chain variable regions of the antibodies described herein is also provided according to the present inventiondisclosure. According to certain embodimentexamples, there is a hinge region between the variable regions.

According to some embodimentexamples, the amino acid sequence of the scFv is set forth in a sequence selected from SEQ ID NO: 56, SEQ ID NO: 57, and an analog thereof having at least 90% sequence similarity to any of said sequences.

According to some embodimentexamples, the CAR comprises an amino acid sequences set forth in any one of SEQ ID NO: 56 and SEQ ID NO: 57.

According to some embodimentexamples, the CAR comprises a scFv sequence and at least one protein domain selected from the group consisting of a CD8 Stalk domain, a CD28 TM domain, a 41BB domain, and a CD3ζ (CD3Z, Zetta) domain. According to some embodimentexamples, the CAR comprises a CD8 Stalk domain. According to some embodimentexamples, the CAR comprises a CD28 TM domain. According to some embodimentexamples, the CAR comprises a CD3Z domain. According to some embodimentexamples, the CAR comprises a 41BB domain. According to specific embodimentexamples, the CAR comprises a CD8 Stalk domain, a CD28 TM domain, a 41BB domain, and a CD3Z domain.

According to some embodimentexamples, the CAR comprises a scFv sequence comprising the PVR binding site of any antibody disclosed above and at least one domain selected from the group consisting of: CD8 Stalk domain, a CD28 TM domain, a 41BB domain, and a CD3Z domain. According to specific embodimentexamples, the CAR comprises a scFv sequence comprising the PVR binding site of any antibody disclosed above and a CD8 Stalk domain, a CD28 TM domain, a 41BB domain, and a CD3Z domain.

According to some embodimentexamples, a lymphocyte engineered to express the CAR described herein is provided. According to some embodimentexamples, a T cell engineered to express the CAR described herein is provided. According to additional embodimentexamples, an NK cell engineered to express the CAR described herein is provided.

According to specific embodimentexamples, an engineered T cell is provided, expressing a

scFv sequence selected from the group consisting of: SEQ ID NO: 56, SEQ ID NO: 57, or an analog thereof having at least 90% sequence similarity to any of said sequences; a CD8 Stalk domain, a CD28 TM domain, a 41BB domain, and a CD3Z domain.

According to an [aspectexample](#), the present [inventiondisclosure](#) provides a method of treating cancer in a subject comprising administering a therapeutically effective amount of at least one lymphocyte comprising the CAR as described herein to said subject.

The present [inventiondisclosure](#) provides, according to another [aspectexample](#), a pharmaceutical composition comprising the humanized antibody or antigen binding fragment described herein and a pharmaceutically acceptable excipient, carrier, or diluent.

Any administration mode may be used to deliver the compositions of the present [inventiondisclosure](#) to a subject in need thereof, including parenteral and enteral administration modes.

According to some [embodimentexamples](#), the pharmaceutical composition is formulated for injection or infusion. According to some [embodimentexamples](#), the pharmaceutical composition is formulated for intravenous administration. In certain [embodimentexamples](#), the pharmaceutical composition is formulated for intratumoral administration.

According to some [embodimentexamples](#), the humanized antibody or antigen binding fragment thereof or the pharmaceutical composition is for use in increasing surface expression and/or signaling of CD226 on CD8+ and CD4+ T cells.

According to [embodimentexamples](#), the humanized antibody or antigen binding fragment thereof, or the pharmaceutical composition is for use in treating a cancer in an individual. In certain [embodimentexamples](#), the cancer comprises a solid tumor. In certain [embodimentexamples](#), the cancer is selected from the group consisting of lung cancer, colon cancer, glioblastoma, adrenal cancer, uterine cancer, head and neck cancer, pancreatic cancer, and breast cancer. Each possibility represents a separate [embodimentexample](#) of the [inventiondisclosure](#).

According to some [embodimentexamples](#), the cancer is a hematological cancer.

According to some [embodimentexamples](#), the hematological cancer is selected from leukemia including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), and chronic lymphocytic leukemia (CLL); lymphoma, including

Hodgkin disease, and non-Hodgkin lymphoma; and multiple myeloma.

According to some [embodimentexamples](#), the individual is human.

According to some [embodimentexamples](#) of the [inventiondisclosure](#), the use further comprises the use of an agent that downregulates the activity or expression of an immune co-inhibitory receptor.

According to some [embodimentexamples](#), the immune co-inhibitory receptor is selected from the group consisting of PD-1, PD-L1, TIGIT, CTLA-4, LAG3, TIM3, BTLA, VISTA, B7H4, CD96, BY55 (CD 160), LAIR1, SIGLEC10, CD112R, CD112, ILT-4 and 2B4. Each possibility represents a separate [embodimentexample](#) of the [inventiondisclosure](#).

According to some [embodimentexamples](#) of the [inventiondisclosure](#), the use further comprises the use in a combination with an anti-endothelial growth factor receptor (EGFR) antibody.

The present [inventiondisclosure](#) provides, according to another [aspectexample](#), a method of increasing surface expression and/or signaling of CD226 in the CD8+ and CD4+ T cells of an individual, the method comprising administering to the individual a therapeutically effective amount of the humanized antibody or antigen binding fragment thereof, or the pharmaceutical composition described herein. In certain [embodimentexamples](#), the CD8+ T cells are tumor infiltrating CD8+ T cells.

The present [inventiondisclosure](#) provides, according to another [aspectexample](#), a method of treating a cancer in an individual in need of such treatment, the method comprising administering to the individual a therapeutically effective amount of the humanized antibody or antigen binding fragment thereof or the pharmaceutical composition. In certain [embodimentexamples](#), the cancer comprises a solid tumor. According to additional [embodimentexamples](#), the cancer is a non-solid tumor. In certain [embodimentexamples](#), the cancer is selected from the group consisting of glioblastoma, pancreatic cancer, breast cancer, bladder cancer, kidney cancer, head and neck cancer, ovarian cancer, colon cancer, cervical cancer, prostate cancer, and lung cancer. In certain [embodimentexamples](#), the method of treating cancer involves preventing or reducing formation, growth or spread of metastases in a subject.

The present [inventiondisclosure](#) provides, according to another [aspectexample](#), a method of treating a cancer in an individual afflicted with a cancer comprising administering to the individual a therapeutically effective amount of the humanized antibody or antigen binding fragment

thereof or the pharmaceutical composition, and an inhibitor of PD-1, PD-L1, CTLA-4 or CD112R signaling. In certain [embodimentexamples](#), the cancer comprises a solid tumor. In certain [embodimentexamples](#), the cancer is selected from the group consisting of glioblastoma, pancreatic cancer, breast cancer, bladder cancer, kidney cancer, head and neck cancer, ovarian cancer, colon cancer, cervical cancer, prostate cancer, or lung cancer. In certain [embodimentexamples](#), the inhibitor of PD-1 signaling is an antibody or fragment thereof that binds to PD-1. In certain [embodimentexamples](#), the antibody or fragment thereof that binds to PD-1 is Pembrolizumab, Nivolumab, AMP-514, Tislelizumab, Spartalizumab, or a PD-1 binding fragment thereof. In certain [embodimentexamples](#), the inhibitor of PD-1 signaling is an antibody that specifically binds PD-L1 or PD-L2. In certain [embodimentexamples](#), the antibody that specifically binds PD-L1 or PD-L2 comprises Durvalumab, Atezolizumab, Avelumab, BMS-936559, or FAZ053, or a PD-L1 or PD-L2 binding fragment thereof. In certain [embodimentexamples](#), the inhibitor of PD-1 signaling comprises an Fc-fusion protein that binds PD-1, PD-L1, or PD-L2. In certain [embodimentexamples](#), the Fc-fusion protein comprises AMP-224 or a PD-1 binding fragment thereof. In certain [embodimentexamples](#), the inhibitor of PD-1 signaling comprises a small molecule inhibitor of PD-1, PD-L1, or PD-L2. In certain [embodimentexamples](#), the small molecule inhibitor of PD-1, PD-L1, or PD-L2 signaling comprises one or more of: N-{2-[(2-methoxy-6-[(2-methyl[1,1'-biphenyl]-3-yl)methoxy]pyridin-3-yl)methyl]amino}ethyl}acetamide (BMS 202); (2-((3-cyanobenzyl)oxy)-4-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)-5-methylbenzyl)-D-serine hydrochloride; (2R,4R)-1-(5-chloro-2-((3-cyanobenzyl)oxy)-4-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)benzyl)-4-hydroxypyrrolidine-2-carboxylic acid; 3-(4,6-dichloro-1,3,5-triazin-2-yl)-1-phenylindole; 3-(4,6-dichloro-1,3,5-triazin-2-yl)-1-phenyl-1h-indole; L- α -Glutamine, N2,N6-bis(L-seryl-L-asparaginyl-L-threonyl-L-seryl-L- α -glutamyl-L-seryl-L-phenylalanyl)-L-lysyl-L-phenylalanyl-L-arginyl-L-valyl-L-threonyl-L-glutamyl-L-leucyl-L-alanyl-L-prolyl-L-lysyl-L-alanyl-L-glutamyl-L-isoleucyl-L-lysyl; (2S)-1-[[2,6-dimethoxy-4-[(2-methyl[1,1'-biphenyl]-3-yl)methoxy]phenyl]methyl]-2-piperidinecarboxylic acid; Glycinamide, N-(2-mercaptoacetyl)-L-phenylalanyl-N-methyl-L-alanyl-L-asparaginyl-L-prolyl-L-histidyl-L-leucyl-N-methylglycyl-L-tryptophyl-L-seryl-L-tryptophyl-N-methyl-L-norleucyl-N-methyl-L-norleucyl-L-arginyl-L-cysteinyl-, cyclic (1 $\rightarrow$ 14)-thioether; or a derivative or analog thereof.

Also described herein is a method of making composition for treating a cancer in an individual afflicted with cancer comprising admixing the humanized antibody or antigen binding fragment thereof and a pharmaceutically acceptable excipient, carrier, or diluent. In

certain ~~embodiment~~examples, the cancer comprises a solid tumor. In certain ~~embodiment~~examples, the cancer is selected from the group consisting of glioblastoma, pancreatic cancer, breast cancer, bladder cancer, kidney cancer, head and neck cancer, ovarian cancer, colon cancer, cervical cancer, prostate cancer, and lung cancer. Also described herein is a method of producing the humanized antibody or antigen binding fragment thereof comprising incubating the cell line described herein in a cell culture medium under conditions sufficient to allow expression and secretion of the humanized antibody or antigen binding fragment thereof.

The present ~~invention~~disclosure further provides, according to an ~~aspect~~example, a method of diagnosing or prognosing cancer in a subject, the method comprises determining the expression level of PVR in a biological sample of said subject using at least one humanized antibody, fragment or scFv as described herein.

The present ~~invention~~disclosure further provides, according to another ~~aspect~~example, a method of determining or quantifying the expression of PVR, the method comprising contacting a biological sample with an antibody or antibody fragment as described herein, and measuring the level of complex formation.

According to some ~~embodiment~~examples, the method for detecting or quantifying the expression of PVR comprises the steps of:

- i. incubating a sample with an antibody specific to PVR or an antibody fragment thereof comprising at least an antigen-binding portion;
- ii. detecting the bound PVR using a detectable probe.

- According to some ~~embodiment~~examples, the method further comprises the steps of:
- iii. comparing the amount of (ii) to a standard curve obtained from a reference sample containing a known amount of PVR; and
 - iv. calculating the amount of the PVR in the sample from the standard curve.

According to some ~~embodiment~~examples, the method comprises indicating a subject as having a PVR positive cancer if the PVR amount is higher than a control or a given reference.

According to some particular ~~embodiment~~examples, the sample is a body fluid or solid tissue. In some ~~embodiment~~examples, the method is performed in-vitro or ex-vivo.

A kit for measuring the expression of PVR in biological sample is also provided

comprising at least one antibody or antibody fragment as described herein and means for measuring PVR expression. In some ~~embodiment~~example, the kit further comprising instruction material directing the use of the kit.

Further ~~embodiment~~examples and the full scope of applicability of the present ~~invention~~disclosure will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred ~~embodiment~~examples of the ~~invention~~disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the ~~invention~~disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The novel features described herein are set forth with particularity in the appended claims. A better understanding of the characteristics and advantages of the features described herein will be obtained by reference to the following detailed description that sets forth illustrative examples, in which the principles of the features described herein are utilized, and the accompanying drawings of which:

FIGs. 1A-1B. Affinity and competition assays of N56 substitutions. **FIG 1A.** Improved affinity for PVR binding by N56E and N56D variants. Biacore assay showing relative and absolute affinity of the N56 substitutions (hIgG4 variants) to PVR-HIS tagged (Sino Cat. no. 10109-H08H). Affinity shift of >25% were considered significant. (*) Relative K_D s for each variant were established by dividing the K_D of the substitution by the K_D of the parental N56 variant (VH0VK0). **FIG. 1B** compares the potency of chimeric antibodies (5B9 wild type - WT, having IgG4 (S241P) HC) with variable regions carrying the LC CDR2 sequence with single amino acid substitutions (to remove a deamidation site). Potency was measured in a competition assay using parental 5B9. Relative IC50 was determined for each variant by dividing its IC50 by that of the chimeric WT antibody tested in parallel.

FIGs. 2A-2B. Improved cross-reactivity for monkey PVR binding by N56E and N56D variants. To assess maximal binding, saturating dose (10ug/ml) of the N56 variant monoclonal antibodies (shown at the X axis) were used to stain PVR-expressing cells, and mean fluorescence intensity (MFI) values were determined by FACS analysis. The fold change was calculated by dividing the MFI of each variant by the MFI of the parental antibody (K0).

THE CLAIMS

1. ~~A humanized antibody or antigen binding fragment thereof, wherein the antibody or antigen binding fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain variable region comprises an amino acid sequence at least about 90% identical to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6; and wherein the light chain variable region comprises an amino acid sequence at least about 90% identical to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9, wherein the antibody or antigen binding fragment thereof binds to human poliovirus receptor (CD155).~~

2. ~~The humanized antibody or antigen binding fragment thereof according to claim 1, wherein the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 47; or.~~

3. ~~The humanized antibody or antigen binding fragment thereof according to claim 1, wherein the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 48; or.~~

4. ~~The~~ A humanized antibody or antigen binding fragment thereof comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain and a light chain ~~according to any one of claims 1 to 3, wherein the~~ heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 47; and the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 48.

5. ~~The~~ The humanized antibody or antigen binding fragment thereof of ~~any one of claims 1 to 4,~~ wherein the heavy chain variable region comprising:

i. a set of three CDR sequences comprising the sequences set forth in SEQ ID Nos. 10-12; and

ii. a set of four heavy chain framework (FR) sequences: (A) FR-H1 selected from the group consisting of SEQ ID NOs: 18, 22, and 26; (B) FR-H2 selected from the group consisting of SEQ ID NOs: 19, 23, and 28; (C) FR-H3 selected from the group consisting of SEQ ID NOs: 20, 24, 27, and 29; and (D) FR-H4 selected from the group consisting of SEQ ID NOs: 21 and 25.

6. ~~The~~ The humanized antibody or antigen binding fragment thereof of any one of claims ~~1 to 5,~~ 1 or 12 to 5, wherein the light chain variable region comprising:

- i. a set of three CDR sequences comprising the sequences set forth in SEQ ID Nos. 13-15; and
- ii. a set of four light chain framework sequences: (A) FR-L1 selected from the group consisting of SEQ ID NOs: 30 and 34; (B) FR-L2 selected from the group consisting of SEQ ID NOs: 31 and 37; (C) FR-L3 selected from the group consisting of SEQ ID NOs: 32, 35, and 36; and (D) FR-L4 is SEQ ID NOs: 33.

~~7.4.~~ The humanized antibody or antigen binding fragment thereof of claim 1, wherein the heavy chain variable region comprises an amino acid sequence at least about 95% identical to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6; and wherein the light chain variable region comprises an amino acid sequence at least about 95% identical to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

~~8. The humanized antibody or antigen binding fragment thereof of claim 1, wherein the heavy chain variable region comprises an amino acid sequence at least about 97% identical to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, and wherein the light chain variable region comprises an amino acid sequence at least about 97% identical to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.~~

~~9.5.~~ The humanized antibody or antigen binding fragment thereof of claim 1, wherein the heavy chain variable region comprises an amino acid sequence identical to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, and wherein the light chain variable region comprises an amino acid sequence identical to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.

~~10.6.~~ The humanized antibody or antigen binding fragment thereof of any one of claims 1 and ~~985~~, wherein the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 1, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 2.

~~11.7.~~ The humanized antibody or antigen binding fragment thereof of any one of claims 1 to ~~1096~~, wherein the humanized antibody or antigen binding fragment thereof is an IgG antibody.

- ~~12.8.~~ The humanized antibody or antigen binding fragment thereof of claim ~~14~~7, wherein the humanized antibody or antigen binding fragment thereof comprises an IgG4 or IgG1 heavy chain constant region.
- 5 ~~13.9.~~ The humanized antibody or antigen binding fragment thereof according to claim ~~12~~1078, wherein the humanized antibody or antigen binding fragment thereof comprises an IgG4 heavy chain constant region having an alteration of a serine residue substituted for proline at position 228 of said IgG4 heavy chain constant region.
- 10 ~~14. The humanized antibody or antigen binding fragment thereof of any one of claims 1 to 10, wherein the humanized antibody or antigen binding fragment thereof is a Fab, F(ab)₂, a single domain antibody, or a single chain variable fragment (scFv).~~
- ~~15.10.~~ A single chain variable fragment (scFv) of a humanized antibody according to any one of claims 1 to ~~14~~189, comprising an amino acid sequence selected from SEQ ID NO: 56, SEQ ID NO: 57, and an analog thereof having at least 90% sequence similarity to any of said sequences.
- 15 ~~16.11.~~ The humanized antibody or antigen binding fragment thereof of any one of claims 1 to ~~15~~2910, wherein the humanized antibody inhibits binding of PVR to at least one of TIGIT, CD96, and CD226.
- ~~17.12.~~ A nucleic acid encoding at least one chain or region of the humanized antibody or antigen binding fragment thereof of any one of claims 1 to ~~166~~1301.
- 20 ~~18.13.~~ A cell line comprising the nucleic acid of claim ~~17~~12.
- ~~19. The cell line of claim 18, wherein the cell line is a Chinese Hamster Ovary cell line.~~
- ~~20.14.~~ A chimeric antigen receptor (CAR) comprising a combination of heavy and light chain variable region sequences of the humanized antibody according to any one of claims 1 to ~~16~~141.
- 25 ~~21. The CAR of claim 20, wherein the heavy chain variable region comprises an amino acid sequence with at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6; and the light chain variable region comprises an amino acid sequence with at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9.~~
- 30 ~~22. The CAR of claim 21, comprising a scFv comprising an amino acid sequence selected from SEQ ID NO: 56, SEQ ID NO: 57, and an analog thereof having at least 90% sequence similarity to any of said sequences.~~

~~23. The CAR of any one of claims 20 to 22, comprising at least one domain selected from the group consisting of: CD8 Stalk domain, a CD28 TM domain, a 41BB domain, and a CD3Z domain.~~

~~24.~~ 15. A pharmaceutical composition comprising the humanized antibody or antigen binding fragment thereof of any one of claims 1 to ~~16~~ 11 or the CAR according to ~~any one of claims 20 and 23~~ 124, and a pharmaceutically acceptable excipient, carrier, or diluent.

~~25. The pharmaceutical composition of claim 24, formulated for intravenous administration.~~

~~26. The pharmaceutical composition of claim 24, formulated for intratumoral administration.~~

~~27.~~ 16. ~~Use of The~~ the humanized antibody or antigen binding fragment thereof of any one of claims 1 to ~~16~~ 11, the CAR of claims ~~124~~ 20 or 23, or the pharmaceutical composition of ~~any one of claims 135~~ 24 to 26 in the manufacture of a medicament for ~~use in~~ increasing surface expression and/or signaling of CD226 on CD8+ T or NK cells.

~~28.~~ 17. ~~Use of T~~ the humanized antibody or antigen binding fragment thereof of any one of claims 1 to ~~16~~ 11, the CAR of claims ~~124~~ 20 or 23, or the pharmaceutical composition of ~~any one of claims 135~~ 24 to 26 in the manufacture of a medicament for ~~use in~~ treating cancer in an individual.

~~29.~~ 18. ~~The humanized antibody or antigen binding fragment thereof or the CAR or the pharmaceutical composition for use of claim 28~~ 17, wherein the cancer comprises a solid tumor.

~~19.~~ The humanized antibody or antigen binding fragment thereof, or the CAR, or the pharmaceutical composition for use of claim 28 17, wherein the cancer is selected from the group consisting of lung cancer, colon cancer, adrenal cancer, uterine cancer, head and neck cancer, pancreatic cancer, and breast cancer.

~~30.~~ 20. ~~The use of claim 17, wherein the medicament is to be administered with an agent that downregulates the activity or expression of an immune co-inhibitory receptor.~~

~~31. A method of increasing surface expression and/or signaling of CD226 in the CD8+ T cells of an individual comprising administering to the individual a therapeutically effective amount of the humanized antibody or antigen binding fragment thereof of any one of claims 1 to 16, the CAR of claims 20 or 23, or the pharmaceutical composition of any one of claims 24 to 26.~~

~~32. The method of claim 31, wherein the CD8+ T cells are tumor infiltrating CD8+ T cells.~~

~~33. A method of treating a cancer in an individual afflicted with a cancer comprising administering to the individual a therapeutically effective amount of the humanized~~

~~antibody or antigen binding fragment thereof of any one of claims 1 to 16, the CAR of claims 20 or 23 or the pharmaceutical composition of any one of 24 to 26.~~

5 ~~34. A method of treating a cancer in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the humanized antibody or antigen binding fragment thereof of any one of claims 1 to 16, the CAR of claims 20 or 23, or the pharmaceutical composition of any one of 24 to 26, and an inhibitor of PD-1, PD-L1, CTLA-4 or CD112R signaling.~~

~~35. The method of any one of claims 33 and 34, wherein the cancer comprises a solid tumor.~~

10 ~~36. The method of claim 33, wherein the cancer is selected from the group consisting of glioblastoma, pancreatic cancer, breast cancer, bladder cancer, kidney cancer, head and neck cancer, ovarian cancer, colon cancer, cervical cancer, prostate cancer, and lung cancer.~~

~~37. The method of any one of claims 33 and 34, wherein the cancer is a hematological cancer.~~

15 ~~38. The method of any one of claims 34 to 37 wherein the inhibitor of PD-1 signaling is an antibody or fragment thereof that binds to PD-1.~~

~~39. The method of claim 38, wherein the antibody or fragment thereof that binds to PD-1 is selected from Pembrolizumab, Nivolumab, AMP-514, Tislelizumab, Spartalizumab, and a PD-1 binding fragment thereof.~~

20 ~~40. The method of any one of claims 34 to 37, wherein the inhibitor of PD-1 signaling is an antibody that specifically binds PD-L1 or PD-L2.~~

~~41. The method of claim 40, wherein the antibody that specifically binds PD-L1 or PD-L2 is selected from Durvalumab, Atezolizumab, Avelumab, BMS-936559, or FAZ053, or a PD-L1 and PD-L2 binding fragment thereof.~~

25 ~~42. The method of any one of claims 34 to 37, wherein the inhibitor of PD-1 signaling comprises a small molecule inhibitor of PD-1, PD-L1, or PD-L2.~~

~~43. A method of preparing a composition for treating a cancer in an individual in need thereof, comprising admixing the humanized antibody or antigen binding fragment thereof of any one of claims 1 to 16 and a pharmaceutically acceptable excipient, carrier, or diluent.~~

~~44. The method of claim 43, wherein the cancer comprises a solid tumor.~~

30 ~~45. The method of claim 44, wherein the cancer is selected from the group consisting of glioblastoma, pancreatic cancer, breast cancer, bladder cancer, kidney cancer, head and neck cancer, ovarian cancer, colon cancer, cervical cancer, prostate cancer, and lung cancer.~~

46. ~~The method of claim 43, wherein the cancer is a hematological cancer.~~
47. ~~A method of producing the humanized antibody or antigen binding fragment thereof of any one of claims 1 to 16 comprising incubating a cell line that contains a nucleic acid encoding the humanized antibody or antigen binding fragment thereof, in a cell culture medium under conditions sufficient to allow expression and secretion of the humanized antibody or antigen binding fragment thereof.~~
48. ~~A method of diagnosing or prognosing cancer in a subject, the method comprises determining the expression level of PVR in a biological sample of said subject using at least one humanized antibody or fragment thereof according to any one of claims 1 to 16.~~
49. ~~The method of claim 48 comprising a step of diagnosing or prognosing cancer in a subject if the expression of PVR is higher than a control or a reference value.~~
50. ~~A kit comprising at least one antibody or antibody fragment according to any one of claims 1 to 16 and means for measuring PVR expression.~~
21. The humanized antibody or antigen binding fragment thereof of any one of claims 1 to 11, the CAR of claim 14, or the pharmaceutical composition of claim 15 for use in therapy.