

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 disclosure thus provides, in some 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 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, wherein the humanized antibody or antigen binding fragment thereof bind to human poliovirus receptor (PVR; CD155).

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.

In one aspect, provided herein is a nucleic acid or combination of nucleic acids encoding the humanized antibody or antigen binding fragment thereof as described herein.

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

In one aspect, provided herein is a chimeric antigen receptor (CAR) NK or T cells 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 NK or T cells as described herein, and a pharmaceutically acceptable excipient, carrier, or diluent.

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

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

The present disclosure provides, according to one 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 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.

25 According to some examples, the humanized antibody or fragment thereof is an IgG

monoclonal antibody. According to some examples, the humanized monoclonal antibody has a heavy chain constant region selected from IgG4 and IgG1. In certain examples, the humanized antibody or fragment thereof is an IgG4 subclass. In certain examples, the humanized antibody or antigen binding fragment thereof is an IgG1 subclass.

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

CLAIMS

1. 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
5 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, wherein the humanized antibody or antigen binding
10 fragment thereof bind to human poliovirus receptor (PVR; CD155).
2. The humanized antibody or antigen binding fragment thereof of claim 1, comprising:
 - a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2; or
 - 15 a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 8; or
 - a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 4, and a light chain variable region comprising the amino acid sequence set forth in
20 SEQ ID NO: 2; or
 - a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 4, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 8; or
 - 25 a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2; or
 - a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5, and a light chain variable region comprising the amino acid sequence set forth in
30 SEQ ID NO: 8; or
 - a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 6, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2; or

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 6, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 8.

3. The humanized antibody or antigen binding fragment thereof of any one of claims 1 and 2,
5 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.
4. The humanized antibody or antigen binding fragment thereof of any one of claims 1 to 3, wherein the humanized antibody or antigen binding fragment thereof is an IgG antibody.
- 10 5. The humanized antibody or antigen binding fragment thereof of claim 4, wherein the humanized antibody or antigen binding fragment thereof comprises an IgG4 or IgG1 heavy chain constant region.
6. The humanized antibody or antigen binding fragment thereof according to claim 5, wherein the humanized antibody or antigen binding fragment thereof comprises an IgG4 heavy
15 chain constant region having an alteration of a serine residue substituted for proline at position 228 of said IgG4 heavy chain constant region.
7. A single chain variable fragment (scFv) of a humanized antibody according to any one of claims 1 to 6, comprising an amino acid sequence selected from SEQ ID NO: 56, SEQ ID NO: 57.
- 20 8. The humanized antibody or antigen binding fragment thereof of any one of claims 1 to 7, wherein the humanized antibody inhibits binding of PVR to at least one of TIGIT, CD96, and CD226.
9. A nucleic acid or combination of nucleic acids encoding the humanized antibody or antigen binding fragment thereof of any one of claims 1 to 8.
- 25 10. A cell line comprising the nucleic acid or combination of nucleic acids of claim 9.
11. A chimeric antigen receptor (CAR) NK or T cells comprising a combination of heavy and light chain variable region sequences of the humanized antibody according to any one of claims 1 to 8.
12. A pharmaceutical composition comprising the humanized antibody or antigen binding
30 fragment thereof of any one of claims 1 to 8 or the CAR NK or T cells according to claim 14, and a pharmaceutically acceptable excipient, carrier, or diluent.

13. Use of the humanized antibody or antigen binding fragment thereof of any one of claims 1 to 8, the CAR NK or T cells of claim 11, or the pharmaceutical composition of claim 12 in the manufacture of a medicament for treating cancer expressing PVR in an individual.
14. The use of claim 13, wherein the cancer comprises a solid tumor.
- 5 15. The use of claim 13, 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.
16. The use of claim 13, wherein the medicament is to be administered with an agent that downregulates the activity or expression of an immune co-inhibitory receptor.
- 10 17. The CAR NK or T cells of claim 11, comprising an amino acid sequence set forth in SEQ ID NO: 56 or SEQ ID NO: 57.
18. The humanized antibody or antigen binding fragment thereof of any one of claims 1 to 8, the CAR NK or T cells of claim 11, or the pharmaceutical composition of claim 12 for use in therapy.

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