SUMMARY

The present disclosure provides antigen binding receptors with improved properties, in particular humanized antigen binding receptors which are stable and highly expressed in transduced cells.

In one aspect, herein provided is an antigen binding receptor comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:129. In one example, the antigen binding receptor comprises the amino acid sequence of SEQ ID NO:129. Therefore, in another aspect, provided is an antigen binding receptor comprising the amino acid sequence of SEQ ID NO:129. In one example, the antigen binding receptor consists of the amino acid sequence of SEQ ID NO:129. Therefore, in another aspect, provided is an antigen binding receptor consisting of the amino acid sequence of SEQ ID NO:129.

In another aspect, provided is an antigen binding receptor comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:132. In one example, the antigen binding receptor comprises the amino acid sequence of SEQ ID NO:132. Therefore, in another aspect, provided is an antigen binding receptor comprising the amino acid sequence of SEQ ID NO:132. In one example, the antigen binding receptor consists of the amino acid sequence of SEQ ID NO:132. Therefore, in another aspect, provided is an antigen binding receptor consisting of the amino acid sequence of SEQ ID NO:132.

In one example, the antigen binding receptor does not comprise the amino acid sequence of SEQ ID NO:19.

In another aspect, provided is an isolated polynucleotide encoding the antigen binding receptor as hereinbefore described.

In one example, the isolated polynucleotide comprises the sequence of SEQ ID NO:130. Therefore, in another aspect, provided is an isolated polynucleotide comprising the sequence of SEQ ID NO:130.

In one example, the isolated polynucleotide comprises the sequence of SEQ ID NO: 133. Therefore, in another aspect, provided is an isolated polynucleotide comprising the sequence of SEQ ID NO: 133.

In another aspect, provided is a polypeptide encoded by the isolated polynucleotide as hereinbefore described.

In another aspect, provided is a vector, particularly an expression vector, comprising the polynucleotide as hereinbefore described.

In another aspect, provided is a transduced T cell comprising the polynucleotide or the vector as hereinbefore described.

In another aspect, provided is a transduced T cell capable of expressing the antigen binding receptor as hereinbefore described.

In another aspect, provided is a kit comprising

(A) a transduced T cell capable of expressing the antigen binding receptor as hereinbefore described; and

(B) an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

In another aspect, provided is a kit comprising

(A) an isolated polynucleotide encoding the antigen binding receptor as hereinbefore described; and

(B) an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

In another aspect, provided is a kit comprising

(A) the isolated polynucleotide or the vector as hereinbefore described; and

(B) an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

In one example, the Fc domain is an IgG1 or an IgG4 Fc domain, particularly a human IgG1 Fc domain.

In one example, the target cell antigen is selected from the group consisting of fibroblast activation protein (FAP), carcinoembryonic antigen (CEA), mesothelin (MSLN), CD20, folate receptor 1 (FOLR1) and tenascin (TNC).

In another aspect, provided is the kit as hereinbefore described for use as a medicament.

In another aspect, provided is the antigen binding receptor or the transduced T cell as hereinbefore described for use as a medicament, wherein a transduced T cell expressing the antigen binding receptor is to be administered before, simultaneously with or after administration of an antibody that binds to a target cell antigen, in particular a cancer cell antigen, and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

In another aspect, provided is the kit as hereinbefore described for use in the treatment of a disease, in particular for use in the treatment of a cancer.

In another aspect, provided is the antigen binding receptor or the transduced T cell as hereinbefore described for use in the treatment of cancer, wherein the transduced T cell expressing the antigen binding receptor is to be administered before, simultaneously with or after administration of an antibody that binds to a cancer cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

In one example, said cancer is selected from cancer of epithelial, endothelial or mesothelial origin and cancer of the blood.

In one example, the cancer antigen is selected from the group consisting of fibroblast activation protein (FAP), carcinoembryonic antigen (CEA), mesothelin (MSLN), CD20, folate receptor 1 (FOLR1) and tenascin (TNC).

In one example, the transduced T cell is derived from a cell isolated from the subject to be treated.

In one example, the transduced T cell is not derived from a cell isolated from the subject to be treated.

Provided is a method of treating a disease in a subject, comprising administering to the subject a transduced T cell capable of expressing the antigen binding receptor as hereinbefore described and administering before, simultaneously with or after administration of the transduced T cell a therapeutically effective amount of an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

In another aspect, provided is use of a transduced T cell capable of expressing the antigen binding receptor as described herein in the manufacture of a medicament for treating a disease in a subject, wherein a therapeutically effective amount of an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering is to be administered before, simultaneously with or after administration of the transduced T cell.

In one example, the method additionally comprises isolating a T cell from the subject and generating the transduced T cell by transducing the isolated T cell with the polynucleotide or the vector as hereinbefore described.

In one example, the T cell is transduced with a retroviral or lentiviral vector construct, or with a non-viral vector construct.

In one example, the transduced T cell is administered to the subject by intravenous infusion.

In one example, the transduced T cell is contacted with anti-CD3 and/or anti-CD28 antibodies prior to administration to the subject.

In one example, the transduced T cell is contacted with at least one cytokine prior to administration to the subject, preferably with interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-15 (IL-15), and/or interleukin-21, or variants thereof.

In one example, the disease is cancer.

In one example, the cancer is selected from cancer of epithelial, endothelial or mesothelial origin and cancer of the blood.

Provided is a method for inducing lysis of a target cell, comprising contacting the target cell with a transduced T cell capable of expressing the antigen binding receptor as hereinbefore described in the presence of an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

In another aspect, provided is a method for inducing lysis of a target cell *in vitro*, comprising contacting the target cell with a transduced T cell capable of expressing the antigen binding receptor as described herein in the presence of an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

In one example, the target cell is a cancer cell.

In one example, the target cell expresses an antigen selected from the group consisting of fibroblast activation protein (FAP), carcinoembryonic antigen (CEA), mesothelin (MSLN), CD20, folate receptor 1 (FOLR1), and tenascin (TNC).

Provided is the use of the antigen binding receptor, the polynucleotide or the transduced T cell as hereinbefore described in the manufacture of a medicament.

In one example, the medicament is for treatment of cancer.

In one example, said cancer is selected from cancer of epithelial, endothelial or mesothelial origin and cancer of the blood.

SHORT DESCRIPTION OF THE FIGURES

FIGURE 1: Schematic representation of second generation chimeric antigen binding receptor with anti-P329G binding moiety in the scFv format. In VH x VL scFv (Figure 1A) orientation and VL x VH (Figure 1B) orientation. Figures 1C and 1D show DNA constructs encoding the antigen binding receptors depicted in Figure 1A and 1B, respectively.

FIGURE 2: depicted is the CAR surface expression of different humanized scFv variants (Figure 2A) and the correlating GFP expression serving as transduction control (Figure 2B)

FIGURE 3: Evaluation of unspecific signaling of anti-P329G CAR Jurkat reporter T cells employing different humanized versions of the P329G binder as binding moiety. Activation was assessed by quantification of the intensity of CD3 downstream signaling using anti-P329G CAR Jurkat-NFAT reporter assay either in the presence of antibodies possessing different Fc variants or with P329G Fc variants but without target cells. Depicted are technical average values from triplicates, error bars indicate SD.

FIGURE 4: Activation of anti-P329G CAR Jurkat reporter T cells employing different humanized versions of the P329G binder in the presence of FolR1^{+} target cells with high (HeLa-FolR1), medium (Skov3) and low (HT29) target expression levels in combination with antibodies that possess high (16D5), medium (16D5 W96Y) or low (16D5 G49S/K53A) affinities towards FolR1. Activation was assessed by quantification of the intensity of CD3 downstream signaling using anti-P329G CAR Jurkat-NFAT reporter assay. Depicted are technical average values from triplicates, error bars indicate SD.

FIGURE 5: Activation of anti-P329G CAR Jurkat NFAT reporter T cells employing different humanized versions of the P329G binder as binding moiety. Activity of the reporter cells was evaluated in the presence of anti-FolR1 (16D5) P329G IgG1 targeting IgG and HeLa (FolR1^{+}) target cells (Figure 5A). Antibody does-dependent activation was assessed by quantification of the intensity of CD3 downstream signaling using anti-P329G CAR Jurkat-NFAT reporter assay and the area under the curve was calculated (Figure 5B). Depicted are technical average values from triplicates, error bars indicate SD.

CLAIMS

1. An antigen binding receptor comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:129.

2. An antigen binding receptor comprising the amino acid sequence of SEQ ID NO:129.

3. An antigen binding receptor consisting of the amino acid sequence of SEQ ID NO:129.

4. An antigen binding receptor comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:132.

5. An antigen binding receptor comprising the amino acid sequence of SEQ ID NO:132.

6. An antigen binding receptor consisting of the amino acid sequence of SEQ ID NO:132.

7. The antigen binding receptor of any one of claims 1-6, wherein the antigen binding receptor does not comprise the amino acid sequence of SEQ ID NO:19

8. An isolated polynucleotide encoding the antigen binding receptor of any one of claims 1-7.

9. An isolated polynucleotide comprising the sequence of SEQ ID NO:130.

10. An isolated polynucleotide comprising the sequence of SEQ ID NO: 133.

11. A polypeptide encoded by the isolated polynucleotide of any one of claims 8-10.

12. A vector, particularly an expression vector, comprising the polynucleotide of any one of claims 8-10.

13. A transduced T cell comprising the polynucleotide of any one of claims 8-10 or the vector of claim 12.

14. A transduced T cell capable of expressing the antigen binding receptor of any one of claims 1-7.

15. A kit comprising

(A) a transduced T cell capable of expressing the antigen binding receptor of any one of claims 1-7; and

(B) an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

16. A kit comprising

(A) an isolated polynucleotide encoding the antigen binding receptor of any one of claims 1-7; and

(B) an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

17. A kit comprising

(A) the isolated polynucleotide of any one of claims 8-10 or the vector of claim 12; and

(B) an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

18. The kit of any one of claims 15-17, wherein the Fc domain is an IgG1 or an IgG4 Fc domain, particularly a human IgG1 Fc domain.

19. The kit of any one of claims 15-18, wherein the target cell antigen selected from the group consisting of fibroblast activation protein (FAP), carcinoembryonic antigen (CEA), mesothelin (MSLN), CD20, folate receptor 1 (FOLR1) and tenascin (TNC).

20. The kit of any one of claims 15-19 for use as a medicament.

21. The antigen binding receptor of any one of claims 1-7 or the transduced T cell of claim 13 or 14 for use as a medicament, wherein a transduced T cell expressing the antigen binding receptor is to be administered before, simultaneously with or after administration of an antibody that binds to a target cell antigen, in particular a cancer cell antigen, and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

22. The kit of any one of claims 15-19 for use in the treatment of a disease, in particular for use in the treatment of a cancer.

23. The antigen binding receptor of any one of claims 1-7 or the transduced T cell of claim 13 or 14 for use in the treatment of cancer, wherein a transduced T cell expressing the antigen binding receptor is to be administered before, simultaneously with or after administration of an antibody that binds to a cancer cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

24. The antigen binding receptor, the transduced T cell or the kit for use according to claim 22 or 23 wherein said cancer is selected from cancer of epithelial, endothelial or mesothelial origin and cancer of the blood.

25. The antigen binding receptor, the transduced T cell or the kit for use according to claim 24, wherein the cancer antigen selected from the group consisting of fibroblast activation protein (FAP), carcinoembryonic antigen (CEA), mesothelin (MSLN), CD20, folate receptor 1 (FOLR1) and tenascin (TNC).

26. The antigen binding receptor, the transduced T cell or the kit for use according to any one of claims 23-25, wherein the transduced T cell is derived from a cell isolated from the subject to be treated.

27. The antigen binding receptor, the transduced T cell or the kit for use according to any one of claims 23-26, wherein the transduced T cell is not derived from a cell isolated from the subject to be treated.

28. Use of a transduced T cell capable of expressing the antigen binding receptor of any one of claims 1-7 in the manufacture of a medicament for treating a disease in a subject, wherein a therapeutically effective amount of an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering is to be administered before, simultaneously with or after administration of the transduced T cell.

29. The use of claim 28, wherein a T cell is to be additionally isolated from the subject and the transduced T cell is to be generated by transducing the isolated T cell with the polynucleotide of any one of claims 8-10, or the vector of claim 12.

30. The use of claim 29, wherein the T cell is to be transduced with a retroviral or lentiviral vector construct, or with a non-viral vector construct.

31. The use of any one of claims 28-30, wherein the transduced T cell is to be administered to the subject by intravenous infusion.

32. The use of any one of claims 28-31, wherein the transduced T cell is to be contacted with anti-CD3 and/or anti-CD28 antibodies prior to administration to the subject.

33. The use of any one of claims 28-32, wherein the transduced T cell is to be contacted with at least one cytokine prior to administration to the subject, preferably with interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-15 (IL-15), and/or interleukin-21, or variants thereof.

34. The use of any one of claims 28-33, wherein the disease is cancer.

35. The use of claim 34, wherein the cancer is selected from cancer of epithelial, endothelial or mesothelial origin and cancer of the blood.

36. A method for inducing lysis of a target cell *in vitro*, comprising contacting the target cell with a transduced T cell capable of expressing the antigen binding receptor of any one of claims 1-7 in the presence of an antibody that binds to a target cell antigen and that comprises an Fc domain comprising the amino acid mutation P329G according to EU numbering.

37. The method of claim 36, wherein the target cell is a cancer cell.

38. The method of claim 36 or 37, wherein the target cell expresses an antigen selected from the group consisting of fibroblast activation protein (FAP), carcinoembryonic antigen (CEA), mesothelin (MSLN), CD20, folate receptor 1 (FOLR1), and tenascin (TNC).

39. Use of the antigen binding receptor of any one of claims 1-7, the polynucleotide of any one of claims 8-10 or the transduced T cell of claim 13 or 14 in the manufacture of a medicament.

40. The use of claim 39, wherein the medicament is for treatment of cancer.

41. The use of claim 40, characterized in that said cancer is selected from cancer of epithelial, endothelial or mesothelial origin and cancer of the blood.