[0015a] In another aspect, provided herein is a prostate specific membrane antigen (PSMA) targeting trispecific protein, wherein said protein comprises (a) a first domain (A) which specifically binds to a human CD3; (b) a second domain (B) which specifically binds to a human serum albumin protein; and (c) a third domain (C) which specifically binds to PSMA, wherein the third domain is a single domain antibody comprising a CDR1, a CDR2, and a CDR3,wherein the CDR1 is selected from the group consisting of SEQ ID Nos. 114-116 and 125, the CDR2 is selected from the group consisting of SEQ ID Nos. 117-123 and 126, and the CDR3 is selected from the group consisting of SEQ ID Nos. 124 and 127, wherein the domains are linked in the order H_2N-(A)-(C)-(B)-COOH, H_2N-(B)-(A)-(C)-COOH, H_2N-(C)-(B)-(A)-COOH, or by linkers L1 and L2 in the order of H_2N-(A)-L1-(C)-L2-(B)-COOH, H_2N-(B)-L1-(A)-L2-(C)-COOH, H_2N-(C)-L1-(B)-L2-(A)-COOH.

[0015b] In another aspect, provided herein is a prostate specific membrane antigen (PSMA) targeting trispecific protein, wherein said protein comprises (a) a first domain (A) which specifically binds to a human CD3; (b) a second domain (B) which specifically binds to a human serum albumin protein and comprises a sequence selected from the group consisting of SEQ ID Nos. 89-95, and 110-112; and (c) a third domain (C) which specifically binds to PSMA, wherein the domains are linked in the order H_2N-(A)-(C)-(B)-COOH, H_2N-(B)-(A)-(C)-COOH, H_2N-(C)-(B)-(A)-COOH, or by linkers L1 and L2 in the order of H_2N-(A)-L1-(C)-L2-(B)-COOH, H_2N-(B)-L1-(A)-L2-(C)-COOH, H_2N-(C)-L1-(B)-L2-(A)-COOH.

[0015c] In another aspect, provided herein is a prostate specific membrane antigen (PSMA) targeting trispecific protein, wherein said protein comprises (a) a first domain (A) which specifically binds to a human CD3 and comprises a sequence selected from the group consisting of SEQ ID Nos. 1-15 and 87-88; (b) a second domain (B) which specifically binds to a human serum albumin protein; and (c) a third domain (C) which specifically binds to PSMA, wherein the domains are linked in the order H_2N-(A)-(C)-(B)-COOH, H_2N-(B)-(A)-(C)-COOH, H_2N-(C)-(B)-(A)-COOH, or by linkers L1 and L2 in the order of H_2N-(A)-L1-(C)-L2-(B)-COOH, H_2N-(B)-L1-(A)-L2-(C)-COOH, H_2N-(C)-L1-(B)-L2-(A)-COOH.

[0015d] In another aspect, provided herein is a prostate specific membrane antigen (PSMA) targeting trispecific protein, wherein said protein comprises (a) a first domain (A) which specifically binds to a human CD3 and comprises a sequence selected from the group consisting of SEQ ID Nos. 1-15 and 87-88; (b) a second domain (B) which specifically binds to a human serum albumin protein and comprises a sequence selected from the group consisting of SEQ ID Nos. 89-95 and 110-112; and (c) a third domain (C) which specifically binds to PSMA and comprises a sequence selected from the group consisting of SEQ ID Nos. 128-140, wherein the

domains are linked in the order H_2N-(A)-(C)-(B)-COOH, H_2N-(B)-(A)-(C)-COOH, H_2N-(C)-(B)-(A)-COOH, or by linkers L1 and L2 in the order of H_2N-(A)-L1-(C)-L2-(B)-COOH, H_2N-(B)-L1-(A)-L2-(C)-COOH, H_2N-(C)-L1-(B)-L2-(A)-COOH.

[0015e] In another aspect, provided herein is prostate specific membrane antigen (PSMA) targeting trispecific protein, wherein said protein comprises (a) a first domain (A) which specifically binds to a human CD3; (b) a second domain (B) which specifically binds to a human serum albumin protein; and (c) a third domain (C) which specifically binds to PSMA, wherein the third domain is a single domain antibody comprising a CDR1, a CDR2, and a CDR3, wherein the CDR1 is selected from the group consisting of SEQ ID Nos. 114-116 and 125, the CDR2 is selected from the group consisting of SEQ ID Nos. 117-123 and 126, and the CDR3 is selected from the group consisting of SEQ ID Nos. 124 and 127, wherein the domains are linked in the order H_2N-(A)-(C)-(B)-COOH, H_2N-(B)-(A)-(C)-COOH, H_2N-(C)-(B)-(A)-COOH, or by linkers L1 and L2 in the order of H_2N-(A)-L1-(C)-L2-(B)-COOH, H_2N-(B)-L1-(A)-L2-(C)-COOH, H_2N-(C)-L1-(B)-L2-(A)-COOH, for use in a therapy.

[0015f] In another aspect, provided herein is a use of an effective amount of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating an individual in need of treatment of cancer.

INCORPORATION BY REFERENCE

[0016] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0018] Figure 1 is schematic representation of an exemplary PMSA targeting trispecific antigen-binding protein where the protein has an constant core element comprising an anti-CD3ε single chain variable fragment (scFv) and an anti-HSA variable heavy chain region; and a PMSA binding domain that can be a VH, scFv, a non-Ig binder, or ligand.

[0019] Figures 2A-B compare the ability of exemplary PSMA targeting trispecific proteins (PSMA targeting TriTAC molecules) with different affinities for CD3 to induce T cells to kill human prostate cancer cells. **Figure 2A** shows killing by different PMSA targeting TriTAC

molecules in prostate cancer model LNCaP. **Figure 2B** shows killing by different PMSA targeting TriTAC molecules in prostate cancer model 22Rv1. **Figure 2C** shows EC50 values for PMSA targeting TriTAC in LNCaP and 22Rv1 prostate cancer models.

[0020] Figure 3 shows the serum concentration of PSMA targeting TriTAC C236 in Cynomolgus monkeys after i.v. administration (100 μg/kg) over three weeks.

[0021] Figure 4 shows the serum concentration of PSMA targeting TriTAC molecules with different CD3 affinities in Cynomolgus monkeys after i.v. administration (100 μg/kg) over three weeks.

[0022] Figures 5A-C show the ability of PSMA targeting TriTAC molecules with different affinities for PSMA to induce T cells to kill the human prostate cancer cell line LNCaP. **Figure 5A** shows the experiment performed in the absence of human serum albumin with a PSMA targeting BiTE as positive control. **Figure 5B** shows the experiment performed in the presence of human serum albumin with a PSMA targeting BiTE as positive control. **Figure 5C** shows EC50 values for PMSA targeting TriTAC in the presence or absence of HSA with a PSMA targeting BiTE as a positive control in LNCaP prostate cancer models.

[0023] Figure 6 demonstrates the ability of PSMA targeting TriTAC molecules to inhibit tumor growth of human prostate cancer cells in a mouse xenograft experiment.

CLAIMS

WHAT IS CLAIMED IS:

1. A prostate specific membrane antigen (PSMA) targeting trispecific protein, wherein said protein comprises

(a) a first domain (A) which specifically binds to a human CD3;

(b) a second domain (B) which specifically binds to a human serum albumin protein; and

(c) a third domain (C) which specifically binds to PSMA, wherein the third domain is a single domain antibody comprising a CDR1, a CDR2, and a CDR3, wherein the CDR1 is selected from the group consisting of SEQ ID Nos. 114-116 and 125, the CDR2 is selected from the group consisting of SEQ ID Nos. 117-123 and 126, and the CDR3 is selected from the group consisting of SEQ ID Nos. 124 and 127,

wherein the domains are linked in the order H_2N-(A)-(C)-(B)-COOH, H_2N-(B)-(A)-(C)-COOH, H_2N-(C)-(B)-(A)-COOH, or by linkers L1 and L2 in the order of H_2N-(A)-L1-(C)-L2-(B)-COOH, H_2N-(B)-L1-(A)-L2-(C)-COOH, H_2N-(C)-L1-(B)-L2-(A)-COOH.

2. The PSMA targeting trispecific protein of claim 1, wherein the first domain comprises a single chain variable fragment (scFv) which is capable of specifically binding to the human CD3.

3. The PSMA targeting trispecific protein of claim 1, wherein the first domain comprises a sequence selected from the group consisting of SEQ ID NO: 1-15 and 87-88.

4. The PSMA targeting trispecific protein of claim 1, wherein the first domain has a K_D binding of 150 nM or less to the human CD3 on CD3 expressing cells.

5. The PSMA targeting trispecific protein of claim 1, wherein the second domain comprises a single domain antibody (sdAb) which specifically binds to the human serum albumin.

6. The PSMA targeting trispecific protein of claim 1, wherein the second domain comprises a sequence selected from the group consisting of SEQ ID NOs: 89-95 and 110-112.

7. The PSMA targeting trispecific protein of claim 1, wherein the third domain comprises a sequence selected from the group consisting of SEQ ID NOs: 128-140.

8. The PSMA targeting trispecific protein of claim 1, wherein linkers L1 and L2 are each independently selected from $(GS)_n$ (SEQ ID NO: 153), $(GGS)_n$ (SEQ ID NO: 154), $(GGGS)_n$ (SEQ ID NO: 155), $(GGSG)_n$ (SEQ ID NO: 156), $(GGSGG)_n$ (SEQ ID NO: 157), or $(GGGGS)_n$ (SEQ ID NO: 158), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

9. The PSMA targeting trispecific protein of claim 1, wherein linkers L1 and L2 are each independently $(GGGGS)_4$ (SEQ ID NO: 159) or $(GGGGS)_3$ (SEQ ID NO: 160).

10. The PSMA targeting trispecific protein of claim 1, wherein the protein is less than about 80 kDa.

11. The PSMA targeting trispecific protein of claim 1, wherein the protein has an elimination half-time of at least about 50 hours.

12. The PSMA targeting trispecific protein of claim 1, wherein the protein has increased tissue penetration as compared to an IgG to the same PSMA.

13. The PSMA targeting trispecific protein of claim 1, wherein the protein comprises a sequence that is at least 80% identical to a sequence selected from the group consisting of SEQ ID NOs: 141-147 and 150-152.

14. A prostate specific membrane antigen (PSMA) targeting trispecific protein, wherein said protein comprises

(a) a first domain (A) which specifically binds to a human CD3;

(b) a second domain (B) which specifically binds to a human serum albumin protein and comprises a sequence selected from the group consisting of SEQ ID Nos. 89-95, and 110-112; and

(c) a third domain (C) which specifically binds to PSMA,

wherein the domains are linked in the order H_2N-(A)-(C)-(B)-COOH, H_2N-(B)-(A)-(C)-COOH, H_2N-(C)-(B)-(A)-COOH, or by linkers L1 and L2 in the order of H_2N-(A)-L1-(C)-L2-(B)-COOH, H_2N-(B)-L1-(A)-L2-(C)-COOH, H_2N-(C)-L1-(B)-L2-(A)-COOH.

15. A prostate specific membrane antigen (PSMA) targeting trispecific protein, wherein said protein comprises

(a) a first domain (A) which specifically binds to a human CD3 and comprises a sequence selected from the group consisting of SEQ ID Nos. 1-15 and 87-88;

(b) a second domain (B) which specifically binds to a human serum albumin protein; and

(c) a third domain (C) which specifically binds to PSMA,

wherein the domains are linked in the order H_2N-(A)-(C)-(B)-COOH, H_2N-(B)-(A)-(C)-COOH, H_2N-(C)-(B)-(A)-COOH, or by linkers L1 and L2 in the order of H_2N-(A)-L1-(C)-L2-(B)-COOH, H_2N-(B)-L1-(A)-L2-(C)-COOH, H_2N-(C)-L1-(B)-L2-(A)-COOH.

16. A prostate specific membrane antigen (PSMA) targeting trispecific protein, wherein said protein comprises

(a) a first domain (A) which specifically binds to a human CD3 and comprises a sequence selected from the group consisting of SEQ ID Nos. 1-15 and 87-88;

(b) a second domain (B) which specifically binds to a human serum albumin protein and comprises a sequence selected from the group consisting of SEQ ID Nos. 89-95 and 110-112; and

(c) a third domain (C) which specifically binds to PSMA and comprises a sequence selected from the group consisting of SEQ ID Nos. 128-140,

wherein the domains are linked in the order H_2N-(A)-(C)-(B)-COOH, H_2N-(B)-(A)-(C)-COOH, H_2N-(C)-(B)-(A)-COOH, or by linkers L1 and L2 in the order of H_2N-(A)-L1-(C)-L2-(B)-COOH, H_2N-(B)-L1-(A)-L2-(C)-COOH, H_2N-(C)-L1-(B)-L2-(A)-COOH.

17. A pharmaceutical composition comprising (i) the PSMA targeting trispecific protein according to any one of claims 1 to 16 and (ii) a pharmaceutically acceptable carrier.

18. Use of an effective amount of the pharmaceutical composition of claim 17 in the manufacture of a medicament for treating an individual in need of treatment of cancer.

19. The use of claim 18, wherein the cancer is prostate cancer or renal cancer.

20. A prostate specific membrane antigen (PSMA) targeting trispecific protein, wherein said protein comprises

(a) a first domain (A) which specifically binds to a human CD3;

(b) a second domain (B) which specifically binds to a human serum albumin protein; and

(c) a third domain (C) which specifically binds to PSMA, wherein the third domain is a single domain antibody comprising a CDR1, a CDR2, and a CDR3, wherein the CDR1 is selected from the group consisting of SEQ ID Nos. 114-116 and 125, the CDR2 is selected from the group consisting of SEQ ID Nos. 117-123 and 126, and the CDR3 is selected from the group consisting of SEQ ID Nos. 124 and 127,

wherein the domains are linked in the order H_2N-(A)-(C)-(B)-COOH, H_2N-(B)-(A)-(C)-COOH, H_2N-(C)-(B)-(A)-COOH, or by linkers L1 and L2 in the order of H_2N-(A)-L1-(C)-L2-(B)-COOH, H_2N-(B)-L1-(A)-L2-(C)-COOH, H_2N-(C)-L1-(B)-L2-(A)-COOH, for use in a therapy.