

TRISPECIFIC PROTEINS AND METHODS OF USE

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/572,381 filed October 13, 2017 which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 11, 2018, is named 47517-723_601_SL.txt and is 752,020 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Cancer is the second leading cause of human death next to coronary disease. Worldwide, millions of people die from cancer every year. In the United States alone, cancer causes the death of well over a half-million people each year, with some 1.4 million new cases diagnosed per year. While deaths from heart disease have been declining significantly, those resulting from cancer generally are on the rise. In the early part of the next century, cancer is predicted to become the leading cause of death.

[0004] Moreover, even for those cancer patients that initially survive their primary cancers, common experience has shown that their lives are dramatically altered. Many cancer patients experience strong anxieties driven by the awareness of the potential for recurrence or treatment failure. Many cancer patients experience significant physical debilitations following treatment.

[0005] Generally speaking, the fundamental problem in the management of the deadliest cancers is the lack of effective and non-toxic systemic therapies. Cancer is a complex disease characterized by genetic mutations that lead to uncontrolled cell growth. Cancerous cells are present in all organisms and, under normal circumstances, their excessive growth is tightly regulated by various physiological factors.

SUMMARY OF THE DISCLOSURE

[0006] The selective destruction of an individual cell or a specific cell type is often desirable in a variety of clinical settings. For example, it is a primary goal of cancer therapy to specifically destroy tumor cells, while leaving healthy cells and tissues intact and undamaged. One such method is by inducing an immune response against the tumor, to make immune effector cells such as natural killer (NK) cells or cytotoxic T lymphocytes (CTLs) attack and destroy tumor cells.

[0006a] In one aspect, provided herein is a B cell maturation agent (BCMA) binding trispecific protein that comprises:

(a) a first domain (A) which is a single chain variable fragment (scFv) that specifically binds to a human CD3;

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

(c) a third domain (C) which is a single domain antibody that specifically binds to a human BCMA comprising the sequence of SEQ ID NO: 468,

wherein the third domain comprises complementarity determining regions CDR1, CDR2, and CDR3 and wherein the CDR1 comprises the amino acid sequence of SEQ ID No. 76, the CDR2 comprises the amino acid sequence of SEQ ID No. 190, and the CDR3 comprises the amino acid sequence of SEQ ID No. 304.

[0006b] In one aspect, provided herein is a use of a pharmaceutical composition that comprises the BCMA binding trispecific protein as described herein in the manufacture of a medicament for the treatment or amelioration of a tumorous disease associated with BCMA in a subject having the tumorous disease.

[0006c] In one aspect, provided herein is a use of a pharmaceutical composition that comprises the BCMA binding trispecific protein as described herein in the manufacture of a medicament for the treatment or amelioration of a tumorous disease associated with BCMA in a subject having the tumorous disease.

[0006d] In one aspect, provided herein is the BCMA binding trispecific protein as described herein for use in therapy.

[0007] Provided herein is a B cell maturation agent (BCMA) binding trispecific protein that comprises: (a) a first domain (A) which specifically binds to human CD3; (b) a second domain

(B) which is a half-life extension domain; and (c) a third domain (C) which specifically binds to BCMA, wherein the domains are linked in the order H2N-(A)-(C)-(B)-COOH, H2N-(B)-(A)-(C)-COOH, H2N-(C)-(B)-(A)-COOH, H2N-(C)-(A)-(B)-COOH, H2N-(A)-(B)-(C)-COOH, or H2N-(B)-(C)-(A)-COOH, wherein the domains are linked by linkers L1 and L2.

[0008] In some instances, the first domain can comprise a variable light domain and variable heavy domain, each of which is capable of specifically binding to human CD3. The first domain can be humanized or human.

[0009] In some instances, the second domain binds albumin. The second domain can comprise a single chain variable fragment (scFv), a variable heavy domain (VH), a variable light domain (VL), a single chain antibody binding domain devoid of light chain (a VHH domain), a peptide, a ligand, or a small molecule.

[0010] The third domain is, in some cases, a single chain antibody binding domain devoid of light chain (a VHH domain), a scFv, a VH domain, a VL domain, a non-Ig domain, a ligand, a knottin, or a small molecule entity that specifically binds to BCMA. In some non-limiting instances, the third domain comprises a VHH domain.

[0011] Provided herein is a BCMA binding trispecific protein where the VHH domain comprises complementarity determining regions CDR1, CDR2, and CDR3, wherein (a) the amino acid sequence of CDR1 is as set forth in $X_1X_2X_3X_4X_5X_6X_7PX_8G$ (SEQ ID NO: 1), wherein X_1 is T or S; X_2 is N, D, or S; X_3 is I, D, Q, H, V, or E; X_4 is F, S, E, A, T, M, V, I, D, Q, P, R, or G; X_5 is S, M, R, or N; X_6 is I, K, S, T, R, E, D, N, V, H, L, A, Q, or G; X_7 is S, T, Y, R, or N; and X_8 is M, G, or Y; (b) the amino acid sequence of CDR2 is as set forth in $AIX_9GX_{10}X_{11}TX_{12}YADSVK$ (SEQ ID NO: 2), wherein X_9 is H, N, or S; X_{10} is F, G, K, R, P, D, Q, H, E, N, T, S, A, I, L, or V; X_{11} is S, Q, E, T, K, or D; and X_{12} is L, V, I, F, Y, or W; and (c) the amino acid sequence of CDR3 is as set forth in $VPWGX_{13}YHPX_{14}X_{15}VX_{16}$ (SEQ ID NO: 3), wherein X_{13} is D, I, T, K, R, A, E, S, or Y; X_{14} is R, G, L, K, T, Q, S, or N; X_{15} is N, K, E, V, R, M, or D; and X_{16} is Y, A, V, K, H, L, M, T, R, Q, C, S, or N.

[0012] In one example, the CDR1 does not comprise an amino acid sequence of SEQ ID NO: 599. In one example, the CDR2 does not comprise an amino acid sequence of SEQ ID NO: 600. In one example, the CDR3 does not comprise an amino acid sequence of SEQ ID NO: 601. In one example, the CDR1 and CDR2 do not comprise amino acid sequences of SEQ ID NO: 599 and 600, respectively. In one example, the CDR1 and CDR3 do not comprise amino acid sequences of SEQ ID NO: 599 and 601, respectively. In one example, the CDR2 and CDR3 do not comprise amino acid sequences of SEQ ID NO: 600 and 601, respectively. In one example, the CDR1, CDR2 and CDR3 do not comprise amino acid sequences of SEQ ID NO: 599, 600 and 601, respectively.

[0013] Provided herein is a BCMA binding trispecific protein, where the VHH domain comprises the following formula: f1-r1-f2-r2-f3-r3-f4; wherein, r1 is SEQ ID NO: 1; r2 is SEQ ID NO: 2; and r3 is SEQ ID NO: 3; and wherein f₁, f₂, f₃ and f₄ are framework residues selected so that said protein is from about eighty percent (80%) to about 99% identical to the amino acid sequence set forth in SEQ ID NO: 598 or 346. Provided herein is a BCMA binding trispecific protein, where the VHH domain comprises the following formula: f1-r1-f2-r2-f3-r3-f4; wherein, r1 is SEQ ID NO: 1; r2 is SEQ ID NO: 2; and r3 is SEQ ID NO: 3; and wherein f₁, f₂, f₃ and f₄ are framework residues selected so that said protein is from about 80% to about 90% identical to the amino acid sequence set forth in SEQ ID NO: 598 or 346. In one example, the amino acid sequence of the single domain BCMA binding protein does not comprise SEQ ID NO: 598.

[0014] In some non-limiting examples, r1 comprises an amino acid sequence set forth as any one of SEQ ID NOs: 4-117.

[0015] In some non-limiting examples, r2 comprises an amino acid sequence set forth as any one of SEQ ID NOs: 118-231.

[0016] In some non-limiting examples, r3 comprises an amino acid sequence set forth as any one of SEQ ID NOs: 232-345.

[0017] In other non-limiting examples, the protein comprises an amino sequence set forth as any one of SEQ ID NOs: 346-460.

[0018] In a single domain BCMA binding protein, f1 comprises, in some instances, SEQ ID NO: 461 or 462.

[0019] In a single domain BCMA binding protein, f2 comprises, in some instances, SEQ ID NO: 463.

[0020] In a single domain BCMA binding protein, f3 comprises, in some instances, SEQ ID NO: 464 or 465.

[0021] In a single domain BCMA binding protein, wherein f4 comprise, in some instances, SEQ ID NO: 466 or 467.

[0022] In one non-limiting example, r1 comprises SEQ ID NO: 76, 114, 115, 116 or 117. In one non-limiting example, r1 comprises SEQ ID NO: 76.

[0023] In one non-limiting example, r1 comprises SEQ ID NO: 76, r2 is SEQ ID NO: 190, and r3 is SEQ ID NO: 304.

[0024] In one non-limiting example, r1 comprises SEQ ID NO: 114, r2 comprises SEQ ID NO: 228 and r3 comprises SEQ ID NO: 342.

[0025] In one non-limiting example, r1 comprises SEQ ID NO: 115, r2 comprises SEQ ID NO: 229 and r3 comprises SEQ ID NO: 343.

[0026] In one non-limiting example, r1 comprises SEQ ID NO: 117, r2 comprises SEQ ID NO: 231 and r3 comprises SEQ ID NO: 345.

[0027] In one non-limiting example, r1 comprises SEQ ID NO: 116, r2 comprises SEQ ID NO: 230 and r3 comprises SEQ ID NO: 344.

[0028] The third domain, in some cases, is a human VHH domain, a humanized VHH domain, an affinity matured VHH domain, or a combination thereof.

[0029] The BCMA binding trispecific protein, in some instances, has an elimination half-time of at least 12 hours, at least 20 hours, at least 25 hours, at least 30 hours, at least 35 hours, at least 40 hours, at least 45 hours, at least 50 hours, or at least 100 hours.

[0030] Provided herein is a BCMA binding trispecific protein that is a VHH domain, where the VHH domain comprises a CDR1, a CDR2, and a CDR3, and wherein the protein comprises the sequence set forth as SEQ ID NO: 346 or 598, wherein one or more amino acid residues selected from amino acid positions 26, 27, 28, 29, 30, 31, 32 and/or 34 of CDR1; positions 52, 54, 55 and/or 57 of CDR2; and positions 101, 105, 106 and/or 108 of CDR3 are substituted, wherein amino acid position 26, if substituted, is substituted with S; amino acid position 27, if substituted, is substituted with D or S; amino acid position 28, if substituted, is substituted with D, Q, H, V, or E; amino acid position 29, if substituted, is substituted with S, E, A, T, M, V, I, D, Q, P, R, or G; amino acid position 30, if substituted, is substituted with M, R, or N; amino acid position 31, if substituted, is substituted with K, S, T, R, E, D, N, V, H, L, A, Q, or G; amino acid position 32, if substituted, is substituted with T, Y, R, or N; amino acid position 34, if substituted, is substituted with G or Y; amino acid position 52, if substituted, is substituted with N or S; amino acid position 54, if substituted, is substituted with G, K, R, P, D, Q, H, E, N, T, S, A, I, L, or V; amino acid position 55, if substituted, is substituted with Q, E, T, K, or D; amino acid position 57, if substituted, is substituted with V, I, F, Y, or W; amino acid position 101, if substituted, is substituted with I, T, K, R, A, E, S, or Y; amino acid position 105, if substituted, is substituted with G, L, K, T, Q, S, or N; amino acid position 106, if substituted, is substituted with K, E, V, R, M, or D; and amino acid position 108, if substituted, is substituted with A, V, K, H, L, M, T, R, Q, C, S, or N. In one non-limiting example, the VHH domain is human, humanized, affinity matured, or a combination thereof.

[0031] Provided herein is a BCMA binding trispecific protein, where the third domain binds to a human BCMA protein that comprises a sequence set forth as SEQ ID NO: 468. In some instances, the third domain binds to an epitope of BCMA, wherein said epitope comprises the extracellular domain of BCMA. In some instances, the third domain binds to an epitope of BCMA, wherein said epitope comprises amino acid residues 5-51 of SEQ ID NO: 468.

[0032] In such BCMA binding trispecific proteins, linkers L1 and L2 are each independently selected from (GS)_n (SEQ ID NO: 472), (GGS)_n (SEQ ID NO: 473), (GGGS)_n (SEQ ID NO: 474), (GGSG)_n (SEQ ID NO: 475), (GGSGG)_n (SEQ ID NO: 476), (GGGGS)_n (SEQ ID NO: 477), (GGGGG)_n (SEQ ID NO: 478) or (GGG)_n (SEQ ID NO: 479) wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0033] In one non-limiting example, in such BCMA binding trispecific proteins the linkers L1 and L2 are each independently (GGGGS)₄ (SEQ ID NO: 480) or (GGGGS)₃ (SEQ ID NO: 481).

[0034] The domains of a BCMA binding trispecific protein can be linked in the order H₂N-(C)-(B)-(A)-COOH.

[0035] In some instances, a BCMA binding trispecific protein is less than about 80 kDa. In other instances, a BCMA binding trispecific protein can be about 50 to about 75 kDa. In other instances, a BCMA binding trispecific protein is less than about 60 kDa.

[0036] A BCMA binding trispecific protein described herein, in some instances, have an elimination half-time of at least about 50 hours, about 100 hours or more.

[0037] A BCMA binding trispecific protein, in some instances, exhibit increased tissue penetration as compared to an IgG to the same BCMA.

[0038] A BCMA binding trispecific protein, in some instances, comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 483-597. A BCMA binding trispecific protein, in some instances, comprises an amino acid sequence as set forth in SEQ ID NO: 520.

[0039] Provided herein in one example is a B cell maturation agent (BCMA) binding trispecific protein comprising: (a) a first domain (A) which specifically binds to human CD3; (b) a second domain (B) which is a half-life extension domain; and (c) a third domain (C) which specifically binds to BCMA, wherein the third domain comprises an amino sequence set forth as any one of SEQ ID NOS: 346-460.

[0040] Provided herein in one example is a B cell maturation agent (BCMA) binding trispecific protein comprising: (a) a first domain (A) which specifically binds to human CD3; (b) a second domain (B) which is a half-life extension domain; and (c) a third domain (C) which specifically binds to BCMA, wherein the third domain comprises complementarity determining regions CDR1, CDR2, and CDR3, wherein CDR1 comprises an amino acid sequence set forth as any one of SEQ ID NOS: 4-117, CDR2 comprises an amino acid sequence set forth as any one of SEQ ID NOS: 118-231, and CDR3 comprises an amino acid sequence set forth as any one of SEQ ID NOS: 232-345.

[0041] Provided herein is a pharmaceutical composition comprising a BCMA binding trispecific protein as described herein and a pharmaceutically acceptable carrier.

[0042] Also provided herein is a process for the production of a BCMA binding trispecific protein described herein, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding a BCMA binding trispecific protein under conditions allowing the expression of the BCMA binding trispecific protein and recovering and purifying the produced protein from the culture.

[0043] One example provides a method for the treatment or amelioration of a tumorous disease, an autoimmune disease or an infection disease associated with BCMA in a subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising a BCMA binding trispecific protein, wherein the BCMA binding protein comprises

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

(b) a second domain (B) which is a half-life extension domain; and

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

wherein the domains are linked in the order H2N-(A)-(C)-(B)-COOH, H2N-(B)-(A)-(C)-COOH, H2N-(C)-(B)-(A)-COOH, H2N-(C)-(A)-(B)-COOH, H2N-(A)-(B)-(C)-COOH, H2N-(B)-(C)-(A)-COOH, wherein the domains are linked by linkers L1 and L2.

[0044] Provided herein is a method for the treatment or amelioration of a tumorous disease, an autoimmune disease or an infection disease associated with BCMA in a subject in need thereof, comprising administering to the subject a pharmaceutical composition as described herein.

[0045] A subject to be treated is, in some instances, a human.

[0046] In some instances, the method further comprises administration of one or more additional agents in combination with the BCMA binding trispecific protein.

[0047] The methods described herein are useful for treatment or amelioration of a tumorous disease, wherein the BCMA binding trispecific protein selectively binds to tumor cells expressing BCMA.

[0048] A tumorous disease to be treated with the described methods comprises a primary cancer or a metastasis thereof. In one instance, the tumorous disease comprises a B cell lineage cancer.

[0049] A B cell lineage cancer to be treated with the recited methods includes, but is not limited to, a multiple myeloma, a leukemia, a lymphoma, or a metastasis thereof.

INCORPORATION BY REFERENCE

[0050] 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.

CLAIMS

What is claimed is:

1. A B cell maturation agent (BCMA) binding trispecific protein that comprises:
 - (a) a first domain (A) which is a single chain variable fragment (scFv) that specifically binds to a human CD3;
 - (b) a second domain (B) which is a single domain antibody that specifically binds to a human serum albumin protein; and
 - (c) a third domain (C) which is a single domain antibody that specifically binds to a human BCMA comprising the sequence of SEQ ID NO: 468,wherein the third domain comprises complementarity determining regions CDR1, CDR2, and CDR3 and wherein the CDR1 comprises the amino acid sequence of SEQ ID No. 76, the CDR2 comprises the amino acid sequence of SEQ ID No. 190, and the CDR3 comprises the amino acid sequence of SEQ ID No. 304.
2. The BCMA binding trispecific protein of claim 1, wherein the first domain, the second domain, and the third domain are independently humanized.
3. The BCMA binding trispecific protein of claim 1, wherein the third domain comprises the following formula:
f1-r1-f2-r2-f3-r3-f4
wherein, r1 is the CDR1; r2 is the CDR2; and r3 is the CDR3; and wherein f1, f2, f3 and f4 are framework residues selected so that said protein is from about 80% to about 99% identical to the amino acid sequence set forth in SEQ ID NO: 346 or SEQ ID NO: 598.
4. The BCMA binding trispecific protein of claim 3, wherein f1 comprises SEQ ID NO: 461 or 462, f2 comprises SEQ ID NO: 463, f3 comprises SEQ ID NO: 464 or 465, and f4 comprises SEQ ID NO: 466 or 467.
5. The BCMA binding trispecific protein of claim 1, wherein the third domain comprises the amino sequence of SEQ ID NO: 383.
6. The BCMA binding trispecific protein of claim 1, wherein said protein has an elimination half-time of at least 12 hours, at least 20 hours, at least 25 hours, at least 30 hours, at least 35 hours, at least 40 hours, at least 45 hours, at least 50 hours, or at least 100 hours, when administered to a subject, wherein the subject is a human.
7. The BCMA binding trispecific protein of claim 1, wherein the third domain binds to an extracellular domain of BCMA.
8. The BCMA binding trispecific protein of claim 1, wherein the trispecific protein comprises the sequence of SEQ ID NO: 520.

9. The BCMA binding trispecific protein of claim 1, that further comprises linkers L1 and L2, wherein the domains of the protein are linked in the order H₂N-(A)-L1-(C)-L2-(B)-COOH, H₂N-(B)-L1-(A)-L2-(C)-COOH, H₂N-(C)-L1-(B)-L2-(A)-COOH, H₂N-(C)-L1-(A)-L2-(B)-COOH, H₂N-(A)-L1-(B)-L2-(C)-COOH or H₂N-(B)-L1-(C)-L2-(A)-COOH.
10. The BCMA binding trispecific protein of claim 9, wherein the domains are linked in the order H₂N-(C)-L1-(B)-L2-(A)-COOH.
11. The BCMA binding trispecific protein of claim 10, wherein the linkers L1 and L2 are each independently selected from a group consisting of (GS)_n (SEQ ID NO: 472), (GGS)_n (SEQ ID NO: 473), (GGGS)_n (SEQ ID NO: 474), (GGSG)_n (SEQ ID NO: 475), (GGSGG)_n (SEQ ID NO: 476), (GGGGS)_n (SEQ ID NO: 477), (GGGGG)_n (SEQ ID NO: 478) and (GGG)_n (SEQ ID NO: 479) wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.
12. Use of a pharmaceutical composition that comprises the BCMA binding trispecific protein of claim 1 in the manufacture of a medicament for the treatment or amelioration of a tumorous disease associated with BCMA in a subject having the tumorous disease.
13. Use of a pharmaceutical composition that comprises the BCMA binding trispecific protein of claim 8 in the manufacture of a medicament for the treatment or amelioration of a tumorous disease associated with BCMA in a subject having the tumorous disease.
14. The BCMA binding trispecific protein of claim 1 or claim 8 for use in therapy.