ENGINEERED IMMUNE CELL TARGETING BCMA AND USE THEREOF

TECHNICAL FIELD

[0001] The present invention relates to the field of immunotherapy, and more specifically to an engineered immune cell targeting BCMA and use thereof.

BACKGROUND OF THE INVENTION

[0002] Multiple myeloma (MM) is a malignant plasma cell tumor. The tumor cells originate from plasma cells in the bone marrow, and plasma cells are cells that develop to the final functional stage of B lymphocytes. Multiple myeloma is basically an incurable disease with the characteristics of high morbidity and high mortality. In 2017's statistics, there were 30,000 newly diagnosed multiple myeloma patients in the United States, among them, 12,000 might face death. At present, common therapies for multiple myeloma include cytotoxic drug therapy, protease inhibitors (Bortezomib, etc.), lenalidomide, monoclonal antibodies, corticosteroids, and the like. However, the current therapies are all partially effective, have no lasting alleviation effect, and the high risk of recurrence. Therefore, improvement in the therapies of multiple myeloma appears to be particularly important.

[0003] Therefore, there is an urgent need in the art for an effective, low-recurrence rate, and safe therapy for multiple myeloma.

SUMMARY OF THE DISCLOSURE

[0004] An object of the present disclosure is to provide an engineered immune cell targeting BCMA and use thereof.

[0005] Another object of the present disclosure is to provide an engineered immune cell targeting both CD19 and BCMA and use thereof.

[0005a] In one aspect, the present disclosure provides a bispecific chimeric antigen receptor (CAR) comprising: a first antigen binding domain exhibiting specific binding to a first target antigen, comprising (i) a first heavy chain variable region ($V_{H,1}$) and (ii) a first light chain variable region ($V_{L,1}$); and

a second antigen binding domain exhibiting specific binding to BCMA, comprising (i) a second heavy chain variable region ($V_{H,BCMA}$) comprising the polypeptide sequence of SEQ ID NO: 9 and (ii) a second light chain variable region ($V_{L,BCMA}$) comprising the polypeptide sequence of SEQ ID NO: 10,

wherein the bispecific CAR comprises a loop structure as shown in a formula selected from the group consisting of:

$V_{L,1}$-the second antigen binding domain-$V_{H,1}$ (A),

$V_{H,1}$-the second antigen binding domain-$V_{L,1}$ (B),

$V_{L,BCMA}$-the first antigen binding domain-$V_{H,BCMA}$ (C), and

$V_{H,BCMA}$ -the first antigen binding domain-$V_{L,BCMA}$ (D),

wherein "-" is each independently a linker peptide or a peptide bond.

~~an antigen binding domain that comprises (i) an antibody heavy chain variable region as shown in SEQ ID NO: 9 and (ii) an antibody light chain variable region as shown in SEQ ID NO: 10.~~

~~**[0005b]** In another aspect, the present disclosure provides one or more chimeric antigen receptors (CARs) comprising one or more antigen binding moieties, wherein the one or more binding moieties comprises (i) a first antigen binding domain exhibiting specific binding to a first target and (ii) a second antigen binding domain exhibiting specific binding to BCMA, wherein the second antigen binding domain comprises (a) an antibody heavy chain variable region as shown in SEQ ID NO: 9 and (b) an antibody light chain variable region as shown in SEQ ID NO: 10; and~~

~~wherein the first target is selected from the group consisting of CD138, Kappa Light Chain, NKG2D ligands, TACI, GPRC5D, CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD38, CD40, CD44V6, CD47, CD52, CD56, CD57, CD58, CD79b, CD80, CD86, CD81, CD123, CD133, CD137, CD151, CD171, CD276, CLL1, B7H4, VEGFR-2, EGFR, GPC3, PMSA, CEACAM6, c-Met, EGFRvIII, ErbB2/HER2, ErbB3, HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, Flt3, CEA, CA125, CTLA-4, GITR, BTLA, TGFBR1, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, PSCA, HVEM, MAGE-A, MSLN, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, MUC16, TCRα, TCRβ, TLR7, TLR9, PTCH1, WT-1, Robol, Frizzled, OX40, Notch-1-4, APRIL, CS1, MAGE3, Claudin 18.2, Folate receptor α, Folate receptor β, GPC2, CD70, BAFF-R, and TROP-2.~~

[0005b~~c~~] In one aspect, the present disclosure provides an engineered immune cell comprising the bispecific CAR as described herein.

~~**[0005d]** In one aspect, the present disclosure provides an engineered immune cell comprising the one or more CARs as described herein.~~

~~**[0005c]** In one aspect, the present disclosure provides an engineered immune cell, the immune cell comprising a first expression cassette and a second expression cassette that are exogenous, wherein the first expression cassette is used to express a first CAR targeting a first target, and the second expression cassette is used to express a second CAR targeting BCMA; or~~

~~the immune cell expressing the first CAR targeting the first target and the second CAR targeting the BCMA,~~

~~wherein an antigen binding domain (scFv) targeting the BCMA in the second CAR comprises an antibody heavy chain variable region as shown in SEQ ID NO: 9 and an antibody light chain variable region as shown in SEQ ID NO: 10; and~~

~~wherein the first target is selected from the group consisting of CD138, Kappa Light Chain, NKG2D ligands, TACI, GPRC5D, CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD38, CD40, CD44V6, CD47, CD52, CD56, CD57, CD58, CD79b, CD80, CD86, CD81, CD123, CD133, CD137, CD151, CD171, CD276, CLL1, B7H4, VEGFR-2, EGFR, GPC3, PMSA, CEACAM6, c-Met, EGFRvIII, ErbB2/HER2, ErbB3, HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, Flt3, CEA, CA125, CTLA-4, GITR, BTLA, TGFBR1, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, PSCA, HVEM, MAGE-A, MSLN, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, MUC16, TCRα, TCRβ, TLR7, TLR9, PTCH1, WT-1, Robol, Frizzled, OX40, Notch-1-4, APRIL, CS1, MAGE3, Claudin 18.2, Folate receptor α, Folate receptor β, GPC2, CD70, BAFF-R, and TROP-2~~

[0006] In a first example of the present disclosure, a chimeric antigen receptor (CAR) or TCR is provided, and an antigen binding domain (scFv) of the CAR or TCR comprises an antibody heavy chain variable region as shown in SEQ ID NO: 9 and an antibody light chain variable region as shown in SEQ ID NO: 10.

[0007] In another preferred example, the scFv further comprises a linker peptide located between the heavy chain variable region and the light chain variable region.

[0008] In another preferred example, the scFv is as shown in the following formula A or formula B:

CLAIMS

WHAT IS CLAIMED IS:

1. A bispecific chimeric antigen receptor (CAR) comprising:

a first antigen binding domain exhibiting specific binding to a first target antigen, comprising (i) a first heavy chain variable region ($V_{H,1}$) and (ii) a first light chain variable region ($V_{L,1}$); and

an a second antigen binding domain exhibiting specific binding to BCMA, comprising that comprises (i) an antibody a second heavy chain variable region ($V_{H,BCMA}$) comprising the polypeptide sequence of as shown in SEQ ID NO: 9 and (ii) an antibodya second light chain variable region ($V_{L,BCMA}$) comprising the polypeptide sequence of as shown in SEQ ID NO: 10,

wherein the bispecific CAR comprises a loop structure as shown in a formula selected from the group consisting of:

$V_{L,1}$-the second antigen binding domain-$V_{H,1}$ (A),

$V_{H,1}$-the second antigen binding domain-$V_{L,1}$ (B),

$V_{L,BCMA}$-the first antigen binding domain-$V_{H,BCMA}$ (C), and

$V_{H,BCMA}$ -the first antigen binding domain-$V_{L,BCMA}$ (D),

wherein "-" is each independently a linker peptide or a peptide bond.

~~2. The CAR of claim 1, wherein the antigen binding domain comprises a single chain variable fragment (scFv) comprising the antibody heavy chain and the antibody light chain.~~

~~3. The CAR according to claim 2, wherein the scFv is as shown in the following formula A or formula B:~~

~~V_H-V_L, (A); V_L-V_H, (B),~~

~~wherein:~~

~~V_H is the antibody heavy chain variable region;~~

~~V_L is the antibody light chain variable region; and~~

~~" " is a linker peptide or a peptide bond.~~

~~4~~2. The bispecific CAR of claim 1, ~~One or more chimeric antigen receptors (CARs) comprising one or more antigen binding moieties, wherein the one or more binding moieties comprises (i) a first antigen binding domain exhibiting specific binding to a first target and (ii) a second antigen binding domain exhibiting specific binding to BCMA,~~

~~wherein the second antigen binding domain comprises (a) an antibody heavy chain variable region as shown in SEQ ID NO: 9 and (b) an antibody light chain variable region as shown in SEQ ID NO: 10; and~~

wherein the first target antigen is selected from the group consisting of CD138, Kappa Light Chain, NKG2D-ligands, TACI, GPRC5D, CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD38, CD40, CD44V6, CD47, CD52, CD56, CD57, CD58, CD79b, CD80, CD86, CD81, CD123, CD133, CD137, CD151, CD171, CD276, CLL1, B7H4, VEGFR-2, EGFR, GPC3, PMSA, CEACAM6, c-Met, EGFRvIII, ErbB2/HER2, ErbB3, HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, Flt3, CEA, CA125, CTLA-4, GITR, BTLA, TGFBR1, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, PSCA, HVEM, MAGE-A, MSLN, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, MUC16, TCRα, TCRβ, TLR7, TLR9, PTCH1, WT-1, Robol, Frizzled, OX40, Notch-1-4, APRIL, CS1, MAGE3, Claudin 18.2, Folate receptor α, Folate receptor β, GPC2, CD70, BAFF-R, and TROP-2.

~~5. The one or more CARs of claim 4, wherein a bispecific CAR comprises (i) the first antigen binding domain and (ii) the second antigen binding domain.~~

~~6. The one or more CARs of claim 4, wherein (i) a first CAR comprises the first antigen binding domain but not the second antigen binding domain and (ii) a second CAR comprises the second antigen binding domain but not the first antigen binding domain.~~

~~7. The one or more CARs of claim 4, wherein the second antigen binding domain comprises a single chain variable fragment (scFv) comprising (a) the antibody heavy chain variable region and (b) the antibody light chain variable region.~~

~~8~~3. The ~~one or more CARs~~bispecific CAR of claim ~~4~~2, wherein the first target antigen is CD19.

~~9. The one or more CARs of claim 4, wherein the first antigen binding domain comprises a single chain variable fragment (scFv) comprising (a) an antibody heavy chain variable region and (b) an antibody light chain variable region.~~

~~10~~4. The ~~one or more CARs~~bispecific CAR of claim ~~9~~3, wherein (a) the $V_{H,1}$~~the antibody heavy chain variable region is as shown in any one of~~ comprises the polypeptide sequence selected from the group consisting of SEQ ID NOs: 11 and 21-30, and (b) the $V_{L,1}$~~the antibody light chain variable region is as shown in any one of~~ comprises the polypeptide sequence selected from the group consisting of SEQ ID NOs: 12 and 31-36.

5. The bispecific CAR of claim 4, wherein (a) the $V_{H,1}$ comprises the polypeptide sequence of SEQ ID NO: 11, and (b) the V_{L-1} comprises the polypeptide sequence of SEQ ID NO: 12.

~~11. The one or more CARs of claim 5, wherein the bispecific CAR has a structure as shown in the following formula II:~~

~~L-scFv1-I-scFv2-H-TM-C-CD3ζ (II)~~

~~in the formula,~~

~~"-" is each independently a linker peptide or a peptide bond;~~

~~L is absent or a signal peptide sequence;~~

~~I is a flexible linker;~~

~~H is absent or a hinge region;~~

~~TM is a transmembrane domain;~~

~~C is a costimulatory signal molecule;~~

~~CD3ζ is a cytoplasmic signaling sequence derived from CD3ζ; and~~

~~scFv1 is the antigen binding domain targeting the first target, and scFv2 is the antigen binding domain targeting the BCMA.~~

~~12. The one or more CARs of claim 5, wherein the bispecific CAR has a structure as shown in the following formula II:~~

~~L-scFv1-I-scFv2-H-TM-C-CD3ζ (II)~~

~~in the formula,~~

~~"-" is each independently a linker peptide or a peptide bond;~~

~~L is absent or a signal peptide sequence;~~

~~I is a flexible linker;~~

~~H is absent or a hinge region;~~

~~TM is a transmembrane domain;~~

~~C is a costimulatory signal molecule;~~

~~CD3ζ is a cytoplasmic signaling sequence derived from CD3ζ; and~~

~~scFv2 is the antigen binding domain targeting the first target, and scFv1 is the antigen binding domain targeting the BCMA.~~

6. The bispecific CAR of claim 1, wherein the bispecific CAR comprises the structure as shown in formula (A).

7. The bispecific CAR of claim 6, wherein the bispecific CAR comprises the structure as shown in formula (A'):

~~13. The one or more CARs of claim 5, wherein the bispecific CAR has a structure as shown in the following formula III:~~

L-$V_{L3,1}$-~~scFv3~~the second antigen binding domain-$V_{H3,1}$-H-TM-C-CD3ζ ~~(III)~~(A')

wherein:

"-" is each independently a linker peptide or a peptide bond;

L is absent or a signal peptide sequence;

H is absent or a hinge region;

TM is a transmembrane domain;

C is a costimulatory signal molecule; and

CD3ζ is a cytoplasmic signaling sequence derived from CD3ζ.

~~in the formula,~~

~~" - " is each independently a linker peptide or a peptide bond;~~

~~elements L, H, TM, C and CD3ζ are as described above; and~~

~~scFv3 is the antigen binding domain targeting the BCMA, V_{H3} is the antibody heavy chain variable region against the first target, and V_{L3} is the antibody light chain variable region against the first target.~~

8. The bispecific CAR of claim 1, wherein the bispecific CAR comprises the structure as shown in formula (C).

9. The bispecific CAR of claim 8, wherein the bispecific CAR comprises the structure as shown in formula (C'):

~~14. The one or more CARs of claim 5, wherein the bispecific CAR has a structure as shown in the following formula III:~~

L-$V_{L3,BCMA}$-~~scFv3~~the first antigen binding domain-$V_{H3,BCMA}$-H-TM-C-CD3ζ ~~(III)~~(C')

wherein:

"-" is each independently a linker peptide or a peptide bond;

L is absent or a signal peptide sequence;

H is absent or a hinge region;

TM is a transmembrane domain;

C is a costimulatory signal molecule; and

CD3ζ is a cytoplasmic signaling sequence derived from CD3ζ.

~~in the formula,~~

~~"-" is each independently a linker peptide or a peptide bond;~~

~~elements L, H, TM, C and CD3ζ are as described above; and~~

~~scFv3 is the antigen binding domain targeting the first target, V_{H3} is the antibody heavy chain variable region against the BCMA, and V_{L3} is the antibody light chain variable region against the BCMA.~~

10. The bispecific CAR of claim 1, wherein the bispecific CAR comprises the structure as shown in formula (B).

11. The bispecific CAR of claim 10, wherein the bispecific CAR comprises the structure as shown in formula (B'):

~~15. The one or more CARs of claim 5, wherein the bispecific CAR has a structure as shown in the following formula III':~~

L-$V_{H3,1}$-~~scFv3~~the second antigen binding domain-$V_{L3,1}$-H1-TM-C-CD3ζ ~~(III')~~(B')

wherein:

"-" is each independently a linker peptide or a peptide bond;

L is absent or a signal peptide sequence;

H is absent or a hinge region;

TM is a transmembrane domain;

C is a costimulatory signal molecule; and

CD3ζ is a cytoplasmic signaling sequence derived from CD3ζ.

~~in the formula,~~

~~"-" is each independently a linker peptide or a peptide bond;~~

~~elements L, H, TM, C and CD3ζ are as described above; and~~

~~scFv3 is the antigen binding domain targeting the BCMA, V_{H3} is the antibody heavy chain variable region against the first target, and V_{L3} is the antibody light chain variable region against the first target.~~

12. The bispecific CAR of claim 1, wherein the bispecific CAR comprises the structure as shown in formula (D).

13. The bispecific CAR of claim 12, wherein the bispecific CAR comprises the structure as shown in formula (D'):

~~16. The one or more CARs of claim 5, wherein the bispecific CAR has a structure as shown in the following formula III':~~

L-$V_{H3,BCMA}$-~~scFv3~~the first antigen binding domain-$V_{L3,BCMA}$-H1-TM-C-CD3ζ ~~(III')~~(D')

wherein:

"-" is each independently a linker peptide or a peptide bond;

L is absent or a signal peptide sequence;

H is absent or a hinge region;

TM is a transmembrane domain;

C is a costimulatory signal molecule; and

CD3ζ is a cytoplasmic signaling sequence derived from CD3ζ.

~~in the formula,~~

~~" - " is each independently a linker peptide or a peptide bond;~~

~~elements L, H, TM, C and CD3ζ are as described above; and~~

~~scFv3 is the antigen binding domain targeting the first target, V_{H3} is the antibody heavy chain variable region against the BCMA, and V_{L3} is the antibody light chain variable region against the BCMA.~~

14. The bispecific CAR of claim 7, wherein the bispecific CAR comprises the structure as shown in formula (E):

L-$V_{L,1}$-$V_{H,BCMA}$-$V_{L,BCMA}$-$V_{H,1}$-H-TM-C-CD3ζ (E).

15. The bispecific CAR of claim 14, wherein the first target antigen is CD19, and wherein:

L comprises the polypeptide sequence of SEQ ID NO: 16;

$V_{L,1}$ comprises the polypeptide sequence of SEQ ID NO: 12;

$V_{H,BCMA}$ comprises the polypeptide sequence of SEQ ID NO: 9;

$V_{L,BCMA}$ comprises the polypeptide sequence of SEQ ID NO: 10;

$V_{H,1}$ comprises the polypeptide sequence of SEQ ID NO: 11;

H comprises the polypeptide sequence of SEQ ID NO: 38;

TM comprises the polypeptide sequence of SEQ ID NO: 7;

C comprises the polypeptide sequence of SEQ ID NO: 5; and

CD3ζ comprises the polypeptide sequence of SEQ ID NO: 3.

~~17~~16. An engineered immune cell comprising the bispecific CAR of claim 1.

~~18. An engineered immune cell comprising the one or more CARs of claim 4.~~

~~19. An engineered immune cell, the immune cell comprising a first expression cassette and a second expression cassette that are exogenous, wherein the first expression cassette is used to express a first CAR~~

~~targeting a first target, and the second expression cassette is used to express a second CAR targeting BCMA; or~~

~~the immune cell expressing the first CAR targeting the first target and the second CAR targeting the BCMA,~~

~~wherein an antigen binding domain (scFv) targeting the BCMA in the second CAR comprises an antibody heavy chain variable region as shown in SEQ ID NO: 9 and an antibody light chain variable region as shown in SEQ ID NO: 10; and~~

~~wherein the first target is selected from the group consisting of CD138, Kappa Light Chain, NKG2D-ligands, TACI, GPRC5D, CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD38, CD40, CD44V6, CD47, CD52, CD56, CD57, CD58, CD79b, CD80, CD86, CD81, CD123, CD133, CD137, CD151, CD171, CD276, CLL1, B7H4, VEGFR-2, EGFR, GPC3, PMSA, CEACAM6, c-Met, EGFRvIII, ErbB2/HER2, ErbB3, HER-2, HER3, ErbB4/HER-4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, Flt1, KDR, Flt4, Flt3, CEA, CA125, CTLA-4, GITR, BTLA, TGFBR1, TGFBR2, TGFBR1, IL6R, gp130, Lewis, TNFR1, TNFR2, PD1, PD-L1, PD-L2, PSCA, HVEM, MAGE-A, MSLN, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, TWEAK-R, LTPR, LIFRP, LRP5, MUC1, MUC16, TCRα, TCRβ, TLR7, TLR9, PTCH1, WT-1, Robol, Frizzled, OX40, Notch-1-4, APRIL, CS1, MAGE3, Claudin 18.2, Folate receptor α, Folate receptor β, GPC2, CD70, BAFF-R, and TROP-2.~~