

CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising an extended-release binding protein, wherein the extended-release binding protein comprises a half-life extended immune cell engaging protein, a masking peptide, and a cleavable linker; wherein the half-life extended immune cell engaging protein comprises a first domain (A), a second domain (B), and a third domain (C), wherein:
 - (i) the first domain (A) comprises an immune cell engaging domain,
 - (ii) the second domain (B) comprises a half-life extended domain, and
 - (iii) the third domain (C) specifically binds to a target antigen;wherein the cleavable linker covalently links the masking peptide to the N-terminus or the C-terminus of the half-life-extended immune cell engaging protein, and wherein the cleavable linker is significantly cleaved in systemic circulation.
2. The pharmaceutical composition of claim 1, wherein the domains are linked in one of the following orders: H₂N-(C)-(B)-(A)-COOH, H₂N-(A)-(B)-(C)-COOH, H₂N-(B)-(A)-(C)-COOH, H₂N-(C)-(A)-(B)-COOH, H₂N-(A)-(C)-(B)-COOH, H₂N-(B)-(C)-(A)-COOH, or by linkers L1 and L2 in one of the following orders: H₂N-(C)-L1-(B)-L2(A)-COOH, H₂N-(A)-L1-(B)-L2-(C)-COOH, H₂N-(B)-L1-(A)-L2-(C)-COOH, H₂N-(C)-L1-(A)-L2-(B)-COOH, H₂N-(A)-L1-(C)-L2(B)-COOH, H₂N-(B)-L1-(C)-L2-(A)-COOH.
3. The pharmaceutical composition of claim 1 or 2, wherein the immune cell engaging domain comprises a T cell engaging domain, a natural killer (NK) cell engaging domain, a NK-T cell engaging domain, a B cell engaging domain, a dendritic cell engaging domain, a macrophage cell engaging domain, or a combination thereof.
4. The pharmaceutical composition of claim 3, wherein the immune cell engaging domain comprises the T cell engaging domain.
5. The pharmaceutical composition of claim 4, wherein the T cell engaging domain binds a CD3 molecule, and wherein the CD3 molecule is at least one of: a CD3 γ molecule, a CD3 δ molecule, or a CD3 ϵ molecule.

6. The pharmaceutical composition of any one of claims 1-5, wherein the first domain comprises a single-chain variable fragment (scFv) specific to human CD3;

wherein the scFv comprises a variable heavy chain region (VH), a variable light chain region (VL), and a linker, wherein VH comprises complementarity determining regions HC CDR1, HC CDR2, and HC CDR3, and wherein VL comprises complementarity determining regions LC CDR1, LC CDR2, and LC CDR3; and

wherein HC CDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3081 and 3087-3098, the HC CDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3082 and 3099-3109, the HC CDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3083 and 3110-3119; and

wherein the LC CDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3084 and 3120-3132, the LC CDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3085 and 3099-3109, the LC CDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3086 and 3146-3152.

7. The pharmaceutical composition of claim 6, wherein the HC CDR1 comprises the amino acid sequence of SEQ ID NO: 3097, the HC CDR2 comprises the amino acid sequence of SEQ ID NO: 3108, the HC CDR3 comprises the amino acid sequence of SEQ ID NO: 3110; and wherein the LC CDR1 comprises the amino acid sequence of SEQ ID NO: 3120, the LC CDR2 comprises the amino acid sequence of SEQ ID NO: 3145, the LC CDR3 comprises the amino acid sequence of SEQ ID NO: 3146.

8. The pharmaceutical composition of any one of claims 1-6, wherein the first domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 3153-3169.

9. The pharmaceutical composition of any one of claims 1-8, wherein the second domain comprises a single domain antibody (sdAb) which specifically binds to HSA;

wherein the sdAb comprises complementarity determining regions CDR1, CDR2, and CDR3, wherein the CDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 3170 and 3173-3175, the CDR2 comprises an amino acid sequence selected

from the group consisting of SEQ ID NOS: 3171 and 3176-3181, the CDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 3172 and 8182-3183.

10. The pharmaceutical composition of claim 9, wherein the CDR1 comprises the amino acid sequence of SEQ ID NO: 3174, the CDR2 comprises the amino acid sequence of SEQ ID NO: 3178, and the CDR3 comprises the amino acid sequence of SEQ ID NO: 3183.

11. The pharmaceutical composition of claim 9, wherein the second domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 3184-3193.

12. The pharmaceutical composition of any one of claims 1-11, wherein the masking peptide inhibits or reduces the binding of the first domain (A) to the human CD3 or the binding of the third domain (C) to the target antigen.

13. The pharmaceutical composition of any one of claims 1-12, wherein the masking peptide comprises an amino acid sequence having an amino acid sequence selected from the group consisting of SEQ ID NOS: 3663-3682.

14. The pharmaceutical composition of any one of claims 1-13, wherein the cleavable linker comprises a cleavage site recognizable by a protease, and wherein the protease is selected from the group consisting of a serine protease, a cysteine protease, an aspartate protease, a threonine protease, a glutamic acid protease, a metalloproteinase, a gelatinase, and an asparagine peptide lyase.

15. The pharmaceutical composition of any one of claims 1-14, wherein the cleavable linker comprises an amino acid sequence having at least 80% homology to any one of SEQ ID NOS: 3688-3770 and 3878.

16. The pharmaceutical composition of any one of claims 1-15, wherein the target antigen is a tumor antigen.

17. The pharmaceutical composition of claim 16, wherein the target antigen comprises CD19, CD20, CD33, FLT3, PSMA, MSLN, BCMA, DLL3, EGFR, or EpCAM.

18. The pharmaceutical composition of claim 17, wherein the third domain comprises

(i) a single domain antibody that specifically binds to FLT3, and wherein the third domain comprises a CDR1 comprising an amino acid selected from the group consisting of SEQ ID NOS: 1080-1155 and 3497-3498, a CDR2 comprising an amino acid selected from the group consisting of SEQ ID NOS: 1156-1231 and 3499-3500, a CDR3 comprising an amino acid selected from the group consisting of SEQ ID NOS: 1232-1307 and 3501-3502;

(ii) a single domain antibody that specifically binds to PSMA, and wherein the third domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 462-465, a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 466-472, and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 474-475;

(iii) a single domain antibody that specifically binds to MSLN, and wherein the third domain comprises a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 490-528, a CDR2 comprising an amino acid selected from the group consisting of SEQ ID NOS: 529-567, and a CDR3 comprising an amino acid selected from the group consisting of SEQ ID NOS: 568-606;

(iv) a single domain antibody that specifically binds to BCMA, and wherein the third domain CDR1 comprises comprising an amino acid selected from the group consisting of SEQ ID NOS: 1-115, a CDR2 comprising an amino acid selected from the group consisting of SEQ ID NOS: 116-230, and a CDR3 comprising an amino acid selected from the group consisting of SEQ ID NOS: 231-345;

(v) a single domain antibody that specifically binds to DLL3, and wherein the third domain comprises a CDR1 comprising an amino acid selected from the group consisting of SEQ ID NOS: 1751-2193, a CDR2 comprising an amino acid selected from the group consisting of SEQ ID NOS: 2194-2636, and a CDR3 comprising an amino acid selected from the group consisting of SEQ ID NOS: 2637-3080;

(vi) a single domain antibody that specifically binds to EGFR, and wherein the third domain comprises a CDR1 comprising an amino acid selected from the group consisting of SEQ ID NOS: 651-699, a CDR2 comprising an amino acid selected from the group consisting of SEQ ID NOS: 700-748, a CDR3 comprising an amino acid selected from the group consisting of SEQ ID NOS: 479-797; or

(vii) a single domain antibody that specifically binds to EpCAM, and wherein the third domain comprises a CDR1 comprising an amino acid selected from the group consisting of SEQ ID NOS: 847-884, a CDR2 comprising an amino acid selected from the group consisting of SEQ ID NOS: 885-922, a CDR3 comprising an amino acid selected from the group consisting of SEQ ID NOS: 923-960.

19. The pharmaceutical composition of any one of claims 1-18, wherein the third domain comprises an amino acid sequence having at least 80% homology to any one of SEQ ID NOs: 346-461, 476-489, 607-650, 798-846, 961-1079, 1308-1750, 3495-3496, 3771-3792, 3793-3808 3809-3823, and 3880.

20. The pharmaceutical composition of claim 19, wherein the third domain comprises an amino acid sequence of SEQ ID NO: 383, 489, 647, 999, 1003, 1074, 1076, 1739, 3495, 3496, 3771, or 3809.

21. The pharmaceutical composition of any one of claims 1-20, wherein

(i) the extended-release binding protein has a higher therapeutic index than a corresponding half-life-extended immune cell engaging protein without the masking peptide;

(ii) administration of the extended-release binding protein results in a lower C_{max}/C_{min} ratio of an active version of the extended-release binding protein in systemic circulation than the C_{max}/C_{min} ratio when a corresponding half-life extended immune cell engaging protein without the masking peptide is administered;

(iii) multiple administration of the extended-release binding protein results in a more gradual increase of a level of an active version of the extended-release binding protein in systemic circulation than when a corresponding half-life-extended immune cell engaging protein without the masking peptide is administered; or

(iv) administration of the extended-release binding protein results in a lower Cytokine release syndrome (CRS) level than the CRS observed when a corresponding half-life extended immune cell engaging protein without the masking peptide is administered.