

molecule to the same target can help overcome this low expression and improve target binding. Some examples of molecules that utilize multiple binding sites are provided in U.S. Provisional Patent Appl. No. 63/110,957.

[0006] In the biopharmaceutical industry, molecules are typically produced in a large-scale fashion in order to meet the commercial needs of supplying a large number of patients and can be assessed for a number of attributes to mitigate the risk that the molecule is not amenable to large-scale production and purification. Efficient expression of these complex, recombinant polypeptides can be an ongoing challenge. Further, even once expressed the polypeptides are often not as stable as desired for a pharmaceutical composition. Various attempts have been made to successfully alter molecule formats to address these challenges. See, for example, International Patent Application No. PCT/US20/36464 entitled “Bispecific Binding Constructs,” and PCT/US20/36474 entitled “Bispecific Binding Constructs with Selectively Cleavable Linkers.” However, challenges still exist for molecules with multiple binding sites. Accordingly, there is a need in the art for therapeutic molecules with multiple binding sites and with favorable pharmacokinetic properties, therapeutic efficacy, and a format that provides efficient production and increased stability.

SUMMARY

[0006a] In one aspect, the present disclosure relates to 1. A molecule comprising a polypeptide chain having the structure: a. VH1-L1-VH2-L2-VL1-L3-VL2-L4-VH3-L1-VH4-L2-VL3-L3-VL4, or b. VH1-L1-VH2-L2-VL1-L3-VL2-L4-Half-Life Extending moiety-L5-VH3-L1-VH4-L2-VL3-L3-VL4, wherein VH1, VH2, VH3, and VH4 are immunoglobulin heavy chain variable regions, VL1, VL2, VL3, and VL4 are immunoglobulin light chain variable regions, and L1, L2 and L3 are linkers, wherein L1 is at least 10 amino acids, L2 is at least 10 amino acids and L3 is at least 10 amino acids, and wherein the total amino acids of L1, L2 and L3 is at least 35 amino acids, wherein the molecule can bind to an immune effector cell and a target cell, and wherein the half-life extending moiety is a single chain immunoglobulin Fc region (“scFc”).

[0006b] In another aspect, the present disclosure refers to a method of manufacturing the molecule as disclosed herein comprising (1) culturing a host cell under conditions so as to express the molecule and (2) recovering the molecule from the cell mass or cell culture supernatant, wherein the host cell comprises one or more nucleic acid(s) encoding molecule as disclosed herein.

[0006c] In another aspect, the present disclosure refers to use of the molecule as disclosed herein in the manufacture of a medicament for the prevention, treatment or amelioration of cancer.

[0006d] In another aspect, the present disclosure refers to use of the molecule as disclosed herein in the manufacture of a medicament for the prevention, treatment or amelioration of an infectious disease.

[0006e] In another aspect, the present disclosure refers to use of the molecule of any of claims 1-14 in the manufacture of a medicament for the prevention, treatment or amelioration of an autoimmune, inflammatory, or fibrotic condition.

[0007] Described herein are novel formats of binding molecules that comprise multiple binding domains. In one **embodiment example**, the **invention disclosure** provides a molecule comprising a polypeptide chain having the structure:

VH1-L1-VH2-L2-VL1-L3-VL2-L4-VH3-L1-VH4-L2-VL3-L3-VL4, or

VH1-L1-VH2-L2-VL1-L3-VL2-L4-Half Life Extending moiety-L5-VH3-L1-VH4-L2-VL3-L3-VL4,

wherein VH1, VH2, VH3, and VH4 are immunoglobulin heavy chain variable regions, VL1, VL2, VL3, and VL4 are immunoglobulin light chain variable regions, and L1, L2, L3, L4, and L5 are linkers, wherein L1 is at least 10 amino acids, L2 is at least 15 amino acids and L3 is at least 10 amino acids, and wherein the molecule can bind to an immune effector cell and a target cell.

[0008] In another **embodiment example**, the **invention disclosure** provides a molecule comprising a polypeptide chain having the structure:

VH1-L1-VH2-L2-VL1-L3-VL2-L4-VH3-L1-VH4-L2-VL3-L3-VL4, or

VH1-L1-VH2-L2-VL1-L3-VL2-L4-Half-Life Extending moiety-L5-VH3-L1-VH4-L2-VL3-L3-VL4,

wherein VH1, VH2, VH3, and VH4 are immunoglobulin heavy chain variable regions, VL1, VL2, VL3, and VL4 are immunoglobulin light chain variable regions, and L1, L2 and L3 are linkers, wherein L1 is at least 10 amino acids, L2 is at least 10 amino acids and L3 is at least 10 amino acids, and wherein the total amino acids of L1, L2 and L3 is at least 35 amino acids, and wherein the molecule can bind to an immune effector cell and a target cell.

[0009] The [inventiondisclosure](#) further provides nucleic acids encoding the molecule described herein, vectors comprising these nucleic acids, and host cells comprising these vectors.

[0010] In yet other [embodimentexamples](#), the [inventiondisclosure](#) provides methods of manufacturing the molecules describe herein comprising (1) culturing a host cell under conditions so as to express the molecule and (2) recovering the molecule from the cell mass or cell culture supernatant, wherein the host cell comprises one or more nucleic acid(s) encoding any of the molecules provided herein.

[0011] In yet other [embodimentexamples](#), the [inventiondisclosure](#) provides a method of treating a cancer patient comprising administering to the patient a therapeutically effective amount of the molecules provided herein.

[0012] In other [embodimentexamples](#), the [inventiondisclosure](#) provides a method for treating a patient having an infectious disease comprising administering to the patient a therapeutically effective dose of the molecules provided herein.

[0013] In a further [embodimentexample](#), the [inventiondisclosure](#) provides a method for treating a patient having an autoimmune, inflammatory, or fibrotic condition comprising administering to the patient a therapeutically effective dose of the molecules provided herein.

[0014] In another [embodimentexample](#), the [inventiondisclosure](#) further provides a pharmaceutical composition comprising the molecules provided herein.

[0015] In another [embodimentexample](#), the [inventiondisclosure](#) provides for the use of the molecules provided herein in the manufacture of a medicament for the prevention, treatment or amelioration of a disease.

CLAIMS

What is claimed is:

~~1. A molecule comprising a polypeptide chain having the structure:~~

- ~~a. VH1-L1-VH2-L2-VL1-L3-VL2-L4-VH3-L1-VH4-L2-VL3-L3-VL4, or~~
- ~~b. VH1-L1-VH2-L2-VL1-L3-VL2-L4-Half Life Extending moiety-L5-VH3-L1-VH4-L2-VL3-L3-VL4,~~

~~wherein VH1, VH2, VH3, and VH4 are immunoglobulin heavy chain variable regions, VL1, VL2, VH3, and VL4 are immunoglobulin light chain variable regions, and L1, L2, L3, L4, and L5 are linkers, wherein L1 is at least 10 amino acids, L2 is at least 15 amino acids and L3 is at least 10 amino acids, and wherein the molecule can bind to an immune effector cell and a target cell.~~

~~2.~~1. A molecule comprising a polypeptide chain having the structure:

- a. VH1-L1-VH2-L2-VL1-L3-VL2-L4-VH3-L1-VH4-L2-VL3-L3-VL4, or
- b. VH1-L1-VH2-L2-VL1-L3-VL2-L4-Half-Life Extending moiety-L5-VH3-L1-VH4-L2-VL3-L3-VL4,

wherein VH1, VH2, VH3, and VH4 are immunoglobulin heavy chain variable regions, VL1, VL2, VL3, and VL4 are immunoglobulin light chain variable regions, and L1, L2 and L3 are linkers, wherein L1 is at least 10 amino acids, L2 is at least 10 amino acids and L3 is at least 10 amino acids, and wherein the total amino acids of L1, L2 and L3 is at least 35 amino acids, and wherein the molecule can bind to an immune effector cell and a target cell, and wherein the half-life extending moiety is a single chain immunoglobulin Fc region ("scFc").

~~3. The molecule of claim 1 or 2, wherein the half life extending moiety is a single chain immunoglobulin Fc region ("scFc").~~

~~4.~~2. The molecule of claim 1~~3~~, wherein the half-life extending moiety is an scFc from a human IgG1, IgG2, or IgG4 antibody.

- ~~5.3.~~ The molecule of claim ~~2~~4, wherein the scFc polypeptide chain comprises one or more alterations that inhibit Fc gamma receptor (FcγR) binding and/or one or more alterations that extends half life
- ~~6.4.~~ The molecule of claim 1-~~or 2~~2, wherein the VH1, VH2, VH3, VH4, VL1, VL2, VL3, and VL4 all have different sequences.
- ~~7.5.~~ The molecule of claim 1-~~or 2~~2, wherein the VH2 and VH4 sequence comprise SEQ ID NO: 41 and the VL2 and VH4 sequence comprise SEQ ID NO: 42.
- ~~8.6.~~ The molecule of claim 1-~~or 2~~2, wherein L1, L2 and L3 are different or the same lengths.
- ~~9. The molecule of claim 1 or 2, wherein L1, L2, and L3 are the same length.~~
- ~~10.7.~~ The molecule of claim 1-~~or 2~~2, wherein L1 and L2 are the same length.
- ~~11.8.~~ The molecule of claim 1-~~or 2~~2, wherein L1 and L3 are the same length.
- ~~12.9.~~ The molecule of claim 1-~~or 2~~2, wherein L2 and L3 are the same length.
- ~~13.10.~~ The molecule of claim 1-~~or 2~~2, wherein the amino acid sequence of L1 is at least 10 amino acids long, the amino acid sequence of L2 is at least 15 amino acids long, and the amino acid sequence of L3 is at least 15 amino acids long.
- ~~14.11.~~ The molecule of claim 1-~~or 2~~2, wherein the molecule exhibits enhanced stability as compared to a molecule having a structure of VH1-Linker-VL1-Linker-VH2-Linker-VL2-Linker-VH3-Linker-VL3-Linker-VH4-Linker-VL4, or VH1-Linker-VL1-Linker-VH2-Linker-VL2-Linker-Half-Life Extending Moiety-Linker-VH3-Linker-VL3-Linker-VH4-Linker-VL4.
- ~~15.12.~~ The molecule of claim 1-~~or 2~~2, wherein the molecule exhibits enhanced *in vitro* expression as compared to a molecule having a structure of VH1-Linker-VL1-Linker-VH2-Linker-VL2-Linker-VH3-Linker-VL3-Linker-VH4-Linker-VL4 or VH1-Linker-VL1-Linker-VH2-Linker-VL2-Linker-Half-Life Extending Moiety-Linker-VH3-Linker-VL3-Linker-VH4-Linker-VL4.

~~16.~~13. The molecule of claim 1 ~~or 2~~, wherein the effector cell expresses an effector cell protein that is part of a human T cell receptor (TCR)-CD3 complex.

~~17.~~14. The molecule of claim 1 ~~3~~6, wherein the effector cell protein is the CD3ε chain

~~18. A nucleic acid encoding the molecule of claims 1-17.~~

~~19. A vector comprising the nucleic acid of claim 18.~~

~~20. A host cell comprising the vector of claim 19.~~

~~21.~~15. A method of manufacturing the molecule of claim 1 comprising (1) culturing a host cell under conditions so as to express the molecule and (2) recovering the molecule from the cell mass or cell culture supernatant, wherein the host cell comprises one or more nucleic acid(s) encoding molecule of any of claims 1-1~~4~~7.

~~22.~~16. A Use of the molecule of any of claims 1-14 in the manufacture of a medicament for the prevention, treatment or amelioration of ~~method of treating a cancer patient comprising administering to the patient a therapeutically effective amount of the molecule of any of claims 1-17.~~

~~23.~~17. The ~~method use~~ of claim ~~16~~22, wherein a chemotherapeutic agent, a non-chemotherapeutic anti-neoplastic agent, and/or radiation is administered to the patient concurrently with, before, or after administration of the molecule.

~~24.~~18. Use of the molecule of any of claims 1-14 in the manufacture of a medicament for the prevention, treatment or amelioration of ~~A method for treating a patient having an infectious disease comprising administering to the patient a therapeutically effective dose of the molecule of any of claims 1-17.~~

~~25.~~19. Use of the molecule of any of claims 1-14 in the manufacture of a medicament for the prevention, treatment or amelioration of ~~A method for treating a patient having an autoimmune, inflammatory, or fibrotic condition comprising administering to the patient a therapeutically effective dose of the molecule of any of claims 1-17.~~

~~26.~~20. A pharmaceutical composition comprising the molecule of any of claims 1-1~~4~~7.

~~27. The use of the molecule of any of claims 1-17 in the manufacture of a medicament for the prevention, treatment or amelioration of a disease.~~