

CLAIMS

What is claimed is:

1. A multifunctional polypeptide molecule comprising a first polypeptide, a second polypeptide, and at least one cytokine polypeptide or a functional fragment or variant thereof, wherein the first polypeptide and the second polypeptide are non-contiguous, wherein:
 - (i) the first polypeptide comprises a first portion of a dimerization module linked to
 - (A) a TCR β V-binding moiety comprising a heavy chain variable domain (VH) and a light chain variable domain (VL), or a single domain antibody, or
 - (B) a first portion of a TCR β V-binding moiety comprising a VH of the TCR β V-binding moiety, wherein the multifunctional polypeptide molecule further comprises a third polypeptide comprising a second portion of the TCR β V-binding moiety comprising a VL of the TCR β V-binding moiety, wherein the third polypeptide is non-contiguous with the first polypeptide and the second polypeptide; and
 - (ii) the second polypeptide comprises a second portion of the dimerization module, wherein the at least one cytokine polypeptide or a functional fragment or variant thereof is covalently linked to the second polypeptide; and, wherein the at least one cytokine polypeptide or a functional fragment or variant thereof comprises a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3523, optionally, wherein the at least one cytokine polypeptide or a functional fragment or variant thereof comprises the sequence of SEQ ID NO: 3523.
2. The multifunctional polypeptide molecule of claim 1, wherein the at least one cytokine polypeptide or a functional fragment or variant thereof comprises, from N-terminus to C-terminus, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3523 operatively linked to a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 2170, optionally, via the sequence of SEQ ID NO: 3524.
3. The multifunctional polypeptide molecule of claim 1 or 2, wherein the at least one cytokine polypeptide or a functional fragment or variant thereof comprises, from N-terminus to C-terminus, the sequence of SEQ ID NO: 3523 operatively linked to the sequence of SEQ ID NO: 2170, optionally, via the sequence of SEQ ID NO: 3524.
4. The multifunctional polypeptide molecule of any one of claims 1, 2 and 3, wherein the at least one cytokine polypeptide or a functional fragment or variant thereof is within a single contiguous polypeptide chain of the second polypeptide.
5. The multifunctional polypeptide molecule of any one of claims 1-4, wherein the TCR β V-binding moiety binds to one or more of a TCR β V subfamily selected from the group consisting of TCR β V1 subfamily, TCR β V2 subfamily, TCR β V3 subfamily, TCR β V4 subfamily, TCR β V5 subfamily,

TCR β V6 subfamily, TCR β V7 subfamily, TCR β V9 subfamily, TCR β V10 subfamily, TCR β V11 subfamily, TCR β V12 subfamily, TCR β V13 subfamily, TCR β V16 subfamily, TCR β V19 subfamily, TCR β V20 subfamily, TCR β V21 subfamily, TCR β V23 subfamily, TCR β V24 subfamily, TCR β V25 subfamily, TCR β V26 subfamily, TCR β V27 subfamily, TCR β V28 subfamily, TCR β V29 subfamily, and TCR β V30 subfamily.

6. The multifunctional polypeptide molecule of any one of claims 1-5, wherein the TCR β V-binding moiety comprises any one selected from the group consisting of a full antibody, a bivalent antibody, a Fab, a F(ab')₂, an Fv, a single chain Fv (scFv), a single domain antibody, a diabody, and a camelid antibody.
7. The multifunctional polypeptide molecule of any one of claims 1-6, wherein the TCR β V-binding moiety comprises a Fab or an scFv.
8. The multifunctional polypeptide molecule of any one of claims 1-7, wherein the first portion of the dimerization module comprises a first immunoglobulin constant region and the second portion of the dimerization module comprises a second immunoglobulin constant region.
9. The multifunctional polypeptide molecule of claim 8, wherein the first immunoglobulin constant region comprises a first Fc region, and the second immunoglobulin constant region comprises a second Fc region, and
wherein the first Fc region, the second Fc region, or a combination thereof is selected from the group consisting of an IgG1 Fc region or a fragment thereof, an IgG2 Fc region or a fragment thereof, an IgG3 Fc region or a fragment thereof, an IgGA1 Fc region or a fragment thereof, an IgGA2 Fc region or a fragment thereof, an IgG4 Fc region or a fragment thereof, an IgJ Fc region or a fragment thereof, an IgM Fc region or a fragment thereof, an IgD Fc region or a fragment thereof, and an IgE Fc region or a fragment thereof.
10. The multifunctional polypeptide molecule of claim 9, wherein the first Fc region, the second Fc region, or a combination thereof comprises an Fc interface with one or more of: a paired cavity-protuberance, an electrostatic interaction, or a strand-exchange, and
wherein dimerization of the first Fc region and the second Fc region is enhanced as indicated by a greater ratio of heteromultimer:homomultimer forms relative to dimerization of Fc regions with a non-engineered interface.
11. The multifunctional polypeptide molecule of claim 9 or 10, wherein the first Fc region, the second Fc region, or a combination thereof comprises an Asn297Ala (N297A) mutation or a Leu234Ala/Leu235Ala (LALA) mutation according to EU Numbering.
12. The multifunctional polypeptide molecule of any one of claims 9-11, wherein the first Fc region, the second Fc region, or a combination thereof comprises a sequence having at least 75% sequence identity to the sequence of SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 3531,

SEQ ID NO: 3533, SEQ ID NO: 3537, SEQ ID NO: 3645, SEQ ID NO: 3646, SEQ ID NO: 3647, SEQ ID NO: 3648, or SEQ ID NO: 3649,

optionally, wherein the first Fc region, the second Fc region, or a combination thereof comprises the sequence of SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 3531, SEQ ID NO: 3533, SEQ ID NO: 3537, SEQ ID NO: 3645, SEQ ID NO: 3646, SEQ ID NO: 3647, SEQ ID NO: 3648, or SEQ ID NO: 3649.

13. The multifunctional polypeptide molecule of any one of claims 1-12, wherein the first portion of the dimerization module comprises a sequence having at least 75% sequence identity to the sequence of SEQ ID NO: 3649 and the second portion of the dimerization module comprises a sequence having at least 75% sequence identity to the sequence of SEQ ID NO: 3648,

optionally, wherein the first portion of the dimerization module comprises the sequence of SEQ ID NO: 3649 and the second portion of the dimerization module comprises the sequence of SEQ ID NO: 3648.

14. The multifunctional polypeptide molecule of any one of claims 1-13, wherein the multifunctional polypeptide molecule further comprises a light chain constant region operatively linked to the VL of the TCR β V-binding moiety; and

wherein the light chain constant region is selected from the group consisting of a kappa light chain constant region or a fragment thereof and a lambda light chain constant region or a fragment thereof.

15. The multifunctional polypeptide molecule of claim 14, wherein the light chain constant region comprises a sequence having at least 75% sequence identity to the sequence of SEQ ID NO: 3644 or the sequence of SEQ ID NO: 3528,

optionally, wherein the light chain constant region comprises the sequence of SEQ ID NO: 3644 or the sequence of SEQ ID NO: 3528.

16. The multifunctional polypeptide molecule of any one of claims 1-15, wherein the TCR β V-binding moiety comprises:

(i) a VH comprising a heavy chain complementarity determining region 1 (HC CDR1) comprising the sequence of SEQ ID NO: 3650, a heavy chain complementarity determining region 2 (HC CDR2) comprising the sequence of SEQ ID NO: 3651, and a heavy chain complementarity determining region 3 (HC CDR3) comprising the sequence of SEQ ID NO: 5; and/or

(ii) a VL comprising a light chain complementarity determining region 1 (LC CDR1) comprising the sequence of SEQ ID NO: 3655, a light chain complementarity determining region 2 (LC CDR2) comprising the sequence of SEQ ID NO: 3653, and a light chain complementarity determining region 3 (LC CDR3) comprising the sequence of SEQ ID NO: 8.

17. The multifunctional polypeptide molecule of any one of claims 1-16, wherein the TCR β V-binding moiety comprises:
- (i) a VH comprising an amino acid sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1346; and/or
 - (ii) a VL comprising an amino acid sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1349.
18. The multifunctional polypeptide molecule of any one of claims 1-17, wherein the TCR β V-binding moiety comprises:
- (i) a VH comprising the sequence of SEQ ID NO: 1346; and/or
 - (ii) a VL comprising the sequence of SEQ ID NO: 1349.
19. The multifunctional polypeptide molecule of any one of claims 1-18, wherein the multifunctional polypeptide molecule comprises:
- (i) a first polypeptide comprising an anti-TCR β V antibody heavy chain variable region, and a first immunoglobulin heavy chain constant region;
 - (ii) a second polypeptide comprising an IL-15 receptor alpha sushi domain or a functional fragment or variant thereof, an IL-15 molecule or a functional fragment or variant thereof, and a second immunoglobulin heavy chain constant region; and
 - (iii) a third polypeptide comprising an anti-TCR β V antibody light chain variable region, and an immunoglobulin light chain constant region,
- optionally, wherein:
- (i) the first polypeptide comprises, from N-terminus to C-terminus, the anti-TCR β V antibody heavy chain variable region operatively linked to the first immunoglobulin heavy chain constant region;
 - (ii) the second polypeptide comprises, from N-terminus to C-terminus, the IL-15 receptor alpha sushi domain or a functional fragment or variant thereof operatively linked to the IL-15 molecule or a functional fragment or variant thereof operatively linked to the second immunoglobulin heavy chain constant region; and
 - (iii) the third polypeptide comprises, from N-terminus to C-terminus, the anti-TCR β V antibody light chain variable region operatively linked to the immunoglobulin light chain constant region.
20. The multifunctional polypeptide molecule of any one of claims 1-19, wherein the multifunctional polypeptide molecule comprises:
- (i) a first polypeptide comprising, from N-terminus to C-terminus, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1346 operatively linked to a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3649;
 - (ii) a second polypeptide comprising, from N-terminus to C-terminus, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3523 operatively linked to a sequence

- having at least 90% sequence identity to the sequence of SEQ ID NO: 2170, optionally, via the sequence of SEQ ID NO: 3524, operatively linked to a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3648, optionally, via the sequence of SEQ ID NO: 3308; and
- (iii) a third polypeptide comprising, from N-terminus to C-terminus, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1349 operatively linked to a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3644.
21. The multifunctional polypeptide molecule of any one of claims 1-20, wherein the multifunctional polypeptide molecule comprises:
- (i) a first polypeptide comprising, from N-terminus to C-terminus, the sequence of SEQ ID NO: 1346 operatively linked to the sequence of SEQ ID NO: 3649;
 - (ii) a second polypeptide comprising, from N-terminus to C-terminus, the sequence of SEQ ID NO: 3523 operatively linked to the sequence of SEQ ID NO: 2170, optionally, via the sequence of SEQ ID NO: 3524, operatively linked to the sequence of SEQ ID NO: 3648, optionally, via the sequence of SEQ ID NO: 3308; and
 - (iii) the third polypeptide comprises, from N-terminus to C-terminus, the sequence of SEQ ID NO: 1349 operatively linked to the sequence of SEQ ID NO: 3644.
22. The multifunctional polypeptide molecule of any one of claims 1-21, wherein the multifunctional polypeptide molecule comprises:
- (i) a first polypeptide comprising a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3517;
 - (ii) a second polypeptide comprising a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3519; and
 - (iii) a third polypeptide comprising a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3518.
23. The multifunctional polypeptide molecule of any one of claims 1-22, wherein the multifunctional polypeptide molecule comprises:
- (i) a first polypeptide comprising the sequence of SEQ ID NO: 3517;
 - (ii) a second polypeptide comprising the sequence of SEQ ID NO: 3519; and
 - (iii) a third polypeptide comprising the sequence of SEQ ID NO: 3518.
24. A multifunctional polypeptide molecule comprising a first polypeptide, a second polypeptide, and a third polypeptide,
- wherein the first polypeptide, the second polypeptide, and the third polypeptide are non-contiguous; and
- wherein:

- (i) the first polypeptide comprises, from N-terminus to C-terminus, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1346 operatively linked to a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3649;
 - (ii) the second polypeptide comprises, from N-terminus to C-terminus, a sequence having at least 90% sequence identity to any one sequence selected from the group consisting of SEQ ID NO: 2170, SEQ ID NO: 2193, SEQ ID NO: 2270, SEQ ID NO: 3540, and SEQ ID NO: 3542 operatively linked to a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3648, optionally, via the sequence of SEQ ID NO: 3308; and
 - (iii) the third polypeptide comprises, from N-terminus to C-terminus, a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1349 operatively linked to a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3644.
25. The multifunctional polypeptide molecule of claim 24, wherein:
- (i) the first polypeptide comprises, from N-terminus to C-terminus, the sequence of SEQ ID NO: 1346 operatively linked to the sequence of SEQ ID NO: 3649;
 - (ii) the second polypeptide comprises, from N-terminus to C-terminus, any one sequence selected from the group consisting of SEQ ID NO: 2170, SEQ ID NO: 2193, SEQ ID NO: 2270, SEQ ID NO: 3540, and SEQ ID NO: 3542 operatively linked to the sequence of SEQ ID NO: 3648, optionally, via the sequence of SEQ ID NO: 3308; and
 - (iii) the third polypeptide comprises, from N-terminus to C-terminus, the sequence of SEQ ID NO: 1349 operatively linked to the sequence of SEQ ID NO: 3644.
26. The multifunctional polypeptide molecule of claim 24 or 25, wherein:
- (i) the first polypeptide comprises a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3517;
 - (ii) the second polypeptide comprises a sequence having at least 90% sequence identity to any one sequence selected from the group consisting of SEQ ID NO: 3520, SEQ ID NO: 3521, SEQ ID NO: 3539, SEQ ID NO: 3541, and SEQ ID NO: 3546; and
 - (iii) the third polypeptide comprises a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 3518.
27. The multifunctional polypeptide molecule of any one of claims 24-26, wherein:
- (i) the first polypeptide comprises the sequence of SEQ ID NO: 3517;
 - (ii) the second polypeptide comprises any one sequence selected from the group consisting of SEQ ID NO: 3520, SEQ ID NO: 3521, SEQ ID NO: 3539, SEQ ID NO: 3541, and SEQ ID NO: 3546; and
 - (iii) the third polypeptide comprises the sequence of SEQ ID NO: 3518.
28. A recombinant polynucleotide comprising a sequence encoding the multifunctional polypeptide molecule of any one of claims 1-27.

29. A method of making the multifunctional polypeptide molecule of any one of claims 1-27 comprising culturing a host cell comprising the recombinant polynucleotide of claim 28 under conditions suitable for expression of the multifunctional polypeptide molecule.
30. A medicament comprising the multifunctional polypeptide molecule of any one of claims 1-27 or the recombinant polynucleotide of claim 28, and a pharmaceutically acceptable carrier, excipient, diluent, or stabilizer.
31. Use of the multifunctional polypeptide molecule of any one of claims 1-27, the recombinant polynucleotide of claim 28, or the medicament of claim 30 in the manufacture of a pharmaceutical composition for treating cancer in a subject in need thereof,
wherein the pharmaceutical composition comprises a therapeutically effective amount of the multifunctional polypeptide molecule, the recombinant polynucleotide, or the medicament for treating cancer in the subject when administered to the subject.
32. The use of claim 31, wherein the cancer is a solid tumor, a hematological cancer, a metastatic cancer, a soft tissue tumor, or any combination thereof.
33. The use of claim 31 or 32, wherein:
- (i) the cancer is a solid tumor selected from the group consisting of melanoma, pancreatic cancer, breast cancer, colorectal cancer, lung cancer, skin cancer, ovarian cancer, liver cancer, and any combination thereof; or
 - (ii) the cancer is a hematological cancer selected from the group consisting of Hodgkin's lymphoma, Non-Hodgkin's lymphoma, acute myeloid leukemia (AML), chronic myeloid leukemia, myelodysplastic syndrome, multiple myeloma, T-cell lymphoma, acute lymphocytic leukemia, and any combination thereof.
34. A pharmaceutical composition comprising the multifunctional polypeptide molecule of any one of claims 1-27, the recombinant polynucleotide of claim 28, or the medicament of claim 30 for use in therapy.