CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant nucleic acid molecule comprising a sequence encoding a T cell receptor (TCR) fusion protein (TFP) wherein the TFP comprises:

   (a) a TCR subunit comprising:

   (i) at least a portion of a TCR extracellular domain, and

   (ii) a TCR transmembrane domain,

   (iii) a TCR intracellular domain, and

   (b) an antigen binding domain that specifically binds CD70; and

   wherein the TCR subunit and the antigen binding domain are operatively linked.

2. The recombinant nucleic acid molecule of claim 1, wherein the TFP functionally interacts with an endogenous TCR complex when expressed in a T cell.

3. The recombinant nucleic acid molecule of claim 1 or 2, wherein the TCR intracellular domain comprises a stimulatory domain from an intracellular signaling domain of CD3 gamma, CD3 delta, or CD3 epsilon.

4. The recombinant nucleic acid molecule of any one of claims 1-3, wherein a T cell expressing the TFP exhibits increased cytotoxicity to a human cell expressing CD70 compared to a T cell not containing the TFP.

5. The recombinant nucleic acid molecule of any one of claims 1-4, wherein the antigen binding domain is connected to the TCR extracellular domain by a linker sequence.

6. The recombinant nucleic acid molecule of claim 5, wherein the linker is 120 amino acids in length or less.

7. The recombinant nucleic acid molecule of claim 5, wherein the linker sequence comprises $(G_4S)_n$, wherein G is glycine, S is serine, and n is an integer from 1 to 10.

8. The recombinant nucleic acid molecule of claim 7, wherein n is an integer from 1 to 4.

9. The recombinant nucleic acid molecule of any one of claims 1-8, wherein at least two of the TCR extracellular domain, the TCR transmembrane domain, and the TCR intracellular domain are from the same TCR subunit.

10. The recombinant nucleic acid molecule of claim 9, wherein at least two of the TCR extracellular domain, the TCR transmembrane domain, and the TCR intracellular domain are from TCR alpha, TCR beta, TCR gamma, TCR delta, CD3 epsilon, CD3 delta, or CD3 gamma.

11. The recombinant nucleic acid molecule of any one of claims 1-10, wherein all three of the TCR extracellular domain, the TCR transmembrane domain, and the TCR intracellular domain are from the same TCR subunit.
12. The recombinant nucleic acid molecule of claim 11, wherein the TCR extracellular domain, the TCR transmembrane domain, and the TCR intracellular domain are from CD3 epsilon, CD3 delta, or CD3 gamma.
13. The recombinant nucleic acid molecule of claim 12, wherein the TCR extracellular domain, the TCR transmembrane domain, and the TCR intracellular domain comprise the constant domain of TCR alpha, the constant domain of TCR beta, the constant domain of TCR gamma, or the constant domain of TCR delta.
14. The recombinant nucleic acid molecule of claim 13, wherein the constant domain of TCR alpha or the constant domain of TCR beta is murine.
15. The recombinant nucleic acid molecule of any one of claims 1-14, wherein the antigen binding domain is a camelid antibody or binding fragment thereof, a murine antibody or binding fragment thereof, or a human or humanized antibody or binding fragment thereof.
16. The recombinant nucleic acid molecule of any one of claims 1-15, wherein the antigen binding domain is a single-chain variable fragment (scFv) or a single domain antibody (sdAb) domain.
17. The recombinant nucleic acid molecule of claim 16, wherein the sdAb is a V_{HH}.
18. The recombinant nucleic acid molecule of any one of claims 1-17, wherein the antigen binding domain binds to human CD70 with a K_D value of 100 nM or less or from about 0.001 nM to about 100 nM.
19. The recombinant nucleic acid molecule of any one of claims 1-18, wherein:
    (i) the antigen binding domain does not compete with CD27 for binding to CD70, does not inhibit CD70 from interacting with CD27, and/or does not bind to the same epitope of CD70 to which CD27 binds; or
    (ii) the antigen binding domain competes with CD27 for binding to CD70, inhibits CD70 from interacting with CD27, and/or binds to the same epitope of CD70 to which CD27 binds.
20. The recombinant nucleic acid molecule of any one of claims 1-18, wherein the antigen binding domain specifically binds to an epitope that is within the amino acid sequence HRDGIYMVHIQVTLAICSSTTAS (SEQ ID NO:1230).

21. The recombinant nucleic acid molecule of claim 20, wherein the antigen binding domain comprises a scFv having at least about 90% sequence identity to any one of sequence of SEQ ID NOs: 1207-1222, 1246, and 1247, a scFv having at least about 90% sequence identity to residues 23-268 of SEQ ID NO: 1236, or a sdAb domain having at least about 90% sequence identity to any one of sequence of SEQ ID NOs: 618, 1224-1227.
22. The recombinant nucleic acid molecule of any one of claims 1-20, wherein the antigen binding domain comprises a variable domain comprising a complementarity determining region 1 (CDR1), a CDR2, and a CDR3, and having at least 90% sequence identity to any one of SEQ ID NOs: 603-620 and 622-688.
23. The recombinant nucleic acid molecule of claim 22, wherein
    (i) CDR1 comprises a sequence of any one of SEQ ID NOs: 87-104 and 107-172;
    (ii) CDR2 comprises a sequence of any one of SEQ ID NOs: 259-276 and 279-344; and
    (iii) CDR3 comprises a sequence of any one of SEQ ID NOs: 431-448 and 451-516.
24. The recombinant nucleic acid molecule of any one of claims 1-21, wherein the antigen binding domain is a single-chain variable fragment (scFv).
25. The recombinant nucleic acid molecule of claim 24, wherein the scFv comprises a heavy chain variable (VH) domain having at least 90%, 95%, or 100% sequence identity to any one of SEQ ID NOs: 783-835, and/or a light chain variable (VL) domain having at least 90%, 95%, or 100% sequence identity to any one of SEQ ID NOs: 995-1047.
26. The recombinant nucleic acid molecule of claim 24 or 25, wherein the VH domain comprises a heavy chain complementary determining region 1 (CDRH1) having a sequence of any one of SEQ ID NOs: 836-888, a CDRH2 having a sequence of any one of SEQ ID NOs: 889-941, and a CDRH3 having a sequence of any one of SEQ ID NOs: 942-994, and/or the VL domain comprises a light chain complementary determining region 1 (CDRL1) having a sequence of any one of SEQ ID NOs: 1048-1100, a CDRL2 having a sequence of any one of SEQ ID NOs: 1101-1153, and a CDRL3 having a sequence of any one of SEQ ID NOs: 1154-1206.
27. The recombinant nucleic acid molecule of claim 24, wherein the scFv comprises a VH domain having at least 90%, 95%, or 100% sequence identity to any one of SEQ ID NOs: 1248, 1250, 1252, and 800, and/or a VL domain having at least 90%, 95%, or 100% sequence identity to any one of SEQ ID NO: 1249, 1251, 1253, or 1012.
28. The recombinant nucleic acid molecule of claim 24 or 27, wherein the scFv comprises:

(i) a VH domain having at least 90%, 95%, or 100% sequence identity to SEQ ID NO: 1248, and a VL domain having at least 90%, 95%, or 100% sequence identity to SEQ ID NO: 1249;

(ii) a VH domain having at least 90%, 95%, or 100% sequence identity to SEQ ID NO: 1250, and a VL domain having at least 90%, 95%, or 100% sequence identity to SEQ ID NO: 1251;

(iii) a VH domain having at least 90%, 95%, or 100% sequence identity to SEQ ID NO: 1252, and a VL domain having at least 90%, 95%, or 100% sequence identity to SEQ ID NO: 1253; or

(iv) a VH domain having at least 90%, 95%, or 100% sequence identity to SEQ ID NO: 800, and a VL domain having at least 90%, 95%, or 100% sequence identity to SEQ ID NO: 1012.

29. The recombinant nucleic acid molecule of claim 28, wherein:

(i) the VH domain of the sequence of SEQ ID NO: 1248 is operably linked via its C-terminus to the N-terminus of the VL domain of the sequence of SEQ ID NO: 1249,

(ii) the VL domain of the sequence of SEQ ID NO: 1249 is operably linked via its C-terminus to the N-terminus of the VH domain of the sequence of SEQ ID NO: 1248,

(iii) the VH domain of the sequence of SEQ ID NO: 1250 is operably linked via its C-terminus to the N-terminus of the VL domain of the sequence of SEQ ID NO: 1251,

(iv) the VL domain of the sequence of SEQ ID NO: 1251 is operably linked via its C-terminus to the N-terminus of the VH domain of the sequence of SEQ ID NO: 1250,

(v) the VH domain of the sequence of SEQ ID NO: 1252 is operably linked via its C-terminus to the N-terminus of the VL domain of the sequence of SEQ ID NO: 1253,

(vi) the VL domain of the sequence of SEQ ID NO: 1253 is operably linked via its C-terminus to the N-terminus of the VH domain of the sequence of SEQ ID NO: 1252, or

(vii) the VH domain of the sequence of SEQ ID NO: 800 is operably linked via its C-terminus to the N-terminus of the VL domain of the sequence of SEQ ID NO: 1012, or

(viii) the VL domain of the sequence of SEQ ID NO: 1012 is operably linked via its C-terminus to the N-terminus of the VH domain of the sequence of SEQ ID NO: 800.

30. The recombinant nucleic acid molecule of claim 28 or 29, wherein the scFv comprises a linker sequence of SEQ ID NO: 1237.

31. The recombinant nucleic acid molecule of claim 29 or 30, wherein the VH domain and the VL domain are operably linked via a linker sequence of SEQ ID NO: 1237, and wherein the VH and VL domains are the sequences of SEQ ID NOs: 1248 and 1249,

respectively; the sequences of SEQ ID NOs: 1250 and 1251, respectively; or the sequences of SEQ ID NOs: 1252 and 1253, respectively.

32. The recombinant nucleic acid molecule of any one of claims 24-31, wherein the antigen binding domain specifically binds to a second epitope is within the amino acid sequence ASRHHPTTLAVGICSPASRSISL (SEQ ID NO:1231).
33. The recombinant nucleic acid molecule of claim 32, wherein the scFv comprises a VH domain that comprises a CDRH1 of SEQ ID NO: 853, a CDRH2 of SEQ ID NO: 906, and a CDRH3 of SEQ ID NO: 959, and a VL domain that comprises a CDRL1 of SEQ ID NO: 1065, a CDRL2 of SEQ ID NO: 1118, and a CDRL3 of SEQ ID NO: 1171.
34. The recombinant nucleic acid molecule of any one of claims 24-33, wherein the scFv comprises a VH domain having at least 90%, 95%, or 100% sequence identity to SEQ ID NO: 800, and/or a VL domain having at least 90%, 95%, or 100% sequence identity to SEQ ID NO: 1012.
35. The recombinant nucleic acid molecule of any one of claims 24-34, wherein the scFv comprises a linker sequence of SEQ ID NO: 782.
36. The recombinant nucleic acid molecule of any one of claims 1-34, wherein:
    (i) a T cell expressing the TFP inhibits tumor growth when expressed in a T cell;
    (ii) a T cell expressing the TFP has increased fratricide relative to a TFP having a different antigen binding domain; or
    (iii) a T cell expressing the TFP has decreased fratricide relative to a TFP having a different antigen binding domain.
37. The recombinant nucleic acid molecule of claim 1, wherein the recombinant nucleic acid molecule encodes any one of the amino acid sequences selected from SEQ ID NOs: 1233, 1236, 1240, and 1264.
38. A recombinant nucleic acid molecule comprising a sequence encoding an antibody or a fragment thereof that specifically binds CD70, wherein the antibody or fragment thereof is the antigen binding domain according to any one of claims 1-37.
39. The recombinant nucleic acid molecule of any one of claims 1-38, further comprising a leader sequence, a promoter, a sequence encoding a poly(A) tail, and/or a 3’UTR sequence.
40. The recombinant nucleic acid molecule of any one of claims 1-39, wherein the nucleic acid is an *in vitro* transcribed nucleic acid.
41. A vector comprising the recombinant nucleic acid molecule of any one of claims 1-40.

42. A cell comprising the recombinant nucleic acid molecule of any one of claims 1-40, or the vector of claim 41.

43. A T cell comprising a recombinant nucleic acid molecule of any one of claims 1-40, wherein the recombinant nucleic acid molecule comprises a sequence encoding a T cell receptor (TCR) fusion protein (TFP), wherein the TFP comprises:

(a) a TCR subunit comprising:

(i) at least a portion of a TCR extracellular domain, and

(ii) a TCR transmembrane domain,

(iii) a TCR intracellular domain, and

(b) an antigen binding domain that specifically binds CD70; and

wherein the TCR subunit and the antigen binding domain are operatively linked.

44. The cell or T cell of claim 42 or 43, wherein the cell or T cell comprises a functional disruption of the endogenous CD70 gene or a functional disruption of the endogenous CIITA gene.

45. A pharmaceutical composition comprising the cell or T cell of any one of claims 42-44 and a pharmaceutically acceptable carrier for use in therapy.

46. A method of producing the cell or T cell of any one of claims 42-44, the method comprising:

(A) (i) disrupting an endogenous CD70 gene, thereby producing a cell or T cell containing a functional disruption of an endogenous CD70 gene; and

(ii) transducing the cell or T cell containing the functional disruption of the endogenous CD70 gene with the recombinant nucleic acid of any one of claims 1-40, or the vector of claim 41;

(B) (i) disrupting an endogenous CIITA gene, thereby producing a cell or T cell containing a functional disruption of an endogenous CIITA gene; and

(ii) transducing the cell or T cell containing the functional disruption of the endogenous CD70 gene or the functional disruption of the endogenous CIITA gene with the recombinant nucleic acid of any one of claims 1-40, or the vector of claim 41;

(C) (i) transducing a cell or T cell with the recombinant nucleic acid of any one of claims 1-40, or the vector of claim 41; and

(ii) contacting the cell or T cell with an anti-CD70 antibody that binds to CD70 on the cell surface; or

(D) transducing a cell or T cell with the recombinant nucleic acid of any one of claims 1-40, or the vector of claim 41, and a sequence encoding a fusion protein comprising an anti-CD70 antibody domain and an ER retention domain.

47. Use of a pharmaceutical composition comprising the cell or T cell of any one of claims 42-44 and a pharmaceutically acceptable carrier, or the pharmaceutical composition of claim 45 in the manufacture of a medicament for treating cancer in a subject in need thereof, wherein an effective amount of the pharmaceutical composition is to be administered to the subject, thereby treating the cancer in the subject.