CLAIMS

What is claimed is:

1. A bispecific molecule comprising:

(a) a first antigen binding domain that binds a p53 R175H mutant peptide presented in a peptide-human leukocyte antigen (HLA) complex (R175H -pHLA) on a target cell, wherein the p53 R175H mutant peptide comprises an amino acid sequence as set forth in SEQ ID NO:7 and the HLA is HLA-A2;

wherein the first antigen binding domain comprises a heavy chain variable region (R175H-pHLA VH) and a light chain variable region (R175H-pHLA VL); wherein the R175H-pHLA VH comprises a HCDR1, HCDR2, and an HCDR2, and wherein the HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO:40, the HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO:42 or SEQ ID NO:47, and the HCDR3 comprises an amino acid sequence as set forth in SEQ ID NO:44; and

wherein the R175H-pHLA VL comprises a LCDR1, a LCDR2, and a LCDR3 and wherein the LCDR1 comprises an amino acid sequence as set forth in SEQ ID NO:30, the LCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 32 or SEQ ID NO:37, and the LCDR3 comprises an amino sequence as set forth in SEQ ID NO:34; and

(b) a second antigen binding domain that binds CD3, wherein the second antigen binding domain comprises a heavy chain variable region (CD3 VH) and a light chain variable region (CD3 VL),

wherein the bispecific molecule further comprises one or more Fc domain modifications, and

wherein the modifications result in (i) an improved half-life, (ii) a reduced risk of complement-dependent cytotoxicity (CDC), (iii) an improved manufacturability, and/or (iv) an improved effector cell engagement of the bispecific molecule.

2. The bispecific molecule of claim 1, wherein the first antigen binding domain does not bind the p53 R175H mutant peptide not presented in an HLA complex or a complex that includes a wild-type version of the p53 peptide.

3. The bispecific molecule of claim 1, comprising

(i) an R175H-pHLA V_H having at least 95% identity to the amino acid sequence of SEQ ID NO:38 and an R175-pHLA V_L having at least 95% identity to the amino acid sequence of SEQ ID NO:28;

(ii) an R175H-pHLA VH having at least 95% identity to the amino acid sequence of SEQ ID NO:46 and an R175-pHLA VL having at least 95% identity to the amino acid sequence of SEQ ID NO:28;

(iii) an R175H-pHLA VH having at least 95% identity to the amino acid sequence of SEQ ID NO:38 and an R175-pHLA VL having at least 95% identity to the amino acid sequence of SEQ ID NO:36; or

(iv) an R175H-pHLA VH having at least 95% identity to the amino acid sequence of SEQ ID NO:46 and an R175-pHLA VL having at least 95% identity to the amino acid sequence of SEQ ID NO:36.

4. The bispecific molecule of claim 1 wherein the CD3 V_H comprises three CDRs having amino acid sequences as set forth in SEQ ID NOs: 61-63; and the CD3 V_L comprises three CDRs having amino acid sequences as set forth in SEQ ID NOs: 57-59.

5. The bispecific molecule of claim 4, wherein the CD3 V_H comprises an amino acid sequence that shares at least 95% identity to SEQ ID NO: 60; and the CD3 V_L comprises an amino acid sequence that shares at least 95% identity to SEQ ID NO: 56.

6. The bispecific molecule of any one of claims 1-5, wherein the bispecific molecule has one or more characteristics selected from the following:

(a) a low target density of less than 2000 targets per cell, less than 1500 targets per cell,

less than 1000 targets per cell, less than 500 targets per cell, less than 100 targets per cell, less than 50 targets per cell, less than 10 targets per cell, or about 1 target per cell;

(b) a binding specificity as measured by half maximal effective concentration (EC50) of 0.001-3000 nM;

(c) low or no cross-reactivity to proteins other than the protein from which the mutant peptide is derived;

(d) a half-life of about 21 days, about 14 days, about 7 days, or about 4 days;

(e) a productibility of at least about 1 g/L, at least about 2 g/L, at least about 3 g/L, at least about 4 g/L, at least about 5 g/L, at least about 10 g/L, at least about 15 g/L, at least about 20 g/L, at least about 25 g/L, or at least about 30 g/L from a cell culture; and

(f) a stability at room temperature of 1-12 weeks.

7. The bispecific molecule of any one of the preceding claims, wherein the bispecific molecule, when engaged with the R175H-pHLA on the target cell and CD3 on the T cell, forms a connection between the target cell and the T cell of about 14 nM in length as measured from the target cell membrane to the T cell membrane.

8. The bispecific molecule of any one of claims 1-7, wherein the domain orientation modification and linker modification comprise the bispecific molecule being configured as a single-chain diabody (scDb) in a configuration of, from N- to C-terminus order:

(i) Mut-pHLA V_L – L1 – CD3 V_H – L2 – CD3 V_L – L3 – Mut-pHLA V_H;

(ii) Mut-pHLA V_H – L1 – CD3 V_L – L2 – CD3 V_H – L3 – Mut-pHLA V_L;

(iii) Mut-pHLA V_L – L1 – CD3 V_L – L2 – CD3 V_H- – L3 – Mut-pHLA V_H;

(iv) Mut-pHLA V_H – L1 – CD3 V_H – L2 – CD3 V_L – L3 – Mut-pHLA V_L;

(v) CD3 V_L – L1 – Mut-pHLA V_H – L2 – Mut-pHLA V_L – L3 – CD3 V_H;

(vi) CD3 V_H – L1 – Mut-pHLA V_L – L2 – Mut-pHLA V_H – L3 – CD3 V_L;

(vii) CD3 V_L – L1 – Mut-pHLA V_L – L2 – Mut-pHLA V_H – L3 – CD3 V_H; or

(viii) CD3 V_H – L1 – Mut-pHLA V_H – L2 – Mut-pHLA V_L – L3 – CD3 V_L,

wherein L1, L2, and L3 are linkers.

9. The bispecific molecule of claim 8, wherein the bispecific molecule is configured in a configuration of, from N- to C-terminus order, CD3 V_L – L1 – Mut-pHLA V_H – L2 – Mut-pHLA V_L – L3 – CD3 V_H.

10. The bispecific molecule of claim 9, wherein L1 is 1-10 amino acids in length, L2 is 5-40 amino acids in length, L3 is 1-10 amino acids in length.

11. The bispecific molecule of claim 9 or 10, wherein the bispecific molecule further comprises one or more Fc domain modifications, wherein the Fc domain modifications comprise two chains of an Fc sequence of immunoglobulin G4 (IgG4) isotype, each peptide chain comprising a hinge, a CH2 domain, and a CH3 domain, and wherein one chain of the Fc sequence comprises S228P, L235E, T366S, L368A, and Y407V mutations, and the other chain of the Fc sequence comprises S228P, L235E, L336W, and H435R mutations in reference to a consensus full-length IgG4 sequence.

12. The bispecific molecule of claim 11, wherein one chain of the Fc sequence comprises an amino acid sequence as set forth in SEQ ID NO: 65, and the other chain of the Fc sequence comprises an amino acid sequence as set forth in SEQ ID NO: 66.

13. The bispecific molecule of claim 11 or claim 12, wherein one chain of the Fc sequence is connected to the first and/or second antigen binding domain at the N-terminal end of the CD3 V_L or the C-terminal end of the CD3 V_H optionally through a linker L4, wherein L4 is about 1-40 amino acids in length.

14. A bispecific molecule comprising:
    (a) a first antigen binding domain that binds a tumor-specific mutant peptide derived from p53 R175H presented in a peptide-human leukocyte antigen (HLA) complex (R175H-pHLA) on a target cell;
    (b) a second antigen binding domain that binds CD3 on a T cell; and
    (c) an Fc domain of an immunoglobulin G4 (IgG4) isotype comprising a first chain of an Fc sequence comprising a hinge, a CH2 domain, and a CH3 domain and a second

chain of an Fc sequence comprising a hinge, a CH2 domain, and a CH3 domain,

wherein the mutant peptide comprises or consists of an amino acid sequence of HMTEVVRHC (SEQ ID NO: 7) and wherein the HLA is HLA-A02;

wherein the first antigen binding domain comprises a heavy chain variable region (R175H-pHLA VH) and a light chain variable region (R175H-pHLA VL);

wherein the R175H-pHLA VH comprises a HCDR1, HCDR2, and an HCDR2, and wherein the HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO:40, the HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO:42 or SEQ ID NO:47, and the HCDR3 comprises an amino acid sequence as set forth in SEQ ID NO:44; and

wherein the R175H-pHLA VL comprises a LCDR1, a LCDR2, and a LCDR3 and wherein the LCDR1 comprises an amino acid sequence as set forth in SEQ ID NO:30, the LCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 32 or SEQ ID NO:37, and the LCDR3 comprises an amino sequence as set forth in SEQ ID NO:34.

15. The bispecific molecule of claim 14, wherein the HLA is HLA-A*02.01, HLA-A*02.06, or HLA-A*02.11.

16. The bispecific molecule of claim 14 or claim 15, wherein the bispecific molecule has one or more characteristics selected from the following:

(a) a low target density of less than 2000 targets per cell, less than 1500 targets per cell, less than 1000 targets per cell, less than 500 targets per cell, less than 100 targets per cell, less than 50 targets per cell, less than 10 targets per cell, or about 1 target per cell;

(b) a binding specificity as measured by half maximal effective concentration (EC50) of 0.001-3000 nM;

(c) low or no cross-reactivity to proteins other than the protein from which the mutant peptide is derived;

(d) a half-life of about 21 days, about 14 days, about 7 days, or about 4 days;

(e) a productibility of at least about 1 g/L, at least about 2 g/L, at least about 3 g/L, at

least about 4 g/L, at least about 5 g/L, at least about 10 g/L, at least about 15 g/L, at least about 20 g/L, at least about 25 g/L, or at least about 30 g/L from a cell culture; and

(f) a stability at room temperature of 1-12 weeks.

17. The bispecific molecule of claim 14, claim 15 or claim 16, wherein the bispecific molecule, when engaged with the R175H-pHLA on the target cell and CD3 on the T cell, forms a connection between the target cell and the T cell of about 14 nM in length as measured from the target cell membrane to the T cell membrane.

18. The bispecific molecule of any one of claims 14-17, wherein:

the second antigen binding domain comprises a heavy chain variable region (CD3 V_H) and light chain variable region (CD3 V_L), and

the bispecific molecule comprises, from N- to C-terminus order, CD3 V_L – L1 – R175H-pHLA V_H – L2 – R175H-pHLA V_L – L3 – CD3 V_H, wherein L1 is 1-10 amino acids in length, L2 is 5-40 amino acids in length, and L3 is 1-10 amino acids in length.

19. The bispecific molecule of claim 14, wherein the R175H-pHLA V_H and the R175H-pHLA V_L comprise:

(v) an R175H-pHLA V_H having at least 95% identity to the amino acid sequence of SEQ ID NO:38 and an R175-pHLA V_L having at least 95% identity to the amino acid sequence of SEQ ID NO:28;

(vi) an R175H-pHLA V_H having at least 95% identity to the amino acid sequence of SEQ ID NO:46 and an R175-pHLA V_L having at least 95% identity to the amino acid sequence of SEQ ID NO:28;

(vii) an R175H-pHLA V_H having at least 95% identity to the amino acid sequence of SEQ ID NO:38 and an R175-pHLA V_L having at least 95% identity to the amino acid sequence of SEQ ID NO:36; or

(viii) an R175H-pHLA V_H having at least 95% identity to the amino acid sequence of SEQ ID NO:46 and an R175-pHLA V_L having at least 95% identity to the amino acid sequence of SEQ ID NO:36.

20. The bispecific molecule of claim 18 or claim 19, wherein the CD3 V_H comprises three CDRs having amino acid sequences as set forth in SEQ ID NOs: 61-63; and the CD3 V_L comprises three CDRs having amino acid sequences as set forth in SEQ ID NOs: 57-59 and wherein the CD3 VH comprises an amino acid sequence that shares at least 95% identity to SEQ ID NO: 60; and the CD3 VL comprises an amino acid sequence that shares about 85%, at least 95% identity to SEQ ID NO: 56.

21. The bispecific molecule of claim 20, wherein the CD3 V_H comprises an amino acid sequence as set forth in SEQ ID NO: 60; and the CD3 V_L comprises an amino acid sequence as set forth in SEQ ID NO: 56.

22. The bispecific molecule of any one of claims 14-21, wherein the first chain of the Fc sequence comprises an amino acid sequence as set forth in SEQ ID NO: 65; and the second chain of the Fc sequence comprises an amino acid sequence as set forth in SEQ ID NO: 66.

23. The bispecific molecule of any one of claims 14-22, wherein the first or second chain of the Fc sequence is connected to the first and/or second antigen binding domain at the N-terminal end of the CD3 V_L or the C-terminal end of the CD3 V_H through a linker L4, wherein L4 is about 1-40 amino acids in length.

24. The bispecific molecule of claim 23, wherein the first or second chain of the Fc sequence is connected to the C-terminal end of the CD3 V_H through L4.

25. The bispecific molecule of claim 24, wherein the bispecific molecule comprises an amino acid sequence that shares at least about 95% identity to SEQ ID NO: 78.

26. A bispecific molecule comprising:

(a) a first polypeptide comprising a first chain of an Fc sequence comprising an amino acid sequence that shares at least about 80%, at least about 85%, at least about 90%, at least about 95%, or 100% identity to SEQ ID NO: 65; and

(b) a second polypeptide comprising, from N- to C-terminus order:

(i) a light chain variable region derived from a first antigen binding domain that binds CD3 (CD3 V_L);

(ii) a linker L1 of about 1-10 amino acids in length;

(iii) a heavy chain variable region derived from a second antigen binding domain that binds a p53 R175H mutant peptide having an amino acid sequence of HMTEVVRHC (SEQ ID NO: 7) presented in a peptide-human leukocyte antigen (HLA) complex (R175H-PHLA) (R175H-pHLA V_H);

(iv) a linker L2 of about 5-40 amino acids in length;

(v) a light chain variable region derived from the second antigen binding domain that binds R175H-pHLA (R175H -pHLA V_L);

(vi) a linker L3 of about 1-10 amino acids in length;

(vii) a heavy chain variable region derived from the first antigen binding domain that binds CD3 (CD3 V_H);

(viii) optionally a linker L4 of about 1-40 amino acids in length; and

(ix) a second chain of an Fc sequence of immunoglobulin G4 (IgG4) isotype comprising an amino acid sequence that shares at least about 80%, at least about 85%, at least about 90%, at least about 95%, or 100% identity to SEQ ID NO: 66.

27. The bispecific molecule of claim 26, wherein:

the R175H-pHLA V_H comprises

(i) three CDRs having amino acid sequences selected from SEQ ID NOs: 40, 42, and 44; or

(ii) three CDRs having amino acid sequences selected from SEQ ID NOs: 40, 47, and 44, and/or

the R175H-pHLA V_L comprises

(i) three CDRs having amino acid sequences selected from SEQ ID NOs: 30, 32, and 34; or

(ii) three CDRs having amino acid sequences selected from SEQ ID NOs: 30, 37, and 34.

28. The bispecific molecule of claim 27, wherein the R175H-pHLA VH comprises an amino acid sequence of SEQ ID NO: 38 or SEQ ID NO: 46; and/or the R175H-pHLA VL comprises an amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 36.

29. The bispecific molecule of any one of claims 26-28, wherein the CD3 V_H comprises one, two, or three CDRs having amino acid sequences selected from SEQ ID NOs: 61-63; and/or the CD3 V_L comprises one, two, or three CDRs having amino acid sequences selected from SEQ ID NOs: 57-59.

30. The bispecific molecule of claim 29, wherein the CD3 V_H comprises an amino acid sequence that shares at least 95% or 100% identity to SEQ ID NO: 60; and/or the CD3 V_L comprises an amino acid sequence that shares at least 95% or 100% identity to SEQ ID NO: 56.

31. The bispecific molecule of any one of claims 26-30, wherein the first polypeptide comprises an amino acid sequence as set forth in_SEQ ID NO: 65; and the second polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 78.

32. A pharmaceutical composition comprising the bispecific molecule of any one of the preceding claims and a pharmaceutically acceptable carrier, excipient, preservative, or combination thereof.

33. The bispecific molecule of any one of claims 1-31 or the pharmaceutical composition of claim 32, for use in diagnosing, treating, or preventing a disease in a subject in need thereof.

34. Use of the bispecific molecule of any one of claims 1-31 or the pharmaceutical composition

of claim 32, in the manufacture of a medicament for diagnosing, treating, or preventing a disease in a subject in need thereof.

35. The bispecific molecule or the pharmaceutical composition for use of claim 33, or the use of claim 34, wherein the subject in need thereof has a disease-associated protein comprising a p53 R175H mutant peptide.

36. The bispecific molecule or the pharmaceutical composition for use of claim 33 or 35, or the use of claim 34 or 35, wherein the disease is cancer.

37. The bispecific molecule or the pharmaceutical composition for use of claim 36, or the use of claim 36, wherein the cancer is a solid cancer.

38. The bispecific molecule or the pharmaceutical composition for use of claim 37, or the use of claim 37, wherein the solid cancer is selected from the group consisting of pancreatic cancer, glioma, glioblastoma, astrocytoma, colorectal cancer, thyroid cancer, gastric cancer, ovarian cancer, melanoma, endometrial cancer, lung cancer, renal cancer, cervical cancer, prostate cancer, breast cancer, urothelial cancer, testicular cancer, head and neck cancer, biliary tract cancer, liver cancer, and esophageal cancer.