

epitope is thought to contribute to high affinity and bioactivity of the TCE molecule. Binding to this unique epitope may also contribute to an acceptable pharmacokinetic profile.

## SUMMARY

**[0010]** The present disclosure provides a T cell engager (TCE) molecule, which may be referred to as an scFab-containing TCE molecule, comprising (i) an scFab that binds a tumor antigen, wherein the scFab comprises a first heavy chain variable region (scFab VH), a CH1 domain, a first light chain variable region (scFab VL), and a C $\kappa$  or C $\lambda$  domain, and (ii) an scFv that binds CD3, comprising a second VL and a second VH, wherein the TCE molecule is a single chain. In some examples, the scFab comprises a C-terminus portion that is connected by a linker to an N-terminal portion of the scFv. In some example the TCE molecule further comprises an scFc. In some examples, the scFc comprises an N-terminus portion that is connected by a linker to the C-terminal portion of the scFv. In a particular example, the scFv binds human CD3. In some examples, the tumor antigen is CCR8.

**[0011]** In some examples, the scFab of a TCE molecule of the present disclosure has an orientation in the following order, from N-terminus to C-terminus, VH, CH1, VL, and either C $\kappa$  or C $\lambda$ . In other examples, the scFab has an orientation in the following order, from N-terminus to C-terminus, VL, either C $\kappa$  or C $\lambda$ , VH, and CH1. In some examples, the scFab comprises a linker that connects the CH1 and VL, wherein the linker is (G4S)<sub>6</sub>, (G4S)<sub>7</sub>, (G4S)<sub>8</sub>, (G4Q)<sub>6</sub>, (G4Q)<sub>7</sub>, or (G4Q)<sub>8</sub>. In some examples, the scFab comprises a linker that connects C $\kappa$  or C $\lambda$  and VH, wherein the linker is (G4S)<sub>6</sub>, (G4S)<sub>7</sub>, (G4S)<sub>8</sub>, (G4Q)<sub>6</sub>, (G4Q)<sub>7</sub>, or (G4Q)<sub>8</sub>. In some examples, the scFab contains a natural cysteine clamp between the heavy and light chain constant domains. In some examples, the TCE molecule comprises an engineered cysteine clamp in the scFab between residue 44 in the VH domain and residue 100 in the VL domain (Kabat numbering). In some examples, the scFab contains a natural cysteine clamp between the heavy and light chain constant domains and an engineered cysteine clamp between residue 44 in the VH domain and residue 100 in the VL domain. In some examples, the TCE molecule CH1, C $\kappa$  and/or C $\lambda$  domains are IgG, IgM, IgA, IgD, or IgE. In a particular example, the domains are IgG. In a more particular example, the domains are IgG1. In some examples, the domains are human. In a particular example, the domains are human IgG1.

**[0012]** The present disclosure provides a single-chain TCE molecule having the following orientation, from N-terminus to C-terminus: scFab (VH, CH1, linker, VL, either C $\kappa$  or C $\lambda$ ), linker, scFv (VH, linker, VL). In an example, the TCE molecule further comprises a scFc, and has the following orientation: scFab (VH, CH1, linker, VL, either C $\kappa$  or C $\lambda$ ), linker, scFv (VH, linker, VL), linker, Fc1 (hinge, CH2, CH3), linker, Fc2 (hinge, CH2, CH3).

**[0013]** The present disclosure provides a single-chain TCE molecule having the following orientation, from N-terminus to C-terminus: scFab (VL, either C $\kappa$  or C $\lambda$ , linker, VH, CH1), linker, scFv (VH, linker, VL). In an example, the TCE molecule further comprises a scFc, and has the following orientation: scFab (VL, either C $\kappa$  or C $\lambda$ , linker, VH, CH1), linker, scFv (VH, linker, VL), linker, Fc1 (hinge, CH2, CH3), linker, Fc2 (hinge, CH2, CH3).

**[0014]** The present disclosure provides a single-chain TCE molecule having the following orientation: scFv that binds CCR8 (VH, linker, VL), linker, scFv that binds CD3 (VH, linker, VL). In an example, the TCE molecule further comprises a scFc, and has the following orientation: scFv that binds CCR8 (VH, linker, VL), linker, scFv that binds CD3 (VH, linker, VL)-Linker- Fc1 (hinge, CH2, CH3), linker, Fc2 (hinge, CH2, CH3).

**[0015]** The present disclosure also provides a TCE molecule having the following orientation from N-terminus to C-terminus: scFv that binds CCR8 (VH, linker, VL)-Linker- scFv that binds CD3 (VH, linker, VL)-Linker-Fc1 (CH2-CH3) -Linker-Fc2 (CH2-CH3). In an example, the TCE molecule binds CCR8 and CD3. The present disclosure provides a TCE molecule having the following orientation from N-terminus to C-terminus: scFv that binds CCR8 (VL-Linker-VH)-Linker-scFv that binds CD3 (VH-Linker-VL)-Linker-Fc1 (CH2-CH3) -Linker-Fc2 (CH2-CH3). In an example, the TCE molecule binds CCR8 and CD3.

**[0016]** The present disclosure provides a single-chain TCE molecule having a scFab-scFv-scFc format. In some examples, the TCE molecule comprises the following orientation: VH-CH1-Linker-VL-C $\kappa$ /C $\lambda$ -Linker-VH-Linker-VL-Linker-VH-Linker-VL-Linker-Fc1-Linker-Fc2. In some examples, the TCE molecule comprises the following orientation: VL-C $\kappa$ /C $\lambda$ -Linker-VH-CH1-Linker-VH-Linker-VL-Linker-VH-Linker-VL-Linker-Fc1-Linker-Fc2. In some examples, the TCE molecule comprises the following orientation: VL-C $\kappa$ /C $\lambda$ -Linker-VH-CH1-Linker-VL-Linker-VH-Linker-VH-Linker-VL-Linker-Fc1-Linker-Fc2. In some examples, the TCE molecule comprises the following orientation: VH-CH1-Linker-VL-C $\kappa$ /C $\lambda$ -Linker-VL-Linker-VH-Linker-VH-Linker-VL-Linker-Fc1-Linker-Fc2. In some examples, the TCE molecule comprises C $\kappa$ . In some examples, the TCE molecule comprises C $\lambda$ .

**[0017]** The present disclosure also provides a single-chain TCE molecule having an scFab-scFab-scFv-scFc format. In some examples, the TCE molecule comprises the following orientation: VH-CH1-Linker-VL- C $\kappa$ /C $\lambda$  -Linker- VH-CH1-Linker-VL- C $\kappa$ /C $\lambda$  -Linker-VH -Linker-VL-Linker-Fc1-Linker-Fc2. In some examples, the TCE molecule comprises the following orientation: VL-C $\kappa$ /C $\lambda$ -Linker-VH-CH1-Linker- VH-CH1-Linker-VL- C $\kappa$ /C $\lambda$  -Linker-VH -Linker-VL-Linker-Fc1-Linker-Fc2. In some examples, the TCE molecule comprises the following orientation: VH-CH1-Linker-VL- C $\kappa$ /C $\lambda$  -Linker- VL-C $\kappa$ /C $\lambda$ -Linker-VH-CH1-Linker-VH -Linker-VL-Linker-Fc1-Linker-Fc2. In some examples, the TCE molecule comprises the following orientation: VL-C $\kappa$ /C $\lambda$ -Linker-VH-CH1-Linker- VL-C $\kappa$ /C $\lambda$ -Linker-VH-CH1-Linker-VH -Linker-VL-Linker-Fc1-Linker-Fc2. In some examples, the TCE molecule comprises C $\kappa$ . In some examples, the TCE molecule comprises C $\lambda$ . In some examples, the TCE molecule comprises C $\kappa$  and C $\lambda$ .

**[0018]** In an example, the scFab VH and CH1 of an scFab-containing TCE molecule of the present disclosure comprise an amino acid sequence given by SEQ ID NO: 12, SEQ ID NO: 28, SEQ ID NO: 44, SEQ ID NO: 60, SEQ ID NO: 76, SEQ ID NO: 92, SEQ ID NO: 108, or SEQ ID NO: 124. In an example, the TCE molecule of the present disclosure comprises a C $\kappa$ . In a particular example, the scFab VL and C $\kappa$  of an scFab-containing TCE molecule of the present disclosure comprise an amino acid sequence given by SEQ ID NO: 13, SEQ ID NO: 29, SEQ ID NO: 45, SEQ ID NO: 61, SEQ ID NO: 77, SEQ ID NO: 93, SEQ ID NO: 109, or SEQ ID NO: 125. In another particular example, the TCE molecule comprises an amino acid sequence given by SEQ ID NO: 14, SEQ ID NO: 30, SEQ ID NO: 46, SEQ ID NO: 62, SEQ ID NO: 78, SEQ ID NO: 94, SEQ ID NO: 110, or SEQ ID NO: 126. In another particular example, the TCE molecule comprises an amino acid sequence given by SEQ ID NO: 15, SEQ ID NO: 31, SEQ ID NO: 47, SEQ ID NO: 63, SEQ ID NO: 79, SEQ ID NO: 95, SEQ ID NO: 111, or SEQ ID NO: 127. In another particular example, the TCE molecule comprises an amino acid sequence given by SEQ ID NO: 16, SEQ ID NO: 32, SEQ ID NO: 48, SEQ ID NO: 64, SEQ ID NO: 80, SEQ ID NO: 96, SEQ ID NO: 112, or SEQ ID NO: 128.

**[0019]** In another example, the scFab VH and CH1 or scFab VL and C $\kappa$  comprise a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of a scFab VH and CH1 or scFab VL and C $\kappa$  sequence listed herein. In another example, the TCE molecule comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of a TCE molecule sequence listed herein.

**[0020]** The present disclosure also provides a TCE molecule comprising (i) a first scFv that binds CCR8, wherein the first scFv comprises a first VH region (CCR8 scFv VH) and a first VL region (CCR8 scFv VL), and (ii) a second scFv that binds CD3, wherein the second scFv comprises a second VH region and a second VL region. A molecule having this structure and that binds CCR8 and CD3 may be referred to as a CCR8 TCE molecule. In a preferred example, the CCR8 TCE molecule is a single chain.

**[0021]** In an example, the CCR8 TCE molecule scFv VH comprises an amino acid sequence given by SEQ ID NO: 7, SEQ ID NO: 23, SEQ ID NO: 39, SEQ ID NO: 55, SEQ ID NO: 71, SEQ ID NO: 87, SEQ ID NO: 103, or SEQ ID NO: 119, and wherein the CCR8 scFv VL comprises an amino acid sequence given by SEQ ID NO: 8, SEQ ID NO: 24, SEQ ID NO: 40, SEQ ID NO: 56, SEQ ID NO: 72, SEQ ID NO: 88, SEQ ID NO: 104, or SEQ ID NO: 120. In another example, the first scFv comprises an amino acid sequence given by SEQ ID NO: 9, 25, 41, 57, 73, 89, 105, or 121. In another example, the TCE molecule comprises an amino acid sequence given by SEQ ID NO: 10, SEQ ID NO: 26, SEQ ID NO: 42, SEQ ID NO: 58, SEQ ID NO: 74, SEQ ID NO: 90, SEQ ID NO: 106, or SEQ ID NO: 122. In another example, the TCE molecule further comprises an scFc, wherein the TCE molecule comprises an amino acid sequence given by SEQ ID NO: 11, SEQ ID NO: 27, SEQ ID NO: 59, SEQ ID NO: 75, SEQ ID NO: 91, SEQ ID NO: 107, or SEQ ID NO: 123. In another example, the CCR8 scFv VH comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to CCR8 scFv VH sequences listed herein. In another example, the CCR8 scFv VL comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the CCR8 scFv VL sequences listed herein.

**[0022]** In another example, the first scFv (that binds CCR8) comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the first scFv sequences listed herein.

**[0023]** In another example, a CCR8 TCE molecule of the present disclosure comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to CCR8 TCE molecule sequences listed herein.

**[0024]** In some examples, the first VH (scFab VH and/or CCR8 scFv VH) of a TCE molecule of the present disclosure comprises HCDR1, HCDR2, HCDR3, and the first VL (scFab VL and/or CCR8 scFv VL) comprises LCDR1, LCDR2, and LCDR3, and wherein:

- a) HCDR1 comprises an amino acid sequence given by SEQ ID NO: 1, SEQ ID NO: 17, SEQ ID NO: 33, SEQ ID NO: 49, SEQ ID NO: 65, SEQ ID NO: 81, SEQ ID NO: 97, or SEQ ID NO: 113;
- b) HCDR2 comprises an amino acid sequence given by SEQ ID NO: 2, SEQ ID NO: 18, SEQ ID NO: 34, SEQ ID NO: 50, SEQ ID NO: 66, or SEQ ID NO: 82;
- c) HCDR3 comprises an amino acid sequence given by SEQ ID NO: 3, SEQ ID NO: 19, SEQ ID NO: 35, SEQ ID NO: 51, SEQ ID NO: 67, or SEQ ID NO: 83;
- d) LCDR1 comprises an amino acid sequence given by SEQ ID NO: 4, SEQ ID NO: 20, SEQ ID NO: 36, SEQ ID NO: 52, SEQ ID NO: 68, or SEQ ID NO: 84;
- e) LCDR2 comprises an amino acid sequence given by SEQ ID NO: 5, SEQ ID NO: 21, SEQ ID NO: 37, SEQ ID NO: 53, SEQ ID NO: 69, or SEQ ID NO: 85, and
- f) LCDR3 comprises an amino acid sequence given by SEQ ID NO: 6, SEQ ID NO: 22, SEQ ID NO: 38, SEQ ID NO: 54, SEQ ID NO: 70, or SEQ ID NO: 86.

**[0025]** In a particular example, HCDR1 comprises an amino acid sequence given by SEQ ID NO: 1, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 2, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 3, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 4, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 5, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 6.

**[0026]** In another particular example, HCDR1 comprises an amino acid sequence given by SEQ ID NO: 17, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 18, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 19, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 20, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 21, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 22.

**[0027]** In another particular example, HCDR1 comprises an amino acid sequence given by SEQ ID NO: 33, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 34, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 35, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 36, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 37, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 38.

**[0028]** In yet another particular example, HCDR1 comprises an amino acid sequence given by SEQ ID NO: 49, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 50, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 51, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 52, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 53, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 54.

**[0029]** In another particular example, HCDR1 comprises an amino acid sequence given by SEQ ID NO: 65, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 66, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 67, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 68, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 69, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 70.

**[0030]** In another particular example, HCDR1 comprises an amino acid sequence given by SEQ ID NO: 81, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 82, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 83, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 84, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 85, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 86.

**[0031]** In yet another particular example, HCDR1 comprises an amino acid sequence given by SEQ ID NO: 97, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 98, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 99, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 100, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 101, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 102.

**[0032]** In another particular example, HCDR1 comprises an amino acid sequence given by SEQ ID NO: 113, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 114, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 115, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 116 or SEQ ID NO: 336 (KSSQSVLYSSNNX1NYLA, wherein X1 is K or R), LCDR2 comprises an amino acid sequence given by SEQ ID NO: 117, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 118.

**[0033]** The present disclosure provides a TCE molecule comprising an orientation, from N-terminus to C-terminus, of an scFv that binds CCR8 (VH, linker, VL), linker, scFv that binds CD3 (VH, linker, VL), wherein the scFv that binds CCR8 comprises CDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising amino acid residues given by SEQ ID NO: 217, SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, and SEQ ID NO: 222, respectively. In an example, the scFv that binds CCR8 comprises a VH and VL given by SEQ ID NO: 223 and SEQ ID NO: 224, respectively. In a particular example, the scFv that binds CCR8 comprises amino acid residues given by SEQ ID NO: 225. In an example, the TCE molecule comprises G4S linkers. In an example, the TCE molecule comprises G4Q linkers. In an example, the CD3-binding scFv is I2E. In another example, the CD3-binding scFv is I2C. In another example, the TCE molecule comprises the amino sequence

given by SEQ ID NO: 226. In a further example, the TCE molecule comprises the amino acid sequence given by SEQ ID NO: 227. In some such examples, the TCE molecule is TCE 1.1. In a preferred example, the TCE molecule is a single chain. In some examples, the TCE molecule may have an orientation such that the VL is N-terminal to the VH.

**[0034]** The present disclosure provides a TCE molecule comprising an orientation, from N-terminus to C-terminus, of an scFv that binds CCR8 (VH, linker, VL), linker, scFv that binds CD3 (VH, linker, VL)-Linker- Fc1 (hinge, CH2, CH3), linker, Fc2 (hinge, CH2, CH3), wherein the scFv that binds CCR8 comprises CDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising amino acid residues given by SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, and SEQ ID NO: 233, respectively. In an example, the scFv that binds CCR8 comprises a VH and VL given by SEQ ID NO: 234 and SEQ ID NO: 235, respectively. In a particular example, the an scFv that binds CCR8 comprises amino acid residues given by SEQ ID NO: 236. In an example, the TCE molecule comprises G4S linkers. In an example, the TCE molecule comprises G4Q linkers. In an example, the CD3-binding scFv is I2E. In another example, the CD3-binding scFv is I2C. In another example, the TCE molecule comprises the amino sequence given by SEQ ID NO: 237. In a further example, the TCE molecule comprises the amino acid sequence given by SEQ ID NO: 238. In some such examples, the TCE molecule is TCE 1.2. In a preferred example, the TCE molecule is a single chain. In some examples, the TCE molecule may have an orientation such that the VL is N-terminal to the VH.

**[0035]** The present disclosure provides a TCE molecule comprising an orientation, from N-terminus to C-terminus, of a scFab that binds CCR8 (VH, CH1, linker, VL, either C $\kappa$  or C $\lambda$ ), linker, an scFv that binds CD3 (VH, linker, VL), wherein the scFab that binds CCR8 comprises CDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising amino acid residues given by SEQ ID NO: 239, SEQ ID NO: 240, SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, and SEQ ID NO: 244, respectively. In an example, the scFab comprises a VH and VL given by SEQ ID NO: 245 and SEQ ID NO: 246, respectively. In a particular example, the scFab comprises amino acid residues given by SEQ ID NO: 247. In an example, the TCE molecule comprises G4S linkers. In an example, the TCE molecule comprises G4Q linkers. In an example, the CD3-binding scFv is I2E. In another example, the CD3-binding scFv is I2C. In another example, the TCE molecule comprises the amino sequence given by SEQ ID NO: 248. In a further example, the TCE molecule comprises the amino acid sequence given by SEQ ID NO: 249. In some such examples, the TCE molecule is TCE 1.3. In a preferred

example, the TCE molecule is a single chain. In some examples, the TCE molecule may have an orientation such that the VL is N-terminal to the VH.

**[0036]** The present disclosure provides a TCE molecule comprising an orientation, from N-terminus to C-terminus, of scFab that binds CCR8 (VH, CH1, linker, VL, either C $\kappa$  or C $\lambda$ ), linker, scFv that binds CD3 (VH, linker, VL), linker, Fc1 (hinge, CH2, CH3), linker, Fc2 (hinge, CH2, CH3), wherein the scFab that binds CCR8 comprises CDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising amino acid residues given by SEQ ID NO: 250, SEQ ID NO: 251, SEQ ID NO: 252, SEQ ID NO: 253, SEQ ID NO: 254, and SEQ ID NO: 255, respectively. In an example, the scFab comprises a VH and VL given by SEQ ID NO: 256 and SEQ ID NO: 257, respectively. In a particular example, the scFab comprises amino acid residues given by SEQ ID NO: 258. In an example, the TCE molecule comprises G4S linkers. In an example, the TCE molecule comprises G4Q linkers. In an example, the CD3-binding scFv is I2E. In another example, the CD3-binding scFv is I2C. In another example, the TCE molecule comprises the amino sequence given by SEQ ID NO: 259. In a further example, the TCE molecule comprises the amino acid sequence given by SEQ ID NO: 260. In some such examples, the TCE molecule is TCE 1.4. In a preferred example, the TCE molecule is a single chain. In some examples, the TCE molecule may have an orientation such that the VL is N-terminal to the VH.

**[0037]** The present disclosure further provides a TCE molecule that binds to human CCR8, which comprises an HCDR1 amino acid sequence of SEQ ID NO: 787; an HCDR2 amino acid sequence of SEQ ID NO: 788; an HCDR3 amino acid sequence of SEQ ID NO: 789; an LCDR1 amino acid sequence of SEQ ID NO: 790; an LCDR2 amino acid sequence of SEQ ID NO: 791; and an LCDR3 amino acid sequence of SEQ ID NO: 792. In some examples, the TCE molecule comprises a VH amino acid sequence of SEQ ID NO: 965 and a VL amino acid sequence of SEQ ID NO: 966.

**[0038]** The present disclosure further provides a TCE molecule that binds to human CCR8, which comprises an HCDR1 amino acid sequence of SEQ ID NO: 787; an HCDR2 amino acid sequence of SEQ ID NO: 788; an HCDR3 amino acid sequence of SEQ ID NO: 789; an LCDR1 amino acid sequence of SEQ ID NO: 336, wherein X<sub>1</sub> is K or R; an LCDR2 amino acid sequence of SEQ ID NO: 791; and an LCDR3 amino acid sequence of SEQ ID NO: 792. In some examples, the TCE molecule comprises a VH amino acid sequence of SEQ ID NO: 965 and a VL amino acid sequence of SEQ ID NO: 342, wherein X<sub>1</sub> is K or R, X<sub>2</sub> is H or Q, and/or X<sub>3</sub> is S or P.

**[0039]** The present disclosure further provides a TCE molecule that binds to human CCR8, which comprises an HCDR1 amino acid sequence of SEQ ID NO: 805, an HCDR2 amino acid sequence of SEQ ID NO: 806, an HCDR3 amino acid sequence of SEQ ID NO: 807, an LCDR1 amino acid sequence of SEQ ID NO: 808, an LCDR2 amino acid sequence of SEQ ID NO: 809, and an LCDR3 amino acid sequence of SEQ ID NO: 810.

**[0040]** The present disclosure further provides a TCE molecule that binds to human CCR8, which comprises: (a) an HCDR1 amino acid sequence of  $X_1X_2GX_4H$ , (SEQ ID NO: 1181), wherein (i)  $X_1$  is N, S, D, G, T, or R, (ii)  $X_2$  is C, N, Y, S, or F, and (iii)  $X_4$  is M or F; (b) an HCDR2 amino acid sequence of SEQ ID NOs: 596, 602, 608, 614, 620, 626, 632, 638, 644, 650, 656, 662, 668, 674, 680, 686, 692, 698, 704, 710, 716, 722, 728, 734, 740, 746, 752, 758, 764, 770, 776, 782, 788, 794, 800, 806, 815, 821, 827, 833, 839, 845, 851, 857, 863, 869, 875, 881, 887, or 893, or a variant thereof that comprises 1-4 amino acid substitutions or is at least 90% identical to any one of the foregoing HCDR2 amino acid sequences; (c) an HCDR3 amino acid sequence of SEQ ID NOs: 597, 603, 609, 615, 621, 627, 633, 639, 645, 651, 657, 663, 669, 675, 681, 687, 693, 699, 705, 711, 717, 723, 729, 735, 741, 747, 753, 759, 765, 771, 777, 783, 795, 801, 807, 816, 822, 828, 834, 840, 846, 852, 858, 864, 870, 876, 882, 888, or 894 or a variant thereof that comprises 1-4 amino acid substitutions or is at least 90% identical to any one of the foregoing HCDR3 amino acid sequences; (d) an LCDR1 amino acid sequence of SEQ ID NOs: 598, 604, 610, 616, 622, 628, 634, 640, 646, 652, 658, 664, 670, 676, 682, 688, 694, 700, 706, 712, 718, 724, 730, 736, 742, 748, 754, 760, 766, 772, 778, 784, 796, 802, 808, 811, 817, 823, 829, 835, 841, 847, 853, 859, 865, 871, 877, 883, or 889 or a variant thereof that comprises 1-4 amino acid substitutions or is at least 90% identical to any one of the foregoing LCDR1 amino acid sequences; (e) an LCDR2 amino acid sequence of  $RX_2X_3X_4RPS$  (SEQ ID NO: 1182), wherein (i)  $X_2$  is A, N, D, S, or Q, (ii)  $X_3$  is S, T, N, I, F, or A, and (iii)  $X_4$  is N or V; and (f) an LCDR3 amino acid sequence of SEQ ID NOs: 600, 606, 612, 618, 624, 630, 636, 642, 648, 654, 660, 666, 672, 678, 684, 690, 696, 702, 708, 714, 720, 726, 732, 738, 744, 750, 756, 762, 768, 774, 780, 786, 798, 804, 810, 813, 819, 825, 831, 837, 843, 849, 855, 861, 867, 873, 879, 885, or 891 or a variant thereof that comprises 1-4 amino acid substitutions or is at least 90% identical to any one of the foregoing LCDR3 amino acid sequences. In some examples, the HCDR1 comprises an amino acid sequence of SEQ ID NOs: 595, 601, 607, 613, 619, 625, 631, 637, 643, 649, 655, 661, 667, 673, 679, 685, 691, 697, 703, 709, 715, 721, 727, 733, 739, 745, 751, 757, 763, 769, 775, 781, 793, 799, 805, 814, 820, 826, 832, 838, 844, 850, 856, 862, 868, 874, 880, 886, or 892. In some examples, the LCDR2 comprises an amino acid sequence of SEQ ID NOs: 599, 605, 611, 617, 623, 629, 635, 641, 647, 653, 659, 665, 671, 677, 683, 689, 695, 701,

707, 713, 719, 725, 731, 737, 743, 749, 755, 761, 767, 773, 779, 785, 797, 803, 809, 812, 818, 824, 830, 836, 842, 848, 854, 860, 866, 872, 878, 884, or 890. In some examples, the VH comprises an amino acid sequence of SEQ ID NOs: 901, 903, 905, 907, 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 967, 969, 971, 974, 976, 978, 980, 982, 984, 986, 988, 990, 992, 994, 996, 998, or 1000. In some examples, the VL comprises an amino acid sequence of SEQ ID NOs: 912, 914, 916, 918, 920, 922, 924, 926, 928, 930, 932, 934, 936, 938, 940, 942, 944, 946, 948, 950, 952, 954, 956, 958, 960, 962, 964, 968, 970, 972, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, or 999.

**[0041]** In some examples, the TCE molecule comprises: (a) a VH comprising an amino acid sequence of SEQ ID NO: 967 and a VL comprising an amino acid sequence of SEQ ID NO: 968; (b) a VH comprising an amino acid sequence of SEQ ID NO: 969 and a VL comprising an amino acid sequence of SEQ ID NO: 970; (c) a VH comprising an amino acid sequence of SEQ ID NO: 971 and a VL comprising an amino acid sequence of SEQ ID NO: 972; (d) a VH comprising an amino acid sequence of SEQ ID NO: 974 and a VL comprising an amino acid sequence of SEQ ID NO: 973; (e) a VH comprising an amino acid sequence of SEQ ID NO: 976 and a VL comprising an amino acid sequence of SEQ ID NO: 975; (f) a VH comprising an amino acid sequence of SEQ ID NO: 978 and a VL comprising an amino acid sequence of SEQ ID NO: 977; (g) a VH comprising an amino acid sequence of SEQ ID NO: 980 and a VL comprising an amino acid sequence of SEQ ID NO: 979; (h) a VH comprising an amino acid sequence of SEQ ID NO: 982 and a VL comprising an amino acid sequence of SEQ ID NO: 981; (i) a VH comprising an amino acid sequence of SEQ ID NO: 984 and a VL comprising an amino acid sequence of SEQ ID NO: 983; (j) a VH comprising an amino acid sequence of SEQ ID NO: 986 and a VL comprising an amino acid sequence of SEQ ID NO: 985; (k) a VH comprising an amino acid sequence of SEQ ID NO: 988 and a VL comprising an amino acid sequence of SEQ ID NO: 987; (l) a VH comprising an amino acid sequence of SEQ ID NO: 990 and a VL comprising an amino acid sequence of SEQ ID NO: 989; (m) a VH comprising an amino acid sequence of SEQ ID NO: 992 and a VL comprising an amino acid sequence of SEQ ID NO: 991; (n) a VH comprising an amino acid sequence of SEQ ID NO: 994 and a VL comprising an amino acid sequence of SEQ ID NO: 993; (o) a VH comprising an amino acid sequence of SEQ ID NO: 996 and a VL comprising an amino acid sequence of SEQ ID NO: 995; (p) a VH comprising an amino acid sequence of SEQ ID NO: 998 and a VL comprising an amino acid sequence of SEQ ID NO: 997; or (q) a VH comprising an amino acid sequence of SEQ ID NO: 1000 and a VL comprising an amino acid sequence of SEQ ID NO: 999.

**[0042]** In some examples, the TCE molecule comprises a heavy chain variable region (VH) amino acid sequence of SEQ ID NO: 965, and a light chain variable region (VL) comprising the amino acid sequence:

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNX1NYLAWYX2QKPGQX3PKLLISWAST  
RESGVPDRFSGSGSGTDFTLTINSLQAEDVAVYYCQYYYSIPITFGGGTKVEIKR (SEQ ID NO:  
342), wherein X1 is K or R, X2 is H or Q, and/or X3 is S or P. In some examples, a TCE molecule of the present disclosure comprises a YTE motif in the Fc region, corresponding to M252Y/S254T/T256E in the constant heavy chain region of IgG1 or IgG4. The YTE extends the half-life of the molecule (see e.g. Booth et al., MABs 2018 Oct; 10(7): 1098-1110). In some examples, the TCE molecule of the present disclosure comprising YTE is a TCE molecule that binds CCR8 and CD3.

**[0043]** In some examples, a TCE molecule of the present disclosure comprises an I2E scFv. In some examples, a TCE molecule of the present disclosure comprises an I2C scFv. The amino acid sequences of I2E are given by SEQ ID NOs. 199 to 206. The amino acid sequences of I2C are given by SEQ ID NOs. 191 to 198.

**[0044]** The present disclosure provides additional TCE molecules described in Table 25. The amino acid sequences of these TCE molecules are given by SEQ ID NOs 261 to 589 in Table 25.

**[0045]** The present disclosure also provides a method of treating cancer in a patient comprising administering an effective amount of a TCE molecule of the present disclosure to the patient. In an example, the cancer is a solid tumor. In a particular example, the cancer is non-small cell lung cancer, gastric cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, triple-negative breast cancer, colorectal cancer, pancreatic cancer, or metastatic castrate-resistant prostate cancer. In some examples, the method further comprises administering to the patient a PD-1 antagonist antibody or a PD-L1 antagonist antibody. In some such examples, the PD-1 antagonist antibody or PD-L1 antagonist antibody is administered prior to, concurrently with, and/or after administration of the TCE molecule. In particular examples, the PD-1 antagonist antibody is pembrolizumab, nivolumab, cemiplimab, or antibody 20C1.009. In other particular examples, the PD-L1 antagonist antibody is atezolizumab, avelumab, or durvalumab. In some examples, the method further comprises administering to the patient a chemotherapeutic agent. In some such examples, the chemotherapeutic agent may be administered prior to, concurrently with, or after administration of the TCE molecule. In some examples, the method comprises administering to the patient a TCE molecule of the present disclosure and a chemotherapeutic

agent. In some examples, the method comprises administering to the patient a TCE molecule of the present disclosure, a PD-1 or PD-L1 antagonist antibody, and a chemotherapeutic agent.

**[0046]** The present disclosure provides a TCE molecule of the present disclosure for use in therapy.

**[0047]** The present disclosure also provides a TCE molecule for use in treating cancer. In an example, the cancer is a solid tumor. In a particular example, the cancer is non-small cell lung cancer, gastric cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, triple-negative breast cancer, colorectal cancer, pancreatic cancer, or metastatic castrate-resistant prostate cancer. In a more particular example, the cancer is non-small cell lung cancer, gastric cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, or triple-negative breast cancer. In some examples, the use further comprises administering to the patient a PD-1 antagonist antibody or PD-L1 antagonist antibody. In some such examples, the PD-1 antagonist antibody or PD-L1 antagonist antibody is administered prior to, concurrently with, and/or after administration of the TCE molecule. In particular examples, the PD-1 antagonist antibody is pembrolizumab, nivolumab, cemiplimab, or antibody 20C1.009. In other particular examples, the PD-L1 antagonist antibody is atezolizumab, avelumab, or durvalumab. In some examples, the use further comprises administering to the patient a chemotherapeutic agent. In some such examples, the chemotherapeutic agent may be administered prior to, concurrently with, or after administration of the TCE molecule. In some examples, the use comprises administering to the patient a TCE molecule of the present disclosure and a chemotherapeutic agent. In some examples, the use comprises administering to the patient a TCE molecule of the present disclosure, a PD-1 or PD-L1 antagonist antibody, and a chemotherapeutic agent.

**[0048]** The present disclosure provides the use of a TCE molecule of the present disclosure for the manufacture of a medicament for the treatment of cancer. In an example, the cancer is a solid tumor. In a particular example, the cancer is non-small cell lung cancer, gastric cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, triple-negative breast cancer, colorectal cancer, pancreatic cancer, or metastatic castrate-resistant prostate cancer. In a more particular example, the cancer is non-small cell lung cancer, gastric cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, or triple-negative breast cancer.

**[0049]** The present disclosure also provides a pharmaceutical composition comprising a TCE molecule of the present disclosure and one or more pharmaceutically acceptable carriers, diluents, or excipients.

**[0050]** The present disclosure also provides a polynucleotide that encodes an amino acid sequence of a TCE molecule of the present disclosure. The term “encoding” or “encodes” refers to a polynucleotide sequence encoding one or more amino acids. The term does not require a start or stop codon. The present disclosure encompasses nucleic acid molecules encoding anti-CCR8 TCE polypeptide sequences.

**[0051]** In an example, the TCE molecule of the present disclosure is encoded by a polynucleotide sequence given by SEQ ID NO: 590. In a particular example, the TCE molecule encoded by the polynucleotide sequence given by SEQ ID NO: 590 comprises the amino acid sequence given by SEQ ID NO: 227.

**[0052]** In an example, the TCE molecule of the present disclosure is encoded by a polynucleotide sequence given by SEQ ID NO: 592. In a particular example, the TCE molecule encoded by the polynucleotide sequence given by SEQ ID NO: 592 comprises the amino acid sequence given by SEQ ID NO: 249.

**[0053]** In an example, the TCE molecule of the present disclosure is encoded by a polynucleotide sequence given by SEQ ID NO: 593. In a particular example, the TCE molecule encoded by the polynucleotide sequence given by SEQ ID NO: 593 comprises the amino acid sequence given by SEQ ID NO: 260.

**[0054]** In an example, the TCE molecule of the present disclosure is encoded by a polynucleotide sequence given by SEQ ID NO: 591. In a particular example, the TCE molecule encoded by the polynucleotide sequence given by SEQ ID NO: 591 comprises the amino acid sequence given by SEQ ID NO: 238.

**[0055]** The present disclosure also provides a DNA molecule comprising a polynucleotide that encodes an amino acid sequence of a TCE molecule of the present disclosure. In an example, the TCE molecule of the present disclosure is encoded by a polynucleotide sequence given by SEQ ID NO: 590. In a particular example, the TCE molecule encoded by the polynucleotide sequence given by SEQ ID NO: 590 comprises the amino acid sequence given by SEQ ID NO: 227. In another example, the TCE molecule of the present disclosure is encoded by a polynucleotide sequence given by SEQ ID NO: 592. In a particular example, the TCE molecule encoded by the polynucleotide sequence given by SEQ ID NO: 592 comprises the amino acid sequence given by SEQ ID NO: 249. In another example, the TCE molecule of the present disclosure is encoded by a polynucleotide sequence given by SEQ ID NO: 593. In a particular example, the TCE molecule encoded by the polynucleotide sequence given by SEQ ID NO: 593 comprises the amino acid sequence given by SEQ ID NO: 260. In another example, the TCE molecule of the present disclosure is encoded by a polynucleotide sequence

given by SEQ ID NO: 591. In a particular example, the TCE molecule encoded by the polynucleotide sequence given by SEQ ID NO: 591 comprises the amino acid sequence given by SEQ ID NO: 238.

**[0056]** The present disclosure further provides a mammalian cell transformed with a DNA molecule of the present disclosure, wherein the transformed mammalian cell is capable of expressing a TCE molecule of the present disclosure.

**[0057]** The present disclosure also provides a process for producing a TCE molecule of the present disclosure, wherein the process comprises cultivating a mammalian cell under conditions such that the TCE molecule is expressed and recovering the expressed TCE molecule. The present disclosure also provides a mammalian cell transformed with a DNA molecule of the present disclosure, wherein the transformed mammalian cell is capable of expressing a TCE molecule of the present disclosure. The present disclosure also provides a TCE molecule obtainable by the process.

**[0058]** In another example, the present disclosure provides a CCR8 TCE molecule that binds human CCR8 at an epitope wherein the epitope comprises at least one residue of SEQ ID NO: 134. In an example, the epitope comprises at least two residues of SEQ ID NO: 134. In an example, the epitope comprises at least three residues of SEQ ID NO: 134. In an example, the epitope comprises at least four residues of SEQ ID NO: 134. In an example, the epitope comprises at least five residues of SEQ ID NO: 134. In an example, the epitope comprises six or more residues of SEQ ID NO: 134. In an example, the epitope comprises seven or more residues of SEQ ID NO: 134. In an example, the epitope comprises eight or more residues of SEQ ID NO: 134. In an example, the epitope comprises nine or more residues of SEQ ID NO: 134. In an example, the epitope comprises ten or more residues of SEQ ID NO: 134. In an example, the epitope comprises eleven or more residues of SEQ ID NO: 134. In an example, the epitope comprises twelve residues of SEQ ID NO: 134. In a particular example, the epitope comprises the threonine residue at position 4 of SEQ ID NO: 134. The term “epitope” as used herein refers to sites of an antigen that are in contact with (e.g. binds) the molecule. The epitope may be determined by a method known to a person of ordinary skill, including flow cytometry of bound TCE molecule to peptides, hydrogen-deuterium exchange, alanine scanning, and/or x-ray crystallography. In an example, the epitope is determined by epitope binning. In an example, the epitope is determined by TCE molecule binding to CCR8 peptide-nanobody complexes. In an example, the epitope is determined by screening TCE molecule binding to CCR8 by phage display. In an example, the epitope is determined by determining binding to a CCR8 peptide expressed in

human cells, wherein the peptide comprises an amino acid sequence given by SEQ ID NO: 134 or amino acid residues 1-12 of SEQ ID NO: 133. In some examples, the epitope is determined by anti-CCR8 TCE molecule binding to the T4R mutation in cynomolgus monkey CCR8. In an example, binding to the T4R mutation is determined in a cell based affinity assay, wherein TCE molecule binding to cells expressing cynomolgus monkey cells CCR8 containing a T4R mutation is compared to TCE molecule binding to cells expressing wild-type cynomolgus monkey CCR8 (comprising a threonine at position four). In some examples, an anti-CCR8 TCE molecule binds threonine at position four if it shows reduced binding to CCR8 comprising a T4R mutation. In particular examples, an anti-CCR8 TCE molecule binds threonine at position four if it shows no detectable binding to CCR8 comprising a T4R mutation. In some examples, wild-type cynomolgus monkey CCR8 comprises an amino acid sequence given by SEQ ID NO: 129. In some examples, cynomolgus monkey CCR8 comprising a T4R mutation comprises an amino acid sequence given by SEQ ID NO: 130.

**[0059]** The present disclosure provides a method of treating cancer in a patient comprising administering to the patient an effective amount of a CCR8 TCE molecule that binds human CCR8 at an epitope wherein the epitope comprises at least one residue of SEQ ID NO: 134. In an example, the epitope comprises at least two residues of SEQ ID NO: 134. In an example, the epitope comprises at least three residues of SEQ ID NO: 134. In an example, the epitope comprises at least four residues of SEQ ID NO: 134. In an example, the epitope comprises at least five residues of SEQ ID NO: 134. In an example, the epitope comprises six or more residues of SEQ ID NO: 134. In an example, the epitope comprises seven or more residues of SEQ ID NO: 134. In an example, the epitope comprises eight or more residues of SEQ ID NO: 134. In an example, the epitope comprises nine or more residues of SEQ ID NO: 134. In an example, the epitope comprises ten or more residues of SEQ ID NO: 134. In an example, the epitope comprises eleven or more residues of SEQ ID NO: 134. In an example, the epitope comprises twelve residues of SEQ ID NO: 134. In an example, the epitope comprises a threonine residue at position 4 of SEQ ID NO: 134. In an example, the epitope is determined by epitope binning. In an example, the epitope is determined by TCE molecule binding to CCR8 peptide-nanobody complexes. In an example, the epitope is determined by screening TCE molecule binding to CCR8 by phage display. In an example, the epitope is determined by determining binding to a CCR8 peptide expressed in human cells, wherein the peptide comprises an amino acid sequence given by SEQ ID NO: 134 or amino acid residues 1-12 of SEQ ID NO: 133. In some examples, the epitope is determined by anti-CCR8 TCE molecule binding to the T4R

mutation in cynomolgus monkey CCR8. In an example, binding to the T4R mutation is determined in a cell based affinity assay, wherein TCE molecule binding to cells expressing cynomolgus monkey cells CCR8 containing a T4R mutation is compared to TCE molecule binding to cells expressing wild-type cynomolgus monkey CCR8 (comprising a threonine at position four). In some examples, an anti-CCR8 TCE molecule binds threonine at position four if it shows reduced binding to CCR8 comprising a T4R mutation. In particular examples, an anti-CCR8 TCE molecule binds threonine at position four if it shows no detectable binding to CCR8 comprising a T4R mutation. In some examples, wild-type cynomolgus monkey CCR8 comprises an amino acid sequence given by SEQ ID NO: 129. In some examples, cynomolgus monkey CCR8 comprising a T4R mutation comprises an amino acid sequence given by SEQ ID NO: 130.

**[0060]** In another example, the present disclosure provides a CCR8 TCE molecule that binds human CCR8 at an epitope wherein the epitope consists of one residue of SEQ ID NO: 134. In an example, the epitope consists of two residues of SEQ ID NO: 134. In an example, the epitope consists of three residues of SEQ ID NO: 134. In an example, the epitope consists of four residues of SEQ ID NO: 134. In an example, the epitope consists of five residues of SEQ ID NO: 134. In an example, the epitope consists of six residues of SEQ ID NO: 134. In an example, the epitope consists of seven residues of SEQ ID NO: 134. In an example, the epitope consists of eight residues of SEQ ID NO: 134. In an example, the epitope consists of nine residues of SEQ ID NO: 134. In an example, the epitope consists of ten residues of SEQ ID NO: 134. In an example, the epitope consists of eleven residues of SEQ ID NO: 134. In an example, the epitope consists of twelve residues of SEQ ID NO: 134. In an example, the epitope consists of a threonine residue at position 4 of SEQ ID NO: 134.

**[0061]** The present disclosure provides a method of treating cancer in a patient comprising administering to the patient an effective amount of a CCR8 TCE molecule that binds human CCR8 at an epitope wherein the epitope consists of one residue of SEQ ID NO: 134. In an example, the epitope consists of two residues of SEQ ID NO: 134. In an example, the epitope consists of three residues of SEQ ID NO: 134. In an example, the epitope consists of four residues of SEQ ID NO: 134. In an example, the epitope consists of five residues of SEQ ID NO: 134. In an example, the epitope consists of six residues of SEQ ID NO: 134. In an example, the epitope consists of seven residues of SEQ ID NO: 134. In an example, the epitope consists of eight residues of SEQ ID NO: 134. In an example, the epitope consists of nine residues of SEQ ID NO: 134. In an example, the epitope consists of

ten residues of SEQ ID NO: 134. In an example, the epitope consists of eleven residues of SEQ ID NO: 134. In an example, the epitope consists of twelve residues of SEQ ID NO: 134. In an example, the epitope consists of a threonine residue at position 4 of SEQ ID NO: 134.

**[0062]** In another example, the present disclosure provides a CCR8 TCE molecule that binds human CCR8 at an epitope wherein the epitope comprises at least one residue of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises at least two residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises at least three residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises at least four residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises at least five residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises six or more residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises seven or more residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises eight or more residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises nine or more residues of amino acid residues 1-12 of SEQ ID NO: 133. In an example, the epitope comprises ten or more residues amino acid residues 1-12 of SEQ ID NO: 133. In an example, the epitope comprises eleven or more residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises twelve residues of amino acid residues 1-12 OF SEQ ID NO: 133. In a particular example, the epitope comprises the threonine residue at position 4 of amino acid residues 1-12 OF SEQ ID NO: 133. The term “epitope” as used herein refers to sites of an antigen that are in contact with (e.g. binds) the molecule. The epitope may be determined by a method known to a person of ordinary skill, including flow cytometry of bound TCE molecule to peptides, hydrogen-deuterium exchange, alanine scanning, and/or x-ray crystallography. In an example, the epitope is determined by epitope binning. In an example, the epitope is determined by TCE molecule binding to CCR8 peptide-nanobody complexes. In an example, the epitope is determined by screening TCE molecule binding to CCR8 by phage display. In an example, the epitope is determined by determining binding to a CCR8 peptide expressed in human cells, wherein the peptide comprises an amino acid sequence given by SEQ ID NO: 134 or amino acid residues 1-12 of SEQ ID NO: 133. In some examples, the epitope is determined by anti-CCR8 TCE molecule binding to the T4R mutation in cynomolgus monkey CCR8. In an example, binding to the T4R mutation is determined in a cell based affinity assay, wherein TCE molecule binding to cells expressing cynomolgus monkey cells CCR8 containing a T4R

mutation is compared to TCE molecule binding to cells expressing wild-type cynomolgus monkey CCR8 (comprising a threonine at position four). In some examples, an anti-CCR8 TCE molecule binds threonine at position four if it shows reduced binding to CCR8 comprising a T4R mutation. In particular examples, an anti-CCR8 TCE molecule binds threonine at position four if it shows no detectable binding to CCR8 comprising a T4R mutation. In some examples, wild-type cynomolgus monkey CCR8 comprises an amino acid sequence given by SEQ ID NO: 129. In some examples, cynomolgus monkey CCR8 comprising a T4R mutation comprises an amino acid sequence given by SEQ ID NO: 130.

**[0063]** The present disclosure provides a method of treating cancer in a patient comprising administering to the patient an effective amount of a CCR8 TCE molecule that binds human CCR8 at an epitope wherein the epitope comprises at least one residue of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises at least two residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises at least three residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises at least four residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises at least five residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises six or more residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises seven or more residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises eight or more residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises nine or more residues of amino acid residues 1-12 of SEQ ID NO: 133. In an example, the epitope comprises ten or more residues amino acid residues 1-12 of SEQ ID NO: 133. In an example, the epitope comprises eleven or more residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises twelve residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope comprises a threonine residue at position 4 of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope is determined by epitope binning. In an example, the epitope is determined by TCE molecule binding to CCR8 peptide-nanobody complexes. In an example, the epitope is determined by screening TCE molecule binding to CCR8 by phage display. In an example, the epitope is determined by determining binding to a CCR8 peptide expressed in human cells, wherein the peptide comprises an amino acid sequence given by SEQ ID NO: 134 or amino acid residues 1-12 of SEQ ID NO: 133. In some examples, the epitope is determined by anti-CCR8 TCE molecule binding to the T4R mutation in cynomolgus monkey CCR8. In an example, binding to the T4R mutation is determined in a cell based affinity

assay, wherein TCE molecule binding to cells expressing cynomolgus monkey cells CCR8 containing a T4R mutation is compared to TCE molecule binding to cells expressing wild-type cynomolgus monkey CCR8 (comprising a threonine at position four). In some examples, an anti-CCR8 TCE molecule binds threonine at position four if it shows reduced binding to CCR8 comprising a T4R mutation. In particular examples, an anti-CCR8 TCE molecule binds threonine at position four if it shows no detectable binding to CCR8 comprising a T4R mutation. In some examples, wild-type cynomolgus monkey CCR8 comprises an amino acid sequence given by SEQ ID NO: 129. In some examples, cynomolgus monkey CCR8 comprising a T4R mutation comprises an amino acid sequence given by SEQ ID NO: 130.

**[0064]** In another example, the present disclosure provides a CCR8 TCE molecule that binds human CCR8 at an epitope wherein the epitope consists of at least one residue of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of two residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of three residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of four residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of five residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of six residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of seven residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of eight residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of nine residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of ten residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of eleven residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of twelve residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of a threonine residue at position 4 of amino acid residues 1-12 OF SEQ ID NO: 133.

**[0065]** The present disclosure provides a method of treating cancer in a patient comprising administering to the patient an effective amount of a CCR8 TCE molecule that binds human CCR8 at an epitope wherein the epitope consists of one residue of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of two residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of three residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of four residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of five residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope

consists of six residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of seven residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of eight residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of nine residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of ten residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of eleven residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of twelve residues of amino acid residues 1-12 OF SEQ ID NO: 133. In an example, the epitope consists of a threonine residue at position 4 of amino acid residues 1-12 OF SEQ ID NO: 133.

**[0066]** In an example, the epitope is determined by epitope binning. In an example, the epitope is determined by TCE molecule binding to CCR8 peptide-nanobody complexes. In an example, the epitope is determined by screening TCE molecule binding to CCR8 by phage display. In an example, the epitope is determined by determining binding to a CCR8 peptide expressed in human cells, wherein the peptide comprises an amino acid sequence given by SEQ ID NO: 134 or amino acid residues 1-12 of SEQ ID NO: 133. In some examples, the epitope is determined by anti-CCR8 TCE molecule binding to the T4R mutation in cynomolgus monkey CCR8. In an example, binding to the T4R mutation is determined in a cell based affinity assay, wherein TCE molecule binding to cells expressing cynomolgus monkey cells CCR8 containing a T4R mutation is compared to TCE molecule binding to cells expressing wild-type cynomolgus monkey CCR8 (comprising a threonine at position four). In some examples, an anti-CCR8 TCE molecule binds threonine at position four if it shows reduced binding to CCR8 comprising a T4R mutation. In particular examples, an anti-CCR8 TCE molecule binds threonine at position four if it shows no detectable binding to CCR8 comprising a T4R mutation. In some examples, wild-type cynomolgus monkey CCR8 comprises an amino acid sequence given by SEQ ID NO: 129. In some examples, cynomolgus monkey CCR8 comprising a T4R mutation comprises an amino acid sequence given by SEQ ID NO: 130.

**[0067]** In some examples, the present disclosure provides a molecule that competes for binding CCR8 with a CCR8 TCE molecule of the present disclosure. Such molecule that competes for binding may be, for example, a TCE molecule, an antibody, antibody fragment, or polypeptide. In some examples, the present disclosure provides a molecule that binds the same epitope as a CCR8 TCE molecule of the present disclosure.

**[0068]** In some examples, a TCE molecule of the present disclosure can be administered concurrently with, before, or after a variety of drugs and treatments widely

employed in cancer treatment such as, for example, chemotherapeutic agents, non-chemotherapeutic agents (e.g. anti-PD-1 or anti-PD-L1 inhibitors, such as antagonist antibodies), anti-neoplastic agents, and/or radiation. For example, administration can occur before, during, and/or after any of the treatments described herein. Examples of chemotherapeutic agents are discussed herein and include, but are not limited to, cisplatin, taxol, etoposide, mitoxantrone (Novantrone®), actinomycin D, cycloheximide, camptothecin (or water soluble derivatives thereof), methotrexate, mitomycin (e.g., mitomycin C), dacarbazine (DTIC), anti-neoplastic antibiotics such as adriamycin (doxorubicin) and daunomycin, and all the chemotherapeutic agents mentioned herein.

**[0069]** In some examples, a TCE molecule of the present disclosure may be administered concurrently with, before, or after a PD-1 antagonist antibody or a PD-L1 antagonist antibody. The term “PD-1 antagonist antibody” refers to an antibody that specifically binds to PD-1 and decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 and one or more of its ligands, such as PD-L1 and PD-L2. In some examples, a PD-1 antagonist antibody inhibits the binding of PD-1 to PD-L1 and/or PD-L2. The term “PD-L1 antagonist antibody” refers to an antibody that specifically binds to PD-L1 and decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with the PD-1 receptor. In some examples, a PD-L1 antagonist antibody inhibits the binding of PD-L1 to PD-1. In some examples, the PD-1 antagonist is any one of Antibody 20C1.006 (SEQ ID NOs: 179-188), Antibody 20C1.009 (SEQ ID NOs: 139-148, or 139-147 and 212), Antibody 20A2.3 (SEQ ID NOs: 149-158), Antibody 20D4.6 (SEQ ID NOs: 159-168), or Antibody 20D4.17 (SEQ ID NOs: 169-178). In one example, the PD-1 antagonist antibody is pembrolizumab. In another example, the PD-1 antagonist antibody is nivolumab. In yet another example, the PD-1 antagonist antibody is cemiplimab. In a particular example, the PD-1 antagonist antibody is antibody 20C1.009, for which the amino acid sequences of the CDRs, variable regions, and full light and heavy chains are provided in SEQ ID NOs: 139-148 and 212. 20C1.009 is also known as AMG 404 and is also known as zelumab. In exemplary aspects, an anti-PD-1 antibody such as 20C1.009 comprises a HC comprising a C-terminal lysine, as in SEQ ID NO: 148. In alternative aspects, the antibody comprises a HC without the C-terminal lysine, as in SEQ ID NO: 212.

**[0070]** In some examples, the 1185-1200

**[0070a]** In one aspect, the present disclosure refers to a T cell engager (TCE) molecule comprising (i) an scFab that binds to a tumor antigen, wherein the scFab comprises a first heavy chain variable region (scFab VH), a CH1 domain, a first light chain variable region (scFab VL), and a Cκ or Cλ domain, and (ii) an scFv that binds CD3, comprising a second VL and a second VH, and wherein the TCE molecule is a single chain.

**[0070b]** In another aspect, the present disclosure refers to a TCE molecule comprising (i) a first scFv that binds CCR8, wherein the first scFv comprises a first VH region (CCR8 scFv VH) and a first VL region (CCR8 scFv VL), and (ii) a second scFv that binds CD3, wherein the second scFv comprises a second VH region and a second VL region, and wherein the TCE molecule is a single chain.

**[0070c]** In another aspect, the present disclosure refers to the TCE molecule as disclosed herein for use in therapy.

**[0070d]** In another aspect, the present disclosure refers to use of the TCE molecule as disclosed herein in the manufacture of a medicament for treating cancer.

**[0070e]** In another aspect, the present disclosure refers to a pharmaceutical composition comprising the TCE molecule as disclosed herein and one or more pharmaceutically acceptable carriers, diluents, or excipients.

**[0070f]** In another aspect, the present disclosure refers to a TCE molecule comprising an scFv that comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, each comprising an amino acid sequence given by SEQ ID NO: 217, 218, 219, 220, 221, and 220, respectively.

**[0070g]** In another aspect, the present disclosure refers to a TCE molecule comprising an scFv that comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, each comprising an amino acid sequence given by SEQ ID NO: 228, 229, 230, 231, 232, and 233, respectively.

**[0070h]** In another aspect, the present disclosure refers to a TCE molecule comprising an scFab that comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, each comprising an amino acid sequence given by SEQ ID NO: 239, 240, 241, 242, 243, and 244, respectively.

**[0070i]** In another aspect, the present disclosure refers to a TCE molecule comprising an scFab that comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, each comprising an amino acid sequence given by SEQ ID NO: 250, 251, 252, 253, 254, and 255, respectively.

**[0070j]** In another aspect, the present disclosure refers to use of an effective amount of a CCR8 TCE molecule that binds human CCR8 at an epitope in the manufacture of a medicament for treating cancer, wherein the epitope comprises at least one residue of SEQ ID NO: 134.

**[0070k]** In another aspect, the present disclosure refers to a TCE molecule that binds to human CCR8, which comprises an HCDR1 amino acid sequence of SEQ ID NO: 787; an HCDR2 amino acid sequence of SEQ ID NO: 788; an HCDR3 amino acid sequence of SEQ ID NO: 789; an LCDR1 amino acid sequence of SEQ ID NO: 336, wherein X<sub>1</sub> is K or R; an LCDR2 amino acid sequence of SEQ ID NO: 791; and an LCDR3 amino acid sequence of SEQ ID NO: 792.

**[0070l]** In another aspect, the present disclosure refers to a TCE molecule that binds to human CCR8, which comprises: (a) an HCDR1 amino acid sequence of  $X_1X_2GX_4H$ , (SEQ ID NO: 1181), wherein (i)  $X_1$  is N, S, D, G, T, or R, (ii)  $X_2$  is C, N, Y, S, or F, and (iii)  $X_4$  is M or F; (b) an HCDR2 amino acid sequence of SEQ ID NOs: 596, 602, 608, 614, 620, 626, 632, 638, 644, 650, 656, 662, 668, 674, 680, 686, 692, 698, 704, 710, 716, 722, 728, 734, 740, 746, 752, 758, 764, 770, 776, 782, 788, 794, 800, 806, 815, 821, 827, 833, 839, 845, 851, 857, 863, 869, 875, 881, 887, or 893; (c) an HCDR3 amino acid sequence of SEQ ID NOs: 597, 603, 609, 615, 621, 627, 633, 639, 645, 651, 657, 663, 669, 675, 681, 687, 693, 699, 705, 711, 717, 723, 729, 735, 741, 747, 753, 759, 765, 771, 777, 783, 795, 801, 807, 816, 822, 828, 834, 840, 846, 852, 858, 864, 870, 876, 882, 888, or 894; (d) an LCDR1 amino acid sequence of any one of SEQ ID NOs: 598, 604, 610, 616, 622, 628, 634, 640, 646, 652, 658, 664, 670, 676, 682, 688, 694, 700, 706, 712, 718, 724, 730, 736, 742, 748, 754, 760, 766, 772, 778, 784, 796, 802, 808, 811, 817, 823, 829, 835, 841, 847, 853, 859, 865, 871, 877, 883, or 889; (e) an LCDR2 amino acid sequence of  $RX_2X_3X_4RPS$  (SEQ ID NO: 1182), wherein (i)  $X_2$  is A, N, D, S, or Q, (ii)  $X_3$  is S, T, N, I, F, or A, and (iii)  $X_4$  is N or V; and (f) an LCDR3 amino acid sequence of SEQ ID NOs: 600, 606, 612, 618, 624, 630, 636, 642, 648, 654, 660, 666, 672, 678, 684, 690, 696, 702, 708, 714, 720, 726, 732, 738, 744, 750, 756, 762, 768, 774, 780, 786, 798, 804, 810, 813, 819, 825, 831, 837, 843, 849, 855, 861, 867, 873, 879, 885, 891.

**[0070m]** In another aspect, the present disclosure refers to a TCE molecule that binds to human CCR8, comprising: (a) a VH comprising an amino acid sequence of SEQ ID NO: 967 and a VL comprising an amino acid sequence of SEQ ID NO: 968; (b) a VH comprising an amino acid sequence of SEQ ID NO: 969 and a VL comprising an amino acid sequence of SEQ ID NO: 970; (c) a VH comprising an amino acid sequence of SEQ ID NO: 971 and a VL comprising an amino acid sequence of SEQ ID NO: 972; (d) a VH comprising an amino acid sequence of SEQ ID NO: 974 and a VL comprising an amino acid sequence of SEQ ID NO: 973; (e) a VH comprising an amino acid sequence of SEQ ID NO: 976 and a VL comprising an amino acid sequence of SEQ ID NO: 975; (f) a VH comprising an amino acid sequence of SEQ ID NO: 978 and a VL comprising an amino acid sequence of SEQ ID NO: 977; (g) a VH comprising an amino acid sequence of SEQ ID NO: 980 and a VL comprising an amino acid sequence of SEQ ID NO: 979; (h) a VH comprising an amino acid sequence of SEQ ID NO: 982 and a VL comprising an amino acid sequence of SEQ ID NO: 981; (i) a VH comprising an amino acid sequence of SEQ ID NO: 984 and a VL comprising an amino acid sequence of SEQ ID NO: 983; (j) a VH comprising an amino acid sequence of SEQ ID NO: 986 and a VL comprising an amino acid sequence of SEQ ID NO: 985; (k) a VH comprising an amino acid sequence of SEQ ID NO: 988 and a VL comprising an amino acid sequence of SEQ ID NO: 987; (l) a VH comprising an amino acid sequence of SEQ ID NO: 990 and a VL comprising an amino acid sequence of SEQ ID NO: 989; (m) a VH comprising an amino acid sequence of SEQ ID NO: 992 and a VL comprising an amino acid sequence of SEQ ID NO: 991; (n) a VH comprising an amino acid sequence of SEQ ID NO: 994 and a VL comprising an amino acid sequence of SEQ ID NO: 993; (o) a VH comprising an amino acid sequence of SEQ ID NO: 996 and a VL comprising an amino acid sequence of SEQ ID NO: 995; (p) a VH comprising an amino acid sequence of SEQ ID NO: 998 and a VL comprising an amino acid sequence of SEQ ID NO: 997; or (q) a VH comprising an amino acid sequence of SEQ ID NO: 1000 and a VL comprising an amino acid sequence of SEQ ID NO: 999.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

## CLAIMS

We claim:

1. A T cell engager (TCE) molecule comprising (i) an scFab that binds to a tumor antigen, wherein the scFab comprises a first heavy chain variable region (scFab VH), a CH1 domain, a first light chain variable region (scFab VL), and a C $\kappa$  or C $\lambda$  domain, and (ii) an scFv that binds CD3, comprising a second VL and a second VH, and wherein the TCE molecule is a single chain.
2. The TCE molecule of Claim 1, wherein the scFab comprises a C-terminus portion that is connected by a linker to an N-terminal portion of the scFv.
3. The TCE molecule of Claim 1 or 2, wherein the TCE molecule further comprises an scFc.
4. The TCE molecule of Claim 3, wherein the scFc comprises an N-terminus portion that is connected by a linker to a C-terminal portion of the scFv.
5. The TCE molecule of any one of Claims 1-4, wherein the scFv binds human CD3.
6. The TCE molecule of any one of Claims 1-5, wherein the scFab has an orientation in the following order, from N-terminus to C-terminus, VH, CH1, VL, and either C $\kappa$  or C $\lambda$ .
7. The TCE molecule of any one of Claims 1-5, wherein the scFab has an orientation in the following order, from N-terminus to C-terminus, VL, either C $\kappa$  or C $\lambda$ , VH, and CH1.
8. The TCE molecule of any one of Claims 1-6, wherein the scFab comprises a linker that connects the CH1 and scFab VL, wherein the linker is (G4S)<sub>6</sub>, (G4S)<sub>7</sub>, (G4S)<sub>8</sub>, (G4Q)<sub>6</sub>, (G4Q)<sub>7</sub>, or (G4Q)<sub>8</sub>.
9. The TCE molecule of any one of Claims 1-5 and 7, wherein the TCE molecule comprises a linker that connects the scFab C $\kappa$  or C $\lambda$  and the scFab VH, wherein the linker is (G4S)<sub>6</sub>, (G4S)<sub>7</sub>, (G4S)<sub>8</sub>, (G4Q)<sub>6</sub>, (G4Q)<sub>7</sub>, or (G4Q)<sub>8</sub>.
10. The TCE molecule of any one of Claims 1-9, wherein CH1, C $\kappa$  and/or C $\lambda$  domains are IgG, IgM, IgA, IgD, or IgE.
11. The TCE molecule of Claim 10, wherein the domains are IgG.
12. The TCE molecule of Claim 10 or 11, wherein the domains are IgG1.
13. The TCE molecule of any one of Claims 1-12, wherein the scFab contains a cysteine clamp between CH1 and either C $\kappa$  or C $\lambda$ .

14. The TCE molecule of any one of Claims 1-13, wherein the orientation of the TCE molecule from N-terminus to C-terminus is: VH-CH1-Linker-VL-Ck or Cλ-Linker-VH-Linker-VL-Linker- Fc1 (CH2-CH3) -Linker-Fc2 (CH2-CH3).
15. The TCE molecule of any one of Claims 1-13, wherein the orientation of the TCE molecule from N-terminus to C-terminus is: VL-CH1-Linker-VH-Ck or Cλ-Linker-VH-Linker-VL-Linker- Fc1 (CH2-CH3) -Linker-Fc2 (CH2-CH3).
16. The TCE molecule of any one of Claims 1-15, wherein the tumor antigen is CCR8.
17. The TCE molecule of any one of Claims 1-16, wherein the scFv that binds CD3 is I2E.
18. The TCE molecule of any one of Claims 1-16, wherein the scFv that binds CD3 is I2C.
19. A TCE molecule comprising (i) a first scFv that binds CCR8, wherein the first scFv comprises a first VH region (CCR8 scFv VH) and a first VL region (CCR8 scFv VL), and (ii) a second scFv that binds CD3, wherein the second scFv comprises a second VH region and a second VL region, and wherein the TCE molecule is a single chain.
20. The TCE molecule of any one of Claims 1-19, wherein the first VH comprises HCDR1, HCDR2, HCDR3, and the first VL comprises LCDR1, LCDR2, and LCDR3, and wherein:
  - a. HCDR1 comprises an amino acid sequence given by SEQ ID NO: 1, SEQ ID NO: 17, SEQ ID NO: 33, SEQ ID NO: 49, SEQ ID NO: 65, SEQ ID NO: 81, SEQ ID NO: 97, or SEQ ID NO: 113;
  - b. HCDR2 comprises an amino acid sequence given by SEQ ID NO: 2, SEQ ID NO: 18, SEQ ID NO: 34, SEQ ID NO: 50, SEQ ID NO: 66, or SEQ ID NO: 82;
  - c. HCDR3 comprises an amino acid sequence given by SEQ ID NO: 3, SEQ ID NO: 19, SEQ ID NO: 35, SEQ ID NO: 51, SEQ ID NO: 67, or SEQ ID NO: 83;
  - d. LCDR1 comprises an amino acid sequence given by SEQ ID NO: 4, SEQ ID NO: 20, SEQ ID NO: 36, SEQ ID NO: 52, SEQ ID NO: 68, or SEQ ID NO: 84;

- e. LCDR2 comprises an amino acid sequence given by SEQ ID NO:5, SEQ ID NO: 21, SEQ ID NO: 37, SEQ ID NO: 53, SEQ ID NO: 69, or SEQ ID NO: 85, and
  - f. LCDR3 comprises an amino acid sequence given by SEQ ID NO: 6, SEQ ID NO: 22, SEQ ID NO: 38, SEQ ID NO: 54, SEQ ID NO: 70, or SEQ ID NO: 86.
21. The TCE molecule of Claim 20, wherein HCDR1 comprises an amino acid sequence given by SEQ ID NO: 1, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 2, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 3, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 4, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 5, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 6.
  22. The TCE molecule of Claim 20, wherein HCDR1 comprises an amino acid sequence given by SEQ ID NO: 17, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 18, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 19, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 20, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 21, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 22.
  23. The TCE molecule of Claim 20, wherein HCDR1 comprises an amino acid sequence given by SEQ ID NO: 33, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 34, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 35, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 36, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 37, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 38.
  24. The TCE molecule of Claim 20, wherein HCDR1 comprises an amino acid sequence given by SEQ ID NO: 49, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 50, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 51, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 52, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 53, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 54.
  25. The TCE molecule of Claim 20, wherein HCDR1 comprises an amino acid sequence given by SEQ ID NO: 65, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 66, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 67, LCDR1 comprises an amino acid sequence given by SEQ ID

- NO: 68, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 69, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 70.
26. The TCE molecule of Claim 20, wherein HCDR1 comprises an amino acid sequence given by SEQ ID NO: 81, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 82, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 83, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 84, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 85, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 86.
  27. The TCE molecule of Claim 20, wherein HCDR1 comprises an amino acid sequence given by SEQ ID NO: 97, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 98, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 99, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 100, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 101, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 102.
  28. The TCE molecule of Claim 20, wherein HCDR1 comprises an amino acid sequence given by SEQ ID NO: 113, HCDR2 comprises an amino acid sequence given by SEQ ID NO: 114, HCDR3 comprises an amino acid sequence given by SEQ ID NO: 115, LCDR1 comprises an amino acid sequence given by SEQ ID NO: 116, LCDR2 comprises an amino acid sequence given by SEQ ID NO: 117, and LCDR3 comprises an amino acid sequence given by SEQ ID NO: 118.
  29. The TCE molecule of any one of Claims 19-28, wherein the first VH comprises an amino acid sequence given by SEQ ID NO: 7, SEQ ID NO: 23, SEQ ID NO: 39, SEQ ID NO: 55, SEQ ID NO: 71, SEQ ID NO: 87, SEQ ID NO: 103, or SEQ ID NO: 119, and wherein the first VL comprises an amino acid sequence given by SEQ ID NO: 8, SEQ ID NO: 24, SEQ ID NO: 40, SEQ ID NO: 56, SEQ ID NO: 72, SEQ ID NO: 88, SEQ ID NO: 104, or SEQ ID NO: 120.
  30. The TCE molecule of any one of Claims 19-29, wherein the first scFv comprises an amino acid sequence given by SEQ ID NO: 9, 25, 41, 57, 73, 89, 105, or 121.
  31. The TCE molecule of any one of Claims 19-30, comprising an amino acid sequence given by SEQ ID NO: 10, SEQ ID NO: 26, SEQ ID NO: 42, SEQ ID NO: 58, SEQ ID NO: 74, SEQ ID NO: 90, SEQ ID NO: 106, or SEQ ID NO: 122.
  32. The TCE molecule of any one of Claims 19-31, further comprising an scFc, wherein the TCE molecule comprises an amino acid sequence given by SEQ ID

- NO: 11, SEQ ID NO: 27, SEQ ID NO: 59, SEQ ID NO: 75, SEQ ID NO: 91, SEQ ID NO: 107, or SEQ ID NO: 123.
33. The TCE molecule of any one of Claims 1-18 and 20-28, wherein the first VH and CH1 comprise an amino acid sequence given by SEQ ID NO: 12, SEQ ID NO: 28, SEQ ID NO: 44, SEQ ID NO: 60, SEQ ID NO: 76, SEQ ID NO: 92, SEQ ID NO: 108, or SEQ ID NO: 124.
34. The TCE molecule of any one of Claims 1-18, 20-28, and 33, comprising a C $\kappa$ , wherein the first VL and C $\kappa$  comprise an amino acid sequence given by SEQ ID NO: 13, SEQ ID NO: 29, SEQ ID NO: 45, SEQ ID NO: 61, SEQ ID NO: 77, SEQ ID NO: 93, SEQ ID NO: 109, or SEQ ID NO: 125.
35. The TCE molecule of any one of Claims 1-18, 20-28, 33, and 34, comprising an amino acid sequence given by SEQ ID NO: 14, SEQ ID NO: 30, SEQ ID NO: 46, SEQ ID NO: 62, SEQ ID NO: 78, SEQ ID NO: 94, SEQ ID NO: 110, or SEQ ID NO: 126.
36. The TCE molecule of any one of Claims 1-18, 20-28, and 33-35, comprising an amino acid sequence given by SEQ ID NO: 15, SEQ ID NO: 31, SEQ ID NO: 47, SEQ ID NO: 63, SEQ ID NO: 79, SEQ ID NO: 95, SEQ ID NO: 111, or SEQ ID NO: 127.
37. The TCE molecule of any one of Claims 3-18, 20-28, 33-36, wherein the TCE molecule comprises an amino acid sequence given by SEQ ID NO: 16, SEQ ID NO: 32, SEQ ID NO: 48, SEQ ID NO: 64, SEQ ID NO: 80, SEQ ID NO: 96, SEQ ID NO: 112, or SEQ ID NO: 128.
38. The TCE molecule of any one of Claims 1-37 for use in therapy.
39. Use of the TCE molecule of any one of Claims 1-37 in the manufacture of a medicament for treating cancer.
40. The use of Claim 39, wherein the cancer is a solid tumor.
41. The use of Claim 39 or 40, wherein the cancer is non-small cell lung cancer, gastric cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, triple-negative breast cancer, colorectal cancer, pancreatic cancer, or metastatic castrate-resistant prostate cancer.
42. The use of Claims 39-41, wherein the cancer is non-small cell lung cancer, gastric cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, or triple-negative breast cancer.

43. The use of Claims 39-43, wherein the medicament further comprises a PD-1 antagonist antibody or PD-L1 antagonist antibody to be administered.
44. The use of Claim 43, wherein the PD-1 antagonist antibody or PD-L1 antagonist antibody is to be administered prior to, concurrently with, and/or after administration of the TCE molecule.
45. The use of Claim 43 or 44, wherein the PD-1 antagonist antibody is pembrolizumab, nivolumab, cemiplimab, or antibody 20C1.009.
46. The use of Claim 43 or 44, wherein the PD-L1 antagonist antibody is atezolizumab, avelumab, or durvalumab.
47. A pharmaceutical composition comprising the TCE molecule of any one of Claims 1-37 and one or more pharmaceutically acceptable carriers, diluents, or excipients.
48. A TCE molecule comprising an scFv that comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, each comprising an amino acid sequence given by SEQ ID NO: 217, 218, 219, 220, 221, and 220, respectively.
49. The TCE molecule of Claim 47, comprising a VH and VL comprising an amino acid sequence given by SEQ ID NO: 223 and SEQ ID NO: 224, respectively.
50. The TCE molecule of Claim 48 or Claim 49, wherein the TCE molecule comprises an scFv comprising an amino acid sequence given by SEQ ID NO: 225.
51. The TCE molecule of any one of Claims 48-50, comprising an amino acid sequence given by SEQ ID NO: 226.
52. The TCE molecule of Claim 51, comprising an amino acid sequence given by SEQ ID NO: 227.
53. A TCE molecule comprising an scFv that comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, each comprising an amino acid sequence given by SEQ ID NO: 228, 229, 230, 231, 232, and 233, respectively.
54. The TCE molecule of Claim 53, comprising a VH and VL comprising an amino acid sequence given by SEQ ID NO: 234 and SEQ ID NO: 235, respectively.
55. The TCE molecule of Claim 53 or Claim 54, wherein the TCE molecule comprises an scFv comprising an amino acid sequence given by SEQ ID NO: 236.
56. The TCE molecule of any one of Claims 53-55 comprising an amino acid sequence given by SEQ ID NO: 237.
57. The TCE molecule of Claim 56, comprising an amino acid sequence given by SEQ ID NO: 238.

58. A TCE molecule comprising an scFab that comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, each comprising an amino acid sequence given by SEQ ID NO: 239, 240, 241, 242, 243, and 244, respectively.
59. The TCE molecule of Claim 58, comprising a VH-CH1 comprising an amino acid sequence given by SEQ ID NO: 245.
60. The TCE molecule of Claim 58 or Claim 59, wherein the TCE molecule comprises a VL-C $\kappa$  comprising an amino acid sequence given by SEQ ID NO: 246.
61. The TCE molecule of any one of Claims 58-60, wherein the scFab comprises an amino acid sequence given by SEQ ID NO: 247.
62. The TCE molecule of any one of Claims 58-61, comprising an amino acid sequence given by SEQ ID NO: 248.
63. The TCE molecule of Claim 73, comprising an amino acid sequence given by SEQ ID NO: 249.
64. A TCE molecule comprising an scFab that comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, each comprising an amino acid sequence given by SEQ ID NO: 250, 251, 252, 253, 254, and 255, respectively.
65. The TCE molecule of Claim 64, comprising a VH-CH1 comprising an amino acid sequence given by SEQ ID NO: 256.
66. The TCE molecule of Claim 64 or Claim 65, wherein the TCE molecule comprises a VL-C $\kappa$  comprising an amino acid sequence given by SEQ ID NO: 257.
67. The TCE molecule of any one of Claims 64-66, wherein the scFab comprises an amino acid sequence given by SEQ ID NO: 258.
68. The TCE molecule of any one of Claims 64-67, comprising an amino acid sequence given by SEQ ID NO: 259.
69. The TCE molecule of Claim 68, comprising an amino acid sequence given by SEQ ID NO: 260.
70. The TCE molecule of any one of Claims 48-69 for use in therapy.
71. Use of the TCE molecule of any one of Claims 48-69 in the manufacture of a medicament for treating cancer.
72. The use of Claims 48-69, wherein the cancer is a solid tumor.

73. The use of Claims 48-69, wherein the cancer is non-small cell lung cancer, gastric cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, triple-negative breast cancer, colorectal cancer, pancreatic cancer, or metastatic castrate-resistant prostate cancer.
74. The use of Claims 48-69, wherein the cancer is non-small cell lung cancer, gastric cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, or triple-negative breast cancer.
75. The use of Claims 48-69, wherein the medicament further comprises a PD-1 antagonist antibody or PD-L1 antagonist antibody to be administered to a patient.
76. The use of Claim 75, wherein the PD-1 antagonist antibody is pembrolizumab, nivolumab, cemiplimab, or antibody 20C1.009.
77. The use of Claim 75 or 76, wherein the PD-L1 antagonist antibody is atezolizumab, avelumab, or durvalumab.
78. A pharmaceutical composition comprising the TCE molecule of any one of Claims 48-69 and one or more pharmaceutically acceptable carriers, diluents, or excipients.
79. Use of an effective amount of a CCR8 TCE molecule that binds human CCR8 at an epitope in the manufacture of a medicament for treating cancer, wherein the epitope comprises at least one residue of SEQ ID NO: 134.
80. The use of Claim 79, wherein the epitope comprises at least two residues of SEQ ID NO: 134.
81. The use of Claim 79 or Claim 80, wherein the epitope comprises at least three residues of SEQ ID NO: 134.
82. The use of any one of Claims 79-81, wherein the epitope comprises at least four residues of SEQ ID NO: 134.
83. The use of any one of Claims 79-82, wherein the epitope comprises at least five residues of SEQ ID NO: 134.
84. The use of any one of Claims 79-82, wherein the epitope comprises a threonine residue at position 4 of SEQ ID NO: 134.
85. A TCE molecule that binds to human CCR8, which comprises an HCDR1 amino acid sequence of SEQ ID NO: 787; an HCDR2 amino acid sequence of SEQ ID NO: 788; an HCDR3 amino acid sequence of SEQ ID NO: 789; an LCDR1 amino acid sequence of SEQ ID NO: 336, wherein X<sub>1</sub> is K or R; an LCDR2 amino acid

sequence of SEQ ID NO: 791; and an LCDR3 amino acid sequence of SEQ ID NO: 792.

86. The TCE molecule of Claim 85, wherein the TCE molecule comprises a VH amino acid sequence of SEQ ID NO: 965 and a VL amino acid sequence of SEQ ID NO: 342, wherein  $X_1$  is K or R,  $X_2$  is H or Q, and/or  $X_3$  is S or P.
87. A TCE molecule that binds to human CCR8, which comprises: (a) an HCDR1 amino acid sequence of  $X_1X_2GX_4H$ , (SEQ ID NO: 1181), wherein (i)  $X_1$  is N, S, D, G, T, or R, (ii)  $X_2$  is C, N, Y, S, or F, and (iii)  $X_4$  is M or F; (b) an HCDR2 amino acid sequence of SEQ ID NOs: 596, 602, 608, 614, 620, 626, 632, 638, 644, 650, 656, 662, 668, 674, 680, 686, 692, 698, 704, 710, 716, 722, 728, 734, 740, 746, 752, 758, 764, 770, 776, 782, 788, 794, 800, 806, 815, 821, 827, 833, 839, 845, 851, 857, 863, 869, 875, 881, 887, or 893; (c) an HCDR3 amino acid sequence of SEQ ID NOs: 597, 603, 609, 615, 621, 627, 633, 639, 645, 651, 657, 663, 669, 675, 681, 687, 693, 699, 705, 711, 717, 723, 729, 735, 741, 747, 753, 759, 765, 771, 777, 783, 795, 801, 807, 816, 822, 828, 834, 840, 846, 852, 858, 864, 870, 876, 882, 888, or 894; (d) an LCDR1 amino acid sequence of any one of SEQ ID NOs: 598, 604, 610, 616, 622, 628, 634, 640, 646, 652, 658, 664, 670, 676, 682, 688, 694, 700, 706, 712, 718, 724, 730, 736, 742, 748, 754, 760, 766, 772, 778, 784, 796, 802, 808, 811, 817, 823, 829, 835, 841, 847, 853, 859, 865, 871, 877, 883, or 889; (e) an LCDR2 amino acid sequence of  $RX_2X_3X_4RPS$  (SEQ ID NO: 1182), wherein (i)  $X_2$  is A, N, D, S, or Q, (ii)  $X_3$  is S, T, N, I, F, or A, and (iii)  $X_4$  is N or V; and (f) an LCDR3 amino acid sequence of SEQ ID NOs: 600, 606, 612, 618, 624, 630, 636, 642, 648, 654, 660, 666, 672, 678, 684, 690, 696, 702, 708, 714, 720, 726, 732, 738, 744, 750, 756, 762, 768, 774, 780, 786, 798, 804, 810, 813, 819, 825, 831, 837, 843, 849, 855, 861, 867, 873, 879, 885, 891.
88. The TCE molecule of Claim 87, wherein HCDR1 comprises an amino acid sequence of SEQ ID NOs: 595, 601, 607, 613, 619, 625, 631, 637, 643, 649, 655, 661, 667, 673, 679, 685, 691, 697, 703, 709, 715, 721, 727, 733, 739, 745, 751, 757, 763, 769, 775, 781, 793, 799, 805, 814, 820, 826, 832, 838, 844, 850, 856, 862, 868, 874, 880, 886, or 892.
89. The TCE molecule of Claim 87 or Claim 88, wherein the LCDR2 comprises an amino acid sequence of SEQ ID NOs: 599, 605, 611, 617, 623, 629, 635, 641, 647, 653, 659, 665, 671, 677, 683, 689, 695, 701, 707, 713, 719, 725, 731, 737,

743, 749, 755, 761, 767, 773, 779, 785, 797, 803, 809, 812, 818, 824, 830, 836, 842, 848, 854, 860, 866, 872, 878, 884, or 890.

90. The TCE molecule of Claims 87-89, wherein the VH comprises an amino acid sequence of SEQ ID NOs: 901, 903, 905, 907, 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 967, 969, 971, 974, 976, 978, 980, 982, 984, 986, 988, 990, 992, 994, 996, 998, or 1000.
91. The TCE molecule of Claims 87-90, the VL comprises an amino acid sequence of SEQ ID NOs: 912, 914, 916, 918, 920, 922, 924, 926, 928, 930, 932, 934, 936, 938, 940, 942, 944, 946, 948, 950, 952, 954, 956, 958, 960, 962, 964, 968, 970, 972, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, or 999.
92. A TCE molecule that binds to human CCR8, comprising: (a) a VH comprising an amino acid sequence of SEQ ID NO: 967 and a VL comprising an amino acid sequence of SEQ ID NO: 968; (b) a VH comprising an amino acid sequence of SEQ ID NO: 969 and a VL comprising an amino acid sequence of SEQ ID NO: 970; (c) a VH comprising an amino acid sequence of SEQ ID NO: 971 and a VL comprising an amino acid sequence of SEQ ID NO: 972; (d) a VH comprising an amino acid sequence of SEQ ID NO: 974 and a VL comprising an amino acid sequence of SEQ ID NO: 973; (e) a VH comprising an amino acid sequence of SEQ ID NO: 976 and a VL comprising an amino acid sequence of SEQ ID NO: 975; (f) a VH comprising an amino acid sequence of SEQ ID NO: 978 and a VL comprising an amino acid sequence of SEQ ID NO: 977; (g) a VH comprising an amino acid sequence of SEQ ID NO: 980 and a VL comprising an amino acid sequence of SEQ ID NO: 979; (h) a VH comprising an amino acid sequence of SEQ ID NO: 982 and a VL comprising an amino acid sequence of SEQ ID NO: 981; (i) a VH comprising an amino acid sequence of SEQ ID NO: 984 and a VL comprising an amino acid sequence of SEQ ID NO: 983; (j) a VH comprising an amino acid sequence of SEQ ID NO: 986 and a VL comprising an amino acid sequence of SEQ ID NO: 985; (k) a VH comprising an amino acid sequence of SEQ ID NO: 988 and a VL comprising an amino acid sequence of SEQ ID NO: 987; (l) a VH comprising an amino acid sequence of SEQ ID NO: 990 and a VL comprising an amino acid sequence of SEQ ID NO: 989; (m) a VH comprising an amino acid sequence of SEQ ID NO: 992 and a VL comprising an amino acid sequence of SEQ ID NO: 991; (n) a VH comprising an amino acid sequence of

SEQ ID NO: 994 and a VL comprising an amino acid sequence of SEQ ID NO: 993; (o) a VH comprising an amino acid sequence of SEQ ID NO: 996 and a VL comprising an amino acid sequence of SEQ ID NO: 995; (p) a VH comprising an amino acid sequence of SEQ ID NO: 998 and a VL comprising an amino acid sequence of SEQ ID NO: 997; or (q) a VH comprising an amino acid sequence of SEQ ID NO: 1000 and a VL comprising an amino acid sequence of SEQ ID NO: 999.