

WHAT IS CLAIMED:

1. An anti-GUCY2C single domain antibody (sdAb) comprising:
 - (i) a CDR1 comprising an amino acid sequence of X_1YGMX_2 , wherein X_1 is A, I or V, and X_2 is D or G (SEQ ID NO: 64);
 - (ii) a CDR2 comprising an amino acid sequence of $X_3IX_4LSGRX_5X_6YX_7DAVX_8G$, wherein X_3 is A, S or T; X_4 is F, W or Y; X_5 is S or T; X_6 is E, N or T; X_7 is A, S or T; and X_8 is K or Q (SEQ ID NO: 65); and
 - (iii) a CDR3 comprising an amino acid sequence of $GX_9X_{10}TAX_{11}SX_{12}RQY$, wherein X_9 is A, E or P; X_{10} is P or T; X_{11} is S or T; and X_{12} is G or V (SEQ ID NO: 66).
2. The anti-GUCY2C sdAb of claim 1, wherein the CDR1 comprises an amino acid sequence selected from a group consisting of SEQ ID NOs: 19 to 26; the CDR2 comprises an amino acid sequence selected from a group consisting of SEQ ID NOs: 27 to 34; and the CDR3 comprises an amino acid sequence selected from a group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.
3. The anti-GUCY2C sdAb of claim 1 or claim 2, comprising:
 - (i) a CDR1 comprising the amino acid sequence of SEQ ID NO: 19; a CDR2 comprising the amino acid sequence of SEQ ID NO: 27; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 35;
 - (ii) a CDR1 comprising the amino acid sequence of SEQ ID NO: 20; a CDR2 comprising the amino acid sequence of SEQ ID NO: 28; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36;
 - (iii) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21; a CDR2 comprising the amino acid sequence of SEQ ID NO: 29; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 37;
 - (iv) a CDR1 comprising the amino acid sequence of SEQ ID NO: 22; a CDR2 comprising the amino acid sequence of SEQ ID NO: 30; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 38;
 - (v) a CDR1 comprising the amino acid sequence of SEQ ID NO: 23; a CDR2 comprising the amino acid sequence of SEQ ID NO: 31; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 39;

(vi) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24; a CDR2 comprising the amino acid sequence of SEQ ID NO: 32; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 40;

(vii) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25; a CDR2 comprising the amino acid sequence of SEQ ID NO: 33; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 41; or

(viii) a CDR1 comprising the amino acid sequence of SEQ ID NO: 26; a CDR2 comprising the amino acid sequence of SEQ ID NO: 34; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 42.

4. An anti-GUCY2C single domain antibody (sdAb) comprising:

(i) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 3;

(ii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 4;

(iii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 5;

(iv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 6;

(v) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 7;

(vi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 8;

(vii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 9; or

(viii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 10.

5. The anti-GUCY2C sdAb of claim 4, wherein the CDR1, CDR2 or CDR3 are determined according to the Kabat numbering scheme, the IMGT numbering scheme, the AbM numbering scheme, the Chothia numbering scheme, the Contact numbering scheme, or a combination thereof.

6. The anti-GUCY2C sdAb of any one of claims 1 to 5, further comprising one or more FR regions as set forth in any one of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID

NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

7. The anti-GUCY2C sdAb of any one of claims 1 to 6, comprising the amino acid sequence of any one of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

8. The anti-GUCY2C sdAb of any one of claims 1 to 6, wherein anti-GUCY2C sdAb comprises or consists of an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more sequence identity with the sequence of any one of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

9. The anti-GUCY2C sdAb of any one of claims 1 to 5, wherein anti-GUCY2C sdAb is a camelid sdAb.

10. The anti-GUCY2C sdAb of any one of claims 1 to 5, wherein anti-GUCY2C sdAb is a humanized sdAb.

11. The anti-GUCY2C sdAb of any one of claims 1 to 10, wherein the anti-GUCY2C sdAb is genetically fused or chemically conjugated to an agent.

12. A chimeric antigen receptor (CAR), comprising:

(a) an extracellular antigen binding domain comprising the anti-GUCY2C sdAb of any one of claims 1 to 10;

(b) a transmembrane domain; and

(c) an intracellular signaling domain.

13. The CAR of claim 12, wherein the extracellular antigen binding domain further comprises one or more additional antigen binding domain(s).

14. The CAR of claim 12 or claim 13, wherein the transmembrane domain is derived from a molecule selected from a group consisting of CD8 α , CD4, CD28, CD137, CD80, CD86, CD152, and PD1.

15. The CAR of claim 14, wherein the transmembrane domain is derived from CD8 α .

16. The CAR of any one of claims 12 to 15, wherein the intracellular signaling domain comprises a primary intracellular signaling domain of an immune effector cell.

17. The CAR of claim 16, wherein the primary intracellular signaling domain is derived from CD3 ζ .

18. The CAR of claim 16 or claim 17, wherein the intracellular signaling domain further comprises a co-stimulatory signaling domain.
19. The CAR of claim 18, wherein the co-stimulatory signaling domain is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, CD137 (4-1BB), OX40, CD30, CD40, CD3, LFA-1, CD2, CD7, LIGHT, NKG2C, B7-H3, ligands of CD83 and combinations thereof.
20. The CAR of claim [2019](#), wherein the co-stimulatory signaling domain is derived from CD137.
21. The CAR of any one of claims 12 to 20, further comprising a hinge domain located between the C-terminus of the extracellular antigen binding domain and the N-terminus of the transmembrane domain.
22. The CAR of claim 21, wherein the hinge domain is derived from CD8 α .
23. The CAR of any one of claims 12 to 22, further comprising a signal peptide located at the N-terminus of the polypeptide.
24. The CAR of claim 23, wherein the signal peptide is derived from CD8 α .
25. A chimeric antigen receptor (CAR), comprising (i) an amino acid sequence selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 55; or (ii) an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more sequence identity with the sequence of any one of SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, and SEQ ID NO: 55.
26. An isolated nucleic acid comprising a nucleic acid sequence encoding the anti-GUCY2C sdAb of any one of claims 1 to 10.
27. The isolated nucleic acid of claim 26, wherein the isolated nucleic acid comprises a nucleic acid sequence of SEQ ID NOs: 11 to 18.
28. A vector comprising the isolated nucleic acid of claim 27.
29. An isolated nucleic acid comprising a nucleic acid sequence encoding the CAR of any one of claims 14 to 25.
30. The isolated nucleic acid of claim 29, wherein the isolated nucleic acid comprises a

nucleic acid sequence of SEQ ID NOs: 56 to 63.

31. A vector comprising the isolated nucleic acid of claim 30.

32. An engineered immune effector cell, comprising the CAR of any one of claims 14 to 25, the isolated nucleic acid of claim 29 or claim 30, or the vector of claim 31.

33. The engineered immune effector cell of claim 32, wherein the immune effector cell is a T cell.

34. A pharmaceutical composition, comprising the anti-GUCY2C sdAb of any one of claims 1 to 10, the engineered immune effector cell of claim 32 or claim 33, or the vector of claim 28 or claim 31, and a pharmaceutically acceptable excipient.

35. Use of an anti-GUCY2C sdAb of any one of claims 1 to 10, an engineered immune effector cell of claim 32 or claim 33, or a pharmaceutical composition of claim 34 in the manufacture of a medicament for ~~A method of~~ treating a disease or disorder in a subject, ~~comprising administering to the subject wherein~~ an effective amount of the anti-GUCY2C sdAb of any one of claims 1 to 10, the engineered immune effector cell of claim 32 or claim 33, or the pharmaceutical composition of claim 34 is to be administered to the subject, and wherein the disease or disorder is a GUCY2C associated disease or disorder or cancer.

~~36.—The method of claim 35, wherein the disease or disorder is a GUCY2C associated disease or disorder.~~

~~37.—The method of claim 35, wherein the disease or disorder is cancer.~~

~~383436.~~ The ~~method~~use of claim ~~3635~~, wherein the ~~disease or disorder cancer~~ is colorectal cancer.

~~393537.~~ The ~~method~~use of claim ~~3635~~, wherein the ~~disease or disorder cancer~~ is gastric cancer.

~~403638.~~ The ~~method~~use of claim ~~3635~~, wherein the ~~disease or disorder cancer~~ is esophageal cancer.