

What is claimed is:

1. An antibody which specifically binds to guanylyl cyclase C (GUCY2c), wherein the antibody comprises:

- a. a heavy chain variable (VH) region comprising a VH complementarity determining region one (VH CDR1), a VH complementarity determining region two (VH CDR2), and a VH complementarity determining region three (VH CDR3) of the VH sequence shown in SEQ ID NO: ~~11, 19, 26, 33, 41, 48, 52, 57, 60, 62, 64, 65, 67, 69, 71, or 73~~; and/or
- b. a light chain variable (VL) region comprising a VL complementarity determining region one (VL CDR1), a VL complementarity determining region two (VL CDR2), and a VL complementarity determining region three (VL CDR3) of the VL sequence shown in SEQ ID NO: ~~92, 100, 104, 106, 112, 119, 125, 129, 134, 136, 137, 138, 140, 143, 145, 147, 150, 152, 156, 158, 160, 162, 166, 170, 171, 172, 173, 174, or 175~~.

~~2.~~ The antibody of claim ~~1~~, wherein:

- ~~c.~~ the VH region comprises (i) a VH CDR1 comprising a sequence of SEQ ID NO: ~~12, 20, 27, 34, 42, 74, 257, 258, 259, 260, or 261~~; (ii) a VH CDR2 comprising a sequence of SEQ ID NO: ~~13, 21, 28, 35, 43, 53, 66, 68, 70, 72, 75, 262, 263, 264, 265, 266, or 267~~; and (iii) a VH CDR3 comprising a sequence of SEQ ID NO: ~~14, 22, 29, 36, or 44~~; and/or
- ~~d.~~ the VL region comprises (i) a VL CDR1 comprising a sequence of SEQ ID NO: ~~93, 101, 105, 107, 113, 120, 148, 153, 163, or 167~~; (ii) a VL CDR2 comprising a sequence of SEQ ID NO: ~~78, 94, 102, 108, 114, 141, 144, 146, 149, 151, 157, 159, 161, 164, or 168~~; and (iii) a VL CDR3 comprising a sequence of SEQ ID NO: ~~95, 109, 115, 121, 142, 154, 165, or 169~~.

~~32.~~ The antibody of claim ~~2~~1, wherein:

- a. the VH region comprises (i) a VH CDR1 comprising the sequence of SEQ ID NO: 74 or 259; (ii) a VH CDR2 comprising the sequence of SEQ ID NO: 75 or 267; and (iii) a VH CDR3 comprising the sequence of SEQ ID NO: 29; and/or

- b. the VL region comprises (i) a VL CDR1 comprising the sequence of SEQ ID NO: 148; (ii) a VL CDR2 comprising the sequence of SEQ ID NO: 149; and (iii) a VL CDR3 comprising the sequence of SEQ ID NO: 142.

~~4. The antibody of claim 1 or 2, wherein:~~

- ~~a. the VH region comprises a sequence of SEQ ID NO: 11, 19, 26, 33, 41, 48, 52, 57, 60, 62, 64, 65, 67, 69, 71, or 73; and/or~~
- ~~b. the VL region comprises a sequence of SEQ ID NO: 92, 100, 104, 106, 112, 119, 125, 129, 134, 136, 137, 138, 140, 143, 145, 147, 150, 152, 156, 158, 160, 162, 166, 170, 171, 172, 173, 174, or 175.~~

~~53.~~ The antibody of any one of claims 1 ~~to 4~~ or 2, wherein the VH region comprises the sequence of SEQ ID NO: 73; and wherein the VL region comprises the sequence of SEQ ID NO: 147.

4. A bispecific antibody that specifically binds to GUCY2c and CD3, wherein the bispecific antibody comprises a first polypeptide chain and a second polypeptide chain, and the first polypeptide chain and the second polypeptide chain together comprise (1) a Gucy2c heavy chain variable region (Gucy2c VH) and a Gucy2c light chain variable region (Gucy2c VL) that form a domain that specifically binds to Gucy2c, and the Gucy2c VH comprises a VH CDR1, a VH CDR2 and a VH CDR3 of the VH sequence shown in SEQ ID NO 73, the Gucy2c VL comprises a VL CDR1, a VL CDR2 and a VL CDR3 of the VL sequence shown in SEQ ID NO 147, and (2) a CD3 VH and a CD3 VL that form a domain that specifically binds to CD3

~~65. A bispecific antibody that specifically binds to GUCY2c and CD3, wherein the bispecific antibody comprises a first polypeptide chain and a second polypeptide chain~~The bispecific antibody of claim 4, wherein:

(I)

- a. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:
 - i. a Domain 1, comprising a VL region of a GUCY2c antibody (GUCY2c VL), and a VH region of a CD3 antibody (CD3 VH), and
 - ii. a first heterodimer-promoting domain; and
- b. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:
 - i. a Domain 2, comprising a VL region of a CD3 antibody (CD3 VL), and a VH region of a GUCY2c antibody (GUCY2c VH), and
 - ii. a second heterodimer-promoting domain;

wherein the GUCY2c VL and the GUCY2c VH form a domain that specifically binds to GUCY2c; and the CD3 VL and the CD3 VH form a domain that specifically binds to CD3;

(II)

- a. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:
 - i. a Domain 1, comprising a CD3 VL, and a GUCY2c VH, and
 - ii. a first heterodimer-promoting domain; and
- b. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:
 - i. a Domain 2 comprising a GUCY2c VL, and a CD3 VH, and
 - ii. a second heterodimer-promoting domain;

wherein the GUCY2c VL and the GUCY2c VH form a domain that specifically binds to GUCY2c; and the CD3 VL and the CD3 VH form a domain that specifically binds to CD3;

(III)

- a. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:
 - i. a Domain 1, comprising a GUCY2c VH, and a CD3 VL, and
 - ii. a first heterodimer-promoting domain; and
- b. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:
 - i. a Domain 2, comprising a CD3 VH, and a GUCY2c VL, and
 - ii. a second heterodimer-promoting domain;

wherein the GUCY2c VL and the GUCY2c VH form a domain that specifically binds to GUCY2c; and the CD3 VL and the CD3 VH form a domain that specifically binds to CD3; or

(IV)

- a. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:
 - i. a Domain 1, comprising a CD3 VH, and a GUCY2c VL, and
 - ii. a first heterodimer-promoting domain; and
- b. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:
 - i. a Domain 2, comprising a GUCY2c VH, and a CD3 VL, and
 - ii. a second heterodimer-promoting domain,

wherein the GUCY2c VL and the GUCY2c VH form a domain that specifically binds to GUCY2c; and the CD3 VL and the CD3 VH form a domain that specifically binds to CD3.

76. The bispecific antibody of claim 65, wherein the first heterodimer-promoting domain and the second heterodimer-promoting domain each comprise an Fc region comprising a CH2 domain and a CH3 domain, wherein the amino acid sequence of each of the CH2 domain and/or the CH3 comprises at least one amino acid modification as compared to wildtype Fc region, to form a knob or a hole.

87. The bispecific antibody of claim 76, wherein the first heterodimer-promoting domain and the second heterodimer-promoting domain are not both knobs or both holes; and/or wherein the first heterodimer-promoting domain and the second heterodimer-promoting domain form an IgG immunoglobulin Fc region.

98. The bispecific antibody of claim 87, wherein the CH3 domain of the first heterodimer-promoting domain comprises mutations Y349C and/or T366W, to form a knob; and the CH3 domain of the second heterodimer-promoting domain comprises

mutations S354C, T366S, L368A, and/or Y407V, to form a hole, (numbering according to the EU index).

~~109~~. The bispecific antibody of claim ~~98~~, wherein the CH2 domain of the first heterodimer-promoting domain and the CH2 domain of the second heterodimer-promoting domain each comprise mutations L234A, L235A and/or G237A, (numbering according to the EU index).

~~1110~~. The bispecific antibody of claim ~~109~~, wherein the first heterodimer-promoting domain comprises a sequence of SEQ ID NO: 188, to form a knob; and wherein the second heterodimer-promoting domain comprises a sequence of SEQ ID NO: 189, to form a hole.

~~1211~~. The bispecific antibody of any one of claims ~~6-5~~ to ~~1110~~, wherein the GUCY2c VL and the CD3 VH are linked by a glycine-serine linker; and the CD3 VL and the GUCY2c VH are linked by a glycine-serine linker; optionally wherein the glycine-serine linker is Linker 1 comprising a sequence of SEQ ID NO: 190 or is Linker 2 comprising a sequence of SEQ ID NO: 191.

~~1312~~. The bispecific antibody of any one of claims ~~6-5~~ to ~~1211~~, wherein Domain 1 is covalently bound to the first heterodimer-promoting domain via a cysteine linker; and Domain 2 is covalently bound to the second heterodimer-promoting domain via a cysteine linker; wherein the cysteine linker comprises at least five amino acids, optionally wherein the cysteine linker is Linker 3 comprising a sequence of SEQ ID NO: 192.

~~1413~~. The bispecific antibody of any one of claims ~~56~~ to ~~1312~~, wherein the first polypeptide chain is covalently bound to the second polypeptide chain by at least one disulfide bond; optionally wherein at least one disulfide bond forms between Linker 3 of the first polypeptide chain and Linker 3 of the second polypeptide chain; further optionally wherein at least one disulfide bond is formed between the first heterodimer-promoting domain and the second heterodimer-promoting domain; further optionally wherein each disulfide bond is formed by linking two cysteine residues.

~~1514~~. The bispecific antibody of any one of claims ~~6-5~~ to ~~1413~~ wherein the antibody comprises:

~~a. a GUCY2c VL CDR1, a GUCY2c VL CDR2, and a GUCY2c VL CDR3 of a GUCY2c VL comprising the sequence shown in SEQ ID NO: 92, 100, 104, 106, 112, 119, 125, 129, 134, 136, 137, 138, 140, 143, 145, 147, 150, 152, 156, 158, 160, 162, 166, 170, 171, 172, 173, 174, or 175;~~

~~b.a. a CD3 VH CDR1, a CD3 VH CDR2, and a CD3 VH CDR3 of a CD3 VH comprising the sequence shown in SEQ ID NOS: 1, 9, or 273; and~~

~~c.b. a CD3 VL CDR1, a CD3 VL CDR2, and a CD3 VL CDR3 of a CD3 VL comprising the sequence shown in SEQ ID NO: 76, 84, 90, 274, 275, or 276; and/or~~

~~d. a GUCY2c VH CDR1, a GUCY2c VH CDR2, and a GUCY2c VH CDR3 of a GUCY2c VH comprising the sequence shown in SEQ ID NO: 11, 19, 26, 33, 41, 48, 52, 57, 60, 62, 64, 65, 67, 69, 71, or 73.~~

16. ~~The bispecific antibody of claim 15, wherein:~~

~~the GUCY2c VL CDR1 comprises a sequence of SEQ ID NO: 93, 101, 105, 107, 113, 120, 148, 153, 163, or 167; the GUCY2c VL CDR2 comprises a sequence of SEQ ID NO: 78, 94, 102, 108, 114, 141, 144, 146, 149, 151, 157, 159, 161, 164, or 168; and the GUCY2c VL CDR3 comprises a sequence of SEQ ID NO: 95, 109, 115, 121, 142, 154, 165, or 169;~~

~~the CD3 VH CDR1 comprises a sequence of SEQ ID NO: 2, 268, or 277; the CD3 VH CDR2 comprises a sequence of SEQ ID NO: 3, 10, 269, or 270; and the CD3 VH CDR3 comprises a sequence of SEQ ID NO: 4;~~

~~the CD3 VL CDR1 comprises a sequence of SEQ ID NO: 77, 85, 91, 278, 279, or 280; the CD3 VL CDR2 comprises a sequence of SEQ ID NO: 78 or 281; and the CD3 VL CDR3 comprises a sequence of SEQ ID NO: 79; and~~

~~the GUCY2c VH CDR1 comprises a sequence of SEQ ID NO: 12, 20, 27, 34, 42, 74, 257, 258, 259, 260, or 261; the GUCY2c VH CDR2 comprises a sequence of SEQ ID NO: 13, 21, 28, 35, 43, 53, 66, 68, 70, 72, 75, 262;~~

~~263, 264, 265, 266, or 267; and the GUCY2c VH CDR3 comprises a sequence of SEQ ID NO: 14, 22, 29, 36, or 44.~~

~~1715.~~ The bispecific antibody of claim ~~1614~~, wherein:

(i)

- a. the GUCY2c VH CDR1 comprises the sequence of SEQ ID NO: 74 or 259;
- b. the GUCY2c VH CDR2 comprises the sequence of SEQ ID NO: 75 or 267;
- c. the GUCY2c VH CDR3 comprises the sequence of SEQ ID NO: 29;
- d. the GUCY2c VL CDR1 comprises the sequence of SEQ ID NO: 148;
- e. the GUCY2c VL CDR2 comprises the sequence of SEQ ID NO: 149; and
- f. the GUCY2c VL CDR3 comprises the sequence of SEQ ID NO: 142;

and/or

(ii)

- a. the CD3 VH CDR1 comprises the sequence of SEQ ID NO: 2 or 268;
- b. the CD3 VH CDR2 comprises the sequence of SEQ ID NO: 10 or 270;
- c. the CD3 VH CDR3 comprises the sequence of SEQ ID NO: 4;
- d. the CD3 VL CDR1 comprises the sequence of SEQ ID NO: 91;
- e. the CD3 VL CDR2 comprises the sequence of SEQ ID NO: 78; and
- f. the CD3 VL CDR3 comprises the sequence of SEQ ID NO: 79.

~~18.~~ The bispecific antibody of any one of claims 6 to 17, wherein:

- a. ~~the GUCY2c VL comprises the sequence shown in SEQ ID NO: 92, 100, 104, 106, 112, 119, 125, 129, 134, 136, 137, 138, 140, 143, 145, 147, 150, 152, 156, 158, 160, 162, 166, 170, 171, 172, 173, 174, or 175;~~

- ~~b. the CD3 VH comprises the sequence shown in SEQ ID NOS: 1 or 9;~~
- ~~c. the CD3 VL comprises the sequence shown in SEQ ID NO: 76, 84 or 90; and/or~~
- ~~d. the GUCY2c comprises the sequence shown in SEQ ID NO: 11, 19, 26, 33, 41, 48, 52, 57, 60, 62, 64, 65, 67, 69, 71, or 73.~~

~~1916.~~ The bispecific antibody of any one of claims 5-15~~18~~, wherein:

(i)

- a. the GUCY2c VH region comprises a sequence of SEQ ID NO: 73; and
- b. the GUCY2c VL region comprises a sequence of SEQ ID NO: 147;

17. The bispecific antibody of any one of claims 5-16, wherein: -and/or

(ii)

- a. the CD3 VH region comprises the sequence of SEQ ID NO: 9; and
- b. the CD3 VL region comprises the sequence of SEQ ID NO: 90.

~~2018.~~ The bispecific antibody of any one of claims ~~6-5~~ to ~~14~~17, wherein the antibody binds to an epitope on GUCY2c extracellular domain, wherein the epitope comprises at least one amino acid residue selected from amino acid residues R73, S74, S75, T76, E78, G79, L80, L82, L83, R84 or I86 of SEQ ID NO: 406.

~~2119.~~ A bispecific antibody that specifically binds to GUCY2c and CD3, wherein the bispecific antibody comprises a first polypeptide chain and a second polypeptide chain, and wherein:

- a. the first polypeptide chain comprises the following regions in the following order in an N-terminal to C-terminal direction:

a VL of a GUCY2c antibody (GUCY2c VL) (SEQ ID NO: 147) – Linker 1 (SEQ ID NO: 190) – a VH of a CD3 antibody (CD3 VH) (SEQ ID NO: 9) – Linker 3 (SEQ ID NO: 192) – a first heterodimer-promoting domain (SEQ ID NO: 188); and

b. the second polypeptide chain comprises the following regions in the following order in an N-terminal to C-terminal direction:

a VL of a CD3 antibody (CD3 VL) (SEQ ID NO: 90) – Linker 2 (SEQ ID NO: 191) – a VH of a GUCY2c antibody (GUCY2c VH) (SEQ ID NO: 73)- Linker 3 (SEQ ID NO: 192) – a second heterodimer-promoting domain (SEQ ID NO: 189).

2220. The bispecific antibody of claim 2119, wherein the GUCY2c VL and the GUCY2c VH form a domain that specifically binds to GUCY2c; and the CD3 VL and the CD3 VH form a domain that specifically binds to CD3; wherein Linker 3 of the first polypeptide chain and Linker 3 of the second polypeptide chain are covalently bound to one another by two disulfide bonds; wherein the first heterodimer-promoting domain and the second heterodimer-promoting domain each comprise a CH2 domain and a CH3 domain; wherein the CH3 domain of the first heterodimer-promoting domain forms a knob, and the CH3 domain of the second heterodimer-promoting domain forms a hole; and wherein at least one disulfide bond is formed between the CH3 domain of the first heterodimer-promoting domain and the CH3 domain of the second heterodimer-promoting domain.

2321. A bispecific antibody that specifically binds to GUCY2c and CD3, wherein the bispecific antibody comprises a first polypeptide chain and a second polypeptide chain; wherein the first polypeptide chain is produced by the expression vector with ATCC Accession No. PTA-124944; and the second polypeptide chain is produced by the expression vector with ATCC Accession No. PTA-124943.

2422. A bispecific antibody capable of specific binding to GUCY2c and to CD3 comprising a first polypeptide chain and a second polypeptide chain, wherein the first polypeptide chain comprises a sequence of SEQ ID NO: 216 and the second polypeptide chain comprises a sequence of SEQ ID NO: 220.

2523. The antibody of any one of claims 1 to 54, or the bispecific antibody of any one of claims 6-5 to 2422, wherein the antibody or bispecific antibody:

- a. binds to extracellular domain of human GUCY2c;
- b. demonstrates an extended serum and tumor half-life of between 30 min to 100 days; and/or
- c. demonstrates a lower EC₅₀ value of between 0.0001 nM and 100 nM in the presence of increased GUCY2c expression levels or increased receptor density levels.

2624. A pharmaceutical composition comprising a therapeutically effective amount of the antibody of any one of claims 1 to 54, or the bispecific antibody of any one of claims 6-5 to 2523, and a pharmaceutically acceptable carrier.

2725. A polynucleotide comprising a nucleotide sequence encoding the antibody of any one of claims 1 to 54, or the bispecific antibody of any one of claims 6-5 to 2523.

2826. A vector comprising the polynucleotide of claim 2725.

2927. A host cell comprising the vector of claim 2826; optionally wherein the host cell recombinantly produces the antibody of any one of claims 1 to 54, or the bispecific antibody of any one of claims 6-5 to 2523; further optionally wherein the host cell is selected from the group consisting of bacterial cell lines, mammalian cell lines, insect cell lines, and yeast cell lines; optionally wherein the mammalian cell line is a CHO cell line.

3028. A method of producing an antibody or a bispecific antibody, comprising culturing the host cell of claim 2927, under conditions that result in production of the antibody of any one of claims 1 to 54, or the bispecific antibody of any of claim 6-5 to 2523, and purifying the antibody or the bispecific antibody from the culture supernatant.

3129. The antibody of any one of claims 1 to 54, the bispecific antibody of any one of claims 6-5 to 2523, or the pharmaceutical composition of claim 2624, for use in therapy.

3230. The antibody of any one of claims 1 to 54, the bispecific antibody of any one of claims 6-5 to 2523, or the pharmaceutical composition of claim 2624, for use in the treatment of a GUCY2c associated disorder.

~~33~~31. The antibody, bispecific antibody or the pharmaceutical composition for use according to claim ~~32~~30, wherein the GUCY2c associated disorder is cancer, optionally wherein the cancer is a cancer of digestive system selected from the group consisting of esophagus, stomach, small intestine, colon, rectum, anus, liver, gall bladder, appendix, bile ducts, and pancreas.

~~34~~32. The antibody, bispecific antibody or the pharmaceutical composition for use according to claim ~~32~~30 or ~~33~~31, wherein the treatment activates a cytolytic T cell response.

~~35~~33. A composition comprising the bispecific antibody of any one of claim ~~6~~5 to ~~25~~23 and a second therapeutic agent; optionally wherein the second agent is an antidiarrheal agent.

~~36~~34. The composition of claim ~~35~~33 for use in the treatment of a GUCY2c associated disorder; optionally wherein the GUCY2c associated disorder is cancer, further optionally wherein the cancer is a cancer of digestive system selected from the group consisting of esophagus, stomach, small intestine, colon, rectum, anus, liver, gall bladder, appendix, bile ducts, and pancreas.