

ANTI-TROP2 ANTIBODIES

TECHNICAL FIELD

5 The present disclosure relates broadly to antibodies and antigen-binding fragments thereof specific to trophoblast cell surface antigen 2 (Trop2). In particular, the present disclosure encompasses the nucleotide and amino-acid sequences of 6 human antibodies, or the antigen-binding portions thereof, that specifically target human Trop2, both in solution and on the surface of cells. Also provided are Trop2 antibody-
10 drug conjugates (ADCs) and multispecific antigen binding proteins, such as bispecific T cell engagers (BiTEs), which bind to Trop2 and also other targets.

BACKGROUND

 In 2020, there was an estimated 19.3 million of new cancers reported worldwide,
15 and the cancer incidence is expected to rise to 28.4 million cases in 2040. Among all adult human cancer cases, approximately 90% are solid tumors. Solid tumors such as lung cancer, liver cancer, stomach cancer and female breast cancer are among the leading causes of cancer-related death. The global high cancer incidence burden, coupled with poor treatment outcomes and survival rates with standard treatment
20 modalities of surgery, chemotherapy and radiotherapy presents a strong need for the development of more effective treatment options for cancer.

 Antibody-drug conjugates (ADCs) are now amongst the fastest growing drug classes in oncology, as they combine the best features of monoclonal antibodies and small molecule drugs to create a single moiety that is highly specific and cytotoxic. Many
25 ADCs have demonstrated impressive activity against treatment-refractory cancers, resulting in their approval for both hematologic malignancies and solid tumor indications.

 Trophoblast cell surface antigen 2 (Trop2), also known as tumor-associated calcium signal transducer 2, is a 36-kDa single pass transmembrane protein that is highly expressed in a variety of epithelial cancers. It has been demonstrated that Trop2 is
30 involved in multiple intracellular signalling including MAPK and PI3K/AKT pathways which are associated with proliferation, migration, and invasion of cancer cells. Overexpression of Trop2 correlates with poor prognosis in several types of cancers, including breast cancer and non-small cell lung cancer (NSCLC). These characteristics

make Trop2 an attractive target for cancer therapy. The anti-Trop2 ADC "Trodelvy" developed by ImmunoMedics is one of the latest ADCs approved in 2021. It is the first and the only ADC to treat the devastating metastatic triple negative breast cancer (TNBC). It was subsequently approved to treat the metastatic urothelial cancer in the same year. Apart from its success in various clinical studies, "Trodelvy" represents one of the largest transactions in the biotech history: a \$21 billion acquisition by Gilead in 2020. AstraZeneca also entered a \$1 billion upfront deal with Daiichi Sankyo to co-develop an ADC against Trop2 DS-1062, with a further \$5 billion in milestones.

Anti-Trop2 ADCs are thus far approved only for TNBC and urothelial cancer, hence there is a need for additional and superior anti-Trop2 ADCs that can treat these indications as well as other cancers.

SUMMARY

The present inventors have successfully identified four anti-Trop2 antibody clones from their naïve human Fab phage libraries, two of them were further engineered to their germline configured counterparts. One or more of these antibodies displayed 1) superior binding affinity and specificity to the Trop2 protein, both in solution and expressed on the cell surface; 2) superior cytotoxic potency when used in ADCC; 3) superior growth inhibition potency when used as ADC; 4) strong cytotoxic potency when used as bi-specific T cell engager (BiTE) antibodies; and 5) strong cytotoxicity potency when used in CAR T cell therapy.

Thus, in one aspect, there is provided an anti-Trop2 antibody or antigen-binding fragment thereof, comprising the CDR sequences selected from the group comprising:

- (i) a CDRH1 sequence of GGSISSGGYY (SEQ ID NO: 1),
a CDRH2 sequence of IYYSGST (SEQ ID NO: 5),
a CDRH3 sequence of AREEGIAAAAFDI (SEQ ID NO: 9),
a CDRL1 sequence of QSVGSF (SEQ ID NO: 13),
a CDRL2 sequence of GAS (SEQ ID NO: 17), and
a CDRL3 sequence of QQSDSSPFT (SEQ ID NO: 19);
- (ii) a CDRH1 sequence of GYTFTSYG (SEQ ID NO: 2),
a CDRH2 sequence of ISAYNGNT (SEQ ID NO: 6),
a CDRH3 sequence of ARKYSGFDY (SEQ ID NO: 10)
a CDRL1 sequence of QSLHNSNGYNY (SEQ ID NO: 14)

- a CDRL2 sequence of LGS (**SEQ ID NO: 18**); and
 a CDRL3 sequence of MQNLQTPWT (**SEQ ID NO: 20**)
- (iii) a CDRH1 sequence of GFTFSSYS (**SEQ ID NO: 3**),
 a CDRH2 sequence of ISSSSSYI (**SEQ ID NO: 7**),
 a CDRH3 sequence of ARDYYDSSGYPPYYGMDV (**SEQ ID NO: 11**),
 a CDRL1 sequence of QSVSSSY (**SEQ ID NO: 15**),
 a CDRL2 sequence of GAS (**SEQ ID NO: 17**), and
 a CDRL3 sequence of HQSGSSLRT (**SEQ ID NO: 21**); and
- (iv) a CDRH1 sequence of GFTFSSYW (**SEQ ID NO: 4**),
 a CDRH2 sequence of IKQDGSEK (**SEQ ID NO: 8**),
 a CDRH3 sequence of ARDFVDWSATPFDY (**SEQ ID NO: 12**),
 a CDRL1 sequence of RSLLHSNGYNY (**SEQ ID NO: 16**),
 a CDRL2 sequence of LGS (**SEQ ID NO: 18**), and
 a CDRL3 sequence of MQALQIPKT (**SEQ ID NO: 22**).
- In one embodiment, the antibody or antigen-binding fragment thereof is a germline configured counterpart thereof.
- In one embodiment, the antibody or antigen-binding fragment thereof comprises a VH domain sequence selected from the group comprising:
- QVQLQESGPGLVKPSQTLSTCAVSGGSISSGGYYWSWIRQPPGKGLEWIGY
 IYYSGSTYYNP SLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREEGIAAA
 AFDIWGQGTM (**SEQ ID NO: 23**),
 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWI
 SAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARKYSGF
 DYWGQGTL (**SEQ ID NO: 24**),
 EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWI
 SAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARKYSGF
 DYWGQGTL (**SEQ ID NO: 25**), and
 EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSI
 SSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDYYDSS
 GYPYYYYGMDVWGQGT (**SEQ ID NO: 26**),
 EVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWWAN
 IKQDGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDFVD
 WSATPFDYWGQGT (**SEQ ID NO: 27**),

or a variant thereof at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

In one embodiment, the antibody or antigen-binding fragment thereof comprises a VL domain sequence selected from the group comprising:

- 5 DIQMTQSPSSLSASVGDRVTITCRASQSVGSFLNWWYQQKPGKAPKLLIYGASS
LQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSDSSPFTFGGGTK
(SEQ ID NO: 28),
EIVLTQSPSSLSASVGDRVTITCRASQSVGSFLNWWYQQKPGKAPQVLIFGASN
LESGVPSRFSGRGSGSEFTLTINSLQPEDFATYYCQQSDSSPFTFGGGTK
10 (SEQ ID NO: 29),
DIVMTQSPSLPVTTPGEPASISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLI
YLGSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQNLQTPWTFG
QGTK (SEQ ID NO: 30),
EIVLTQSPSLPVTTPGEPASISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIY
15 LGSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQNLQTPWTFGQG
TK (SEQ ID NO: 31),
ETTLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGA
SSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQSGSSLRTFGQGTT
(SEQ ID NO: 32), and
20 DVVMTQSPSLPVTTPGEPASISCRSSRSLHNSNGYNYLDWYVQKPGQSPQLLI
YLGSYRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQIPKTFGQ
GTK (SEQ ID NO: 33),

or a variant thereof at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

- 25 In one embodiment, the antibody or antigen-binding fragment thereof comprises a VH domain and a VL domain selected from the group comprising:

- (i) a VH domain sequence of:
QVQLQESGPGLVKPSQTLSTCAVSGGSISGGYYWSWIRQPPGKGL
EWIGYIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYY
30 CAREEGIAAAAFDIWGQGT (SEQ ID NO: 23) or a variant thereof with
at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto,
and
a VL domain sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQSVGSFLNWWYQQKPGKAPKLLI
YGASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSDSSPF
TFGGGTK (SEQ ID NO: 28) or a variant thereof with at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identical thereto;

5 (ii) a VH domain sequence of:
QVQLQESGPGLVKPSQTLSTCAVSGGSISSGGYYWSWIRQPPGKGL
EWIGYIYYSGSTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYY
CARREGIAAAAFDIWGQGT (SEQ ID NO: 23) or a variant thereof at
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto,
10 and
a VL domain sequence of:
EIVLTQSPSSLSASVGDRVTITCRASQSVGSFLNWWYQQKPGKAPQVLI
FGASNLESGVPSRFSGRGSGSEFTLTINSLQPEDFATYYCQQSDSSP
FTFGGGTK (SEQ ID NO: 29) or a variant thereof at least 80%, 85%,
15 90%, 95%, 96%, 97%, 98%, or 99% identical thereto;

(iii) a VH domain sequence of:
QVQLVQSGAEVKKPGASVKVCKASGYTFTSYGISWVRQAPGQGLE
WMGWISAYNGNTNYAQLQGRVTMTTDTSTSTAYMELRSLRSDDTA
VYYCARKYSGFDYWGQGT (SEQ ID NO: 24) or a variant thereof at
20 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto,
and
a VL domain sequence of:
DIVMTQSPLSLPVTPGEPASISCRSSQSLHNSGYNLYLDWYLQKPGQS
PQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQ
25 NLQTPWTFGQGTK (SEQ ID NO: 30) or a variant thereof at least 80%,
85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto;

(iv) a VH domain sequence of:
EVQLVQSGAEVKKPGASVKVCKASGYTFTSYGISWVRQAPGQGLE
WMGWISAYNGNTNYAQLQGRVTMTTDTSTSTAYMELRSLRSDDTA
30 VYYCARKYSGFDYWGQGT (SEQ ID NO: 25) or a variant thereof at
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto,
and
a VL domain sequence of:

- EIVLTQSPLSLPVTGPGEPAISCRSSQSLHNSNGYNYLDWYLQKPGQS
PQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQN
LQTPWTFGQGTK **(SEQ ID NO: 31)** or a variant thereof at least 80%,
85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto;
- 5 (v) a VH domain sequence of:
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLE
WVSSISSSSYIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYY
CARDYYDSSGYPYYYYGMDVWGQGT **(SEQ ID NO: 26)** or a variant
thereof at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical
thereto, and
- 10 a VL domain sequence of:
ETTLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRL
LIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCHQSGSSL
RTFGQGT **(SEQ ID NO: 32)** or a variant thereof at least 80%, 85%,
15 90%, 95%, 96%, 97%, 98%, or 99% identical thereto; and
- (vi) a VH domain sequence of:
EVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLE
WWANIKQDGSEKYYVDSVKGRFTISRDNKNSLYLQMNSLRAEDTAV
YYCARDFVDWSATPFDYWGQGT **(SEQ ID NO: 27)** or a variant thereof
20 at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto,
and
- a VL domain sequence of:
DVVMTQSPLSLPVTGPGEPAISCRSSRSLHNSNGYNYLDWYVQKPGQ
SPQLLIYLGSRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQ
25 ALQIPKTFGQGTK **(SEQ ID NO: 33)** or a variant thereof at least 80%,
85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.
- In one embodiment, the antibody is a full-length antibody.
- In one embodiment, the antibody is an IgG antibody, such as an IgG1 antibody.
- In one embodiment, the antibody or antigen-binding fragment thereof is a fully
30 human antibody or antigen-binding fragment thereof.
- In one embodiment, the antibody or antigen-binding fragment thereof is specific
to human Trop2.

In one embodiment, the antibody or antigen-binding fragment thereof exhibits cross-reactivity with more two or more different species.

In one embodiment, the antibody or antigen-binding fragment thereof binds to human Trop2 and also cynomolgus Trop2.

- 5 In one embodiment, the antibody or antigen-binding fragment thereof is conjugated to a therapeutic agent, for example wherein the antibody or antigen-binding fragment thereof is an antibody-drug conjugate (ADC).

In one embodiment, the therapeutic agent is an anti-cancer agent, for example a chemotherapeutic agent.

- 10 In one embodiment, the therapeutic agent is selected from the group comprising MMAE (vedotin), PNU-159682, DX-8951 (exatecan), PBD (pyrrolobenzodiazepine) and DMDM.

In one aspect, there is provided an antibody-drug conjugate (ADC) comprising the antibody or antigen-binding fragment thereof as described above.

- 15 In one aspect, there is provided a multi-specific antibody comprising an anti-Trop2 binding domain, wherein the anti-Trop 2 binding domain is an antibody or antigen-binding fragment thereof as defined above.

In one embodiment, the multi-specific antibody is a bispecific antibody.

In one embodiment, the multi-specific antibody is an immune cell engager.

- 20 In one embodiment, the immune cell engager binds to an immune marker selected from the group consisting of CD3, NKG2D, CD4, CD8, CD16 and CD64.

In one embodiment, the immune cell engager is selected from the group comprising a T cell engager, an NK cell engager, a monocyte engager and a macrophage engager.

- 25 In one embodiment, the immune cell engager is a bispecific T cell engager (BiTE), such as an inducible BiTE, a non-inducible BiTE, or a constitutive expression BiTE.

In one aspect, there is provided an immune cell comprising an anti-Trop2 antibody or antigen-binding fragment thereof or multi-specific antibody as defined above.

- 30 In one embodiment, the immune cell is selected from the group comprising a T-cell, a CAR T-cell, a natural killer (NK) cell, a monocyte and a macrophage, in particular a CAR T-cell.

In one aspect, there is provided a pharmaceutical composition comprising the antibody or antigen-binding fragment thereof, the antibody drug conjugate, the multi-

specific antibody, or the immune cell as defined above, wherein the composition optionally comprises a pharmaceutically acceptable excipient, buffer and/or additive.

In one aspect, there is provided a polynucleotide encoding an anti-Trop2 antibody or antigen-binding fragment thereof, an antibody drug conjugate or a multi-specific antibody as defined above.

In one embodiment, the polynucleotide comprises a sequence selected from the group comprising:

- i. CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTC
ACAGACCCTGTCCCTCACCTGCGCTGTCTCTGGTGGCTCCATCAG
CAGTGGTGGTTACTACTGGAGTTGGATCCGCCAGCCCCCGGGAA
GGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTA
CTACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACG
TCTAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACTGCCGCG
GACACGGCCGTGTATTACTGTGCGAGAGAAGAGGGTATAGCAGCA
GCTGCTTTTGATATCTGGGGCCAAGGGACAATG (SEQ ID NO: 34) or
a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,
or 99% identity thereto,
- ii. CAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGG
GCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCA
GCTACGGTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTG
AGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAAACTATGC
ACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCAC
GAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC
GGCCGTGTATTACTGTGCGAGAAAATACAGTGGCTTTGACTACTGG
GGCCAGGGCACCCTG (SEQ ID NO: 35) or a variant thereof having at
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
- iii. GAGGTCCAGCTGGTaCAGTCTGGAGCTGAGGTGAAGAAGCCTGGG
GCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCA
GCTACGGTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTG
AGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAAACTATGC
ACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCAC
GAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC
GGCCGTGTATTACTGTGCGAGAAAATACAGTGGCTTTGACTACTGG

- GGCCAGGGCACCCCTG (**SEQ ID NO: 36**) or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
- iv. GAGGTCCAGCTGGTGCAGTCTGGGGGAGGCCTGGTCAAGCCTGG
GGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGT
5 AGCTATAGCATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTG
GAGTGGGTCTCATCCATTAGTAGTAGTAGTTACATATACTACG
CAGACTCAGTGAAGGGCCGATTACCCTCTCCAGAGACAACGCCA
AGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
CGGCTGTTTATTACTGTGCGAGAGATTACTATGATAGTAGTGGCTA
10 TCCCTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC
G (**SEQ ID NO: 37**) or a variant thereof having at least 80%, 85%, 90%,
95%, 96%, 97%, 98%, or 99% identity thereto,
- v. GAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTCCAGCCTGG
GGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTTAGT
15 AGCTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGCTG
GAGTGGGTGGCCAACATAAAGCAAGATGGAAGTGAGAAATACTAT
GTGGACTCTGTGAAGGGCCGATTACCCTCTCCAGAGACAACGCC
AAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
CGGCTGTGTATTACTGTGCGAGGGATTTGTGGACTGGTCCGCCA
20 CACCCTTTGACTACTGGGGCCAGGGCACCCCTG (**SEQ ID NO: 38**) or
a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,
or 99% identity thereto,
- vi. GACATTCAGATGACGCAGTCTCCATCCTCCCTGTCTGCTTCTGTGG
GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCGTTGGCT
25 CTTTTTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCT
CCTGATCTATGGTGCCTCCAGTTTACAGAGTGGGGTCCCATCAAGG
TTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCA
GTCTGCAACCCGAAGATTTTGAACCTTACTACTGTCAACAGAGTGA
CAGTTCCCCCTTCACTTTCCGGCGAGGGACCAAG (**SEQ ID NO: 39**)
30 or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%,
98%, or 99% identity thereto,
- vii. GAAATTGTGTTGACGCAGTCTCCATCCTCCCTGTCTGCTTCTGTGG
GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCGTTGGCT

CTTTTTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTCAGGT
CCTGATCTTTGGTGCCTCCAATTTAGAAAGTGGGGTCCCATCAAGG
TTCAGTGGCAGAGGATCTGGGTCAGAATCACTCTCACCATCAACA
GTCTGCAACCCGAAGATTTTGCAACTTACTACTGTCAACAGAGTGA
5 CAGTTCCCCCTTCACTTTTCGGCGGAGGGACCAAG (**SEQ ID NO: 40**)
or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%,
98%, or 99% identity thereto,

viii. GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGTCTCCTGC
10 ATAGTAATGGATACAACTATTTGGATTGGTACCTGCAGAAGCCAGG
GCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCC
GGGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTT
ACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATT
ACTGCATGCAAAATCTTCAAACCTCCGTGGACGTTTCGGCCAAGGGAC
15 CAAG (**SEQ ID NO: 41**) or a variant thereof having at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identity thereto,

ix. GAAATTGTGCTGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGTCTCCTGC
20 ATAGTAATGGATACAACTATTTGGATTGGTACCTGCAGAAGCCAGG
GCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCC
GGGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTT
ACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGATTTATT
ACTGCATGCAAAATCTTCAAACCTCCGTGGACGTTTCGGCCAAGGGAC
25 CAAG (**SEQ ID NO: 42**) or a variant thereof having at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identity thereto,

x. GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG
GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCA
GCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCA
GGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCAG
30 ACAGGTTCACTGGCAGTGGGTCTGGGACAGACTTCAATCTCACCAT
CAGCAGACTGGAGCCTGAAGATTTTGAGTGTATTACTGTCAACAG
TCTGGCAGTTCACTTCGGACGTTTCGGCCAAGGGACCACG (**SEQ ID**

NO: 43) or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, and

xi. GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
 GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCGAGCCTCCTAC
 5 ATAGTAATGGATACAACCTATTTGGATTGGTACGTGCAGAAGCCAGG
 GCAGTCTCCACAGCTCCTGATCTATTTGGGTTTCATATCGGGCCTCC
 GGGGTCCCTGACAGGTTTAGTGGCAGTGGATCAGGCACAGATTTT
 AACTGAAGATCAGCAGGGTGGAGGCTGAGGATGTTGGAGTTTAT
 TACTGCATGCAAGCTTTACAAATTCCGAAGACGTTCCGCCAAGGGA
 10 CCAAG (**SEQ ID NO: 44**) or a variant thereof having at least 80%, 85%,
 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

In one aspect, there is provided a vector comprising one or more polynucleotides as defined above.

In one embodiment, the vector comprises a polynucleotide selected from the
 15 group comprising:

(i) a polynucleotide having the sequence:
 CAGGTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGCCTTC
 ACAGACCCTGTCCCTCACCTGCGCTGTCTCTGGTGGCTCCATCAG
 CAGTGGTGGTTACTACTGGAGTTGGATCCGCCAGCCCCCGGGAA
 20 GGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTA
 CTACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACG
 TCTAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACTGCCGCG
 GACACGGCCGTGTATTACTGTGCGAGAGAAGAGGGTATAGCAGCA
 GCTGCTTTTGATATCTGGGGCCAAGGGACAATG (**SEQ ID NO: 34**) or
 25 a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,
 or 99% identity thereto, and
 a polynucleotide having the sequence:
 GACATTCAGATGACGCAGTCTCCATCCTCCCTGTCTGCTTCTGTGG
 GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCGTTGGCT
 30 CTTTTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCCTAAGCT
 CCTGATCTATGGTGCCTCCAGTTTACAGAGTGGGGTCCCATCAAGG
 TTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCA
 GTCTGCAACCCGAAGATTTTGCAACTTACTACTGTCAACAGAGTGA

CAGTTCCCCCTTCACTTTTCGGCGGAGGGACCAAG (SEQ ID NO: 39)

or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(ii) a polynucleotide having the sequence:

5 CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTC
ACAGACCCTGTCCCTCACCTGCGCTGTCTCTGGTGGCTCCATCAG
CAGTGGTGGTTACTACTGGAGTTGGATCCGCCAGCCCCCGGGAA
GGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTA
CTACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACG
10 TCTAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACTGCCGCG
GACACGGCCGTGTATTACTGTGCGAGAGAAGAGGGTATAGCAGCA
GCTGCTTTTGATATCTGGGGCCAAGGGACAATG (SEQ ID NO: 34) or
a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,
or 99% identity thereto, and

15 a polynucleotide having the sequence:

GAAATTGTGTTGACGCAGTCTCCATCCTCCCTGTCTGCTTCTGTGG
GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCGTTGGCT
CTTTTTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCCTCAGGT
CCTGATCTTTGGTGCCTCCAATTTAGAAAGTGGGGTCCCATCAAGG
20 TTCAGTGGCAGAGGATCTGGGTCAGAATTCAGTCTACCATCAACA
GTCTGCAACCCGAAGATTTTGCAACTTACTACTGTCAACAGAGTGA
CAGTTCCCCCTTCACTTTTCGGCGGAGGGACCAAG (SEQ ID NO: 40)
or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%,
98%, or 99% identity thereto;

25 (iii) a polynucleotide having the sequence:

CAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGG
GCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCA
GCTACGGTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTG
AGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGC
30 ACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCAC
GAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC
GGCCGTGTATTACTGTGCGAGAAAATACAGTGGCTTTGACTACTGG
GGCCAGGGCACCCCTG (SEQ ID NO: 35) or a variant thereof having at

least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
and

a polynucleotide having the sequence:

5 GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGTCTCCTGC
ATAGTAATGGATACAACTATTTGGATTGGTACCTGCAGAAGCCAGG
GCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCC
GGGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTT
ACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATT
10 ACTGCATGCAAAATCTTCAAACCTCCGTGGACGTTCGGCCAAGGGAC
CAAG (SEQ ID NO: 41) or a variant thereof having at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(iv) a polynucleotide having the sequence:

15 GAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGG
GCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCA
GCTACGGTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTG
AGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGC
ACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCAC
GAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC
20 GGCCGTGTATTACTGTGCGAGAAAATACAGTGGCTTTGACTACTGG
GGCCAGGGCACCCTG (SEQ ID NO: 36) or a variant thereof having at
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
and

a polynucleotide having the sequence:

25 GAAATTGTGCTGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGTCTCCTGC
ATAGTAATGGATACAACTATTTGGATTGGTACCTGCAGAAGCCAGG
GCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCC
GGGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTT
30 ACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGATTTATT
ACTGCATGCAAAATCTTCAAACCTCCGTGGACGTTCGGCCAAGGGAC
CAAG (SEQ ID NO: 42) or a variant thereof having at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

- (v) a polynucleotide having the sequence:
 GAGGTCCAGCTGGTGCAGTCTGGGGGAGGCCTGGTCAAGCCTGG
 GGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGT
 AGCTATAGCATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTG
 5 GAGTGGGTCTCATCCATTAGTAGTAGTAGTAGTTACATATACTACG
 CAGACTCAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCA
 AGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
 CGGCTGTTTATTACTGTGCGAGAGATTACTATGATAGTAGTGGCTA
 TCCCTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC
 10 G (**SEQ ID NO: 37**) or a variant thereof having at least 80%, 85%, 90%,
 95%, 96%, 97%, 98%, or 99% identity thereto, and
 a polynucleotide having the sequence:
 GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG
 GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCA
 15 GCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCA
 GGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCAG
 ACAGGTTCACTGGCAGTGGGTCTGGGACAGACTTCAATCTCACCAT
 CAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAACAG
 TCTGGCAGTTCACTTCGGACGTTCCGCCAAGGGACCACG (**SEQ ID**
 20 **NO: 43**) or a variant thereof having at least 80%, 85%, 90%, 95%, 96%,
 97%, 98%, or 99% identity thereto; and
 (vi) a polynucleotide having the sequence:
 GAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTCCAGCCTGG
 GGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTAGT
 25 AGCTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTG
 GAGTGGGTGGCCAACATAAAGCAAGATGGAAGTGAGAAATACTAT
 GTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCC
 AAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
 CGGCTGTGTATTACTGTGCGAGGGATTTTGTGGACTGGTCCGCCA
 30 CACCCTTTGACTACTGGGGCCAGGGCACCCTG (**SEQ ID NO: 38**) or
 a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,
 or 99% identity thereto, and
 a polynucleotide having the sequence:

GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
 GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCGGAGCCTCCTAC
 ATAGTAATGGATACAACCTATTTGGATTGGTACGTGCAGAAGCCAGG
 GCAGTCTCCACAGCTCCTGATCTATTTGGGTTTCATATCGGGCCTCC
 5 GGGGTCCCTGACAGGTTTAGTGGCAGTGGATCAGGCACAGATTTT
 ACACTGAAGATCAGCAGGGTGGAGGCTGAGGATGTTGGAGTTTATT
 ACTGCATGCAAGCTTTACAAATTCCGAAGACGTTTCGGCCAAGGGAC
 CAAG (SEQ ID NO: 44) or a variant thereof having at least 80%, 85%,
 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

10 In one aspect, there is provided a host cell comprising a vector as defined above.

In one aspect, there is provided a method of treating a patient in need thereof,
 comprising administering the antibody or antigen-binding fragment thereof, the antibody
 drug conjugate, the multi-specific antibody, the immune cell or the pharmaceutical
 composition as defined above.

15 In one aspect, there is provided an antibody or antigen-binding fragment thereof,
 an antibody drug conjugate, a multi-specific antibody, an immune cell or a
 pharmaceutical composition as defined above for use in the treatment of a patient.

In one aspect, there is provided a use of an antibody or antigen-binding fragment
 thereof, an antibody drug conjugate, a multi-specific antibody, an immune cell or a
 20 pharmaceutical composition as defined above in the manufacture of a medicament for
 the treatment of a patient, for example for the treatment of cancer.

In one embodiment, the patient has cancer.

In one embodiment, the cancer is Trop2+.

In one aspect, there is provided a method of diagnosing a tumor expressing Trop2
 25 comprising contacting the tumor with an antibody or antigen-binding fragment thereof,
 multi-specific antibody or a composition as defined above.

In one embodiment, the cancer is selected from the group comprising gastric
 adenocarcinoma, colon cancer, pancreatic cancer and breast cancer.

30 DEFINITIONS

The term "antibody" as used herein relates to whole (i.e., full length) antibodies
 (i.e., comprising the elements of two heavy chains and two light chains) and functionally
 active fragments thereof (i.e., molecules that contain an antigen binding domain that

specifically binds an antigen, also termed antibody fragments or antigen-binding fragments). Features described herein with respect to antibodies also apply to antibody fragments unless context dictates otherwise. The term "antibody" encompasses monovalent, i.e., antibodies comprising only one antigen binding domain (e.g., one-armed antibodies comprising a full-length heavy chain and a full-length light chain interconnected, also termed "half-antibody"), and multivalent antibodies, i.e., antibodies comprising more than one antigen binding domain, e.g., bivalent.

The term "antigen-binding fragment" as employed herein refers to functionally active antibody binding fragments including but not limited to Fab, modified Fab, Fab', modified Fab', F(ab')₂, Fv, single domain antibodies, scFv, Fv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above.

A "binding fragment" as employed herein refers to a fragment capable of binding a target peptide or antigen with sufficient affinity to characterize the fragment as specific for the peptide or antigen.

The term "monoclonal antibody" (or "mAb") refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. each individual of a monoclonal antibody preparation are identical except for possible mutations (e.g., naturally occurring mutations), that may be present in minor amounts. Certain differences in the protein sequences linked to post-translational modifications (for example, cleavage of the heavy chain C-terminal lysine, deamidation of asparagine residues and/or isomerization of aspartate residues) may nevertheless exist between the various different antibody molecules present in the composition. Contrary to polyclonal antibody preparations, each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen.

The term "diabody" as employed herein refers to two Fv pairs, a first VH/VL pair and a further VH/VL pair which have two inter-Fv linkers, such that the VH of a first Fv is linked to the VL of the second Fv and the VL of the first Fv is linked to the VH of the second Fv.

As used herein, the term "nanobody" refers to a single domain antibody (sdAb), with an antibody fragment consisting of a single monomeric variable antibody domain.

The term "triabody" (also referred to a Fab(scFv)₂) as employed herein refers to a Fab fragment with a first scFv appended to the C-terminal of the light chain and a

second scFv appended to the C-terminal of the heavy chain. The term "tetrabody" as employed herein refers to a format similar to the diabody comprising four Fvs and four inter-Fv linkers.

The term "multivalent antibody" refers to an antibody comprising more than one antigen binding domain e.g., bivalent.

The term "Fv" refers to two variable domains of full-length antibodies, for example co-operative variable domains, such as a cognate pair or affinity matured variable domains, i.e., a VH and VL pair. The term "scFv" refers to single chain variable fragment which is a fusion protein of the variable regions of the heavy and light chains of the immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. The term "bis-scFv" as described herein refers to a bispecific scFv.

The term "dsscFv" or "disulphide-stabilised single chain variable fragment" as employed herein refers to a single chain variable fragment which is stabilised by a peptide linker between the VH and VL variable domain and also includes an inter-domain disulphide bond between VH and VL.

The term "DVD-Ig" (also known as dual V domain IgG) refers to a full-length antibody with 4 additional variable domains, one on the N-terminus of each heavy and each light chain.

The term "Fab" refers to as used herein refers to an antibody fragment comprising a light chain fragment comprising a VL (variable light) domain and a constant domain of a light chain (CL), and a VH (variable heavy) domain and a first constant domain (CH1) of a heavy chain. Dimers of a Fab' according to the present disclosure create a F(ab')₂ where, for example, dimerization may be through the hinge. The term "F(ab')₂" refers to a monovalent fragment of a single light chain homodimer, which is obtained by pepsin digestion of IgG, followed by reduction of the light chain disulfide bond. The term "F(ab')₂" as described herein refers to a fragment of IgG that is prepared by pepsin digestion of IgG. The F(ab')₂ fragment is a disulfide-linked homodimer of the two light chain dimers, so it retains bivalent epitope binding like whole IgG, but as it lacks the heavy chains, it is smaller in size compared to a whole IgG. F(ab')₂ and F(ab') fragments do not bind to immunoglobulin receptors on cells, which can be useful for achieving specific staining of the primary antibody target.

The term "DiFab" as employed herein refers to two Fab molecules linked via their C-terminus of the heavy chains or two Fab' molecules linked via one or more disulfide bonds in the hinge region thereof.

The term "antigen binding variant" refers to a polypeptide, for example, an antibody possessing the desired characteristics described herein and comprising a VH and/or a VL that has at least about 80% amino acid sequence identity with a VH and/or a VL of the reference antibody. Such antibody variants include, for instance, antibodies wherein one or more amino acid residues are added to or deleted from the VH and/or a VL domain. Ordinarily, an antibody variant will have at least about 80% amino acid sequence identity, alternatively at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity, to an antibody described herein. Optionally, variant antibodies will have no more than one conservative amino acid substitution as compared to an antibody sequence provided herein, alternatively no more than about any of 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions as compared to an antibody sequence provided herein.

The term "specifically" as employed herein in the context of antibodies is intended to refer to an antibody that only recognizes the antigen to which it is specific or an antibody that has significantly higher binding affinity to the antigen to which it is specific compared to binding to antigens to which it is non-specific, for example at least 5, 6, 7, 8, 9, 10 times higher binding affinity.

The term "epitope" or "binding site" in the context of antibodies refers to a site (or a part) on an antigen to which the paratope of an antibody binds or recognizes. Epitopes can be formed both from contiguous amino acids (also often called "linear epitopes") or noncontiguous amino acids formed by tertiary folding of a protein (often called "conformational epitopes"). Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5-10 amino acids in a unique spatial conformation. Epitopes usually consist of chemically active surface groups of molecules such as amino acids, sugar side chains and usually have specific 3D structural and charge characteristics.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes),

e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called a, d, e, g, and m, respectively.

The term "chimeric antibody" (or antigen-binding fragment thereof) is an antibody molecule (or antigen-binding fragment thereof) in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e. g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity. For example, a mouse antibody can be modified by replacing its constant region with the constant region from a human immunoglobulin. Due to the replacement with a human constant region, the chimeric antibody can retain its specificity in recognizing the antigen while having reduced antigenicity in human as compared to the original mouse antibody.

The term "chimeric antigen receptors" refers to receptor protein that has been engineered to give T cells the new ability to target a specific antigen. The receptors are chimeric in that they combine both antigen-binding and T cell activating functions into a single receptor. CAR T cell therapy uses T cells engineered with CARs to treat cancer. T cells in CAR T immunotherapy are modified to recognize cancer cells in order to more effectively target and destroy them. CAR T cells can be derived either from T cells in a patient's own blood (autologously) or from the T cells of another, healthy, donor (allogeneically). Once isolated from a person, these T cells are genetically engineered to express a specific CAR, which programs them to target an antigen that is present on the surface of tumors. For safety, CAR T cells are engineered to be specific to an antigen that is expressed on a tumor but is not expressed on healthy cells. CAR T cells destroy cells through extensive stimulated cell proliferation, increasing the degree to which they are toxic to other living cells (cytotoxicity) and by causing the increased secretion of factors that can affect other cells such as cytokines, interleukins and growth factors. The surface of CAR T cells can bear either two types of co-receptors, CD4 and CD8, each with different and interacting cytotoxic effects.

The term "human antibody" or "humanized antibody" (or antigen-binding fragment thereof), as used herein, is intended to include antibodies (and antigen-binding

fragments thereof) having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies or immunoglobulins are divided into the classes: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (subtypes), e.g. IgG1, IgG2, IgG3, and IgG4, IgA1, and IgA2. Therefore, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required. Furthermore, if the antibody contains a constant region, the constant region also is derived from such human sequences. A humanized antibody (or antigen-binding fragment thereof) retains the reactivity of a non-human antibody while being less immunogenic in humans. This can be achieved, for instance, by retaining the non-human CDR regions and replacing the remaining parts of the antibody with their human counterparts (i. e., the constant region as well as the framework portions of the variable region). Additional framework region modifications may be made within the human framework sequences as well as within the CDR sequences derived from the germline of another mammalian species. The humanized antibodies of the present disclosure may include amino acid residues not encoded by human sequences (e. g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo, or a conservative substitution to promote stability or manufacturing). This definition of a humanized antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries, administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, e. g., immunized xenomice via a human B-cell hybridoma technology.

The term "recombinant humanized antibody" as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from a host cell transformed to express the humanized antibody, e. g., from a transfectoma, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of all or a portion of a human immunoglobulin gene, sequences to other DNA sequences.

The term "isolated" means, throughout this specification, that the antibody, or polynucleotide, as the case may be, exists in a physical milieu distinct from that in which it may occur in nature. The term "isolated" nucleic acid refers to a nucleic acid molecule that has been isolated from its natural environment or that has been synthetically created.

5 An isolated nucleic acid may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof. An isolated antibody refers to an antibody that is substantially free of other cellular material and/or chemicals.

The term "Complementarity Determining Regions" ("CDRs") refers to amino acid sequences with boundaries determined using any of a number of well-known schemes, including those described by Kabat (i.e., "Kabat" numbering scheme); Al-Lazikani ("Chothia" numbering scheme); ImMunoGenTics (IMGT) numbering ("IMGT" numbering scheme); and the like. The term "Complementarity Determining Regions" ("CDRs") refers to regions of hypervariability that contain the binding domain that interacts with an antigen. Antibodies typically comprise six CDRs: three in the VH (H1, H2, H3), and three
15 in the VL (L1, L2, L3).

As used herein, the term "sequence identity" refers to the percentage sequence identities that are determined with antibody sequences maximally aligned by the Kabat numbering convention. After alignment, if a subject antibody region (e.g., the entire mature variable region of a heavy or light chain) is being compared with the same region
20 of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage.

25 "Conservative substitutions" may be made, for instance, on the basis of similarity in polarity, charge, size, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the amino acid residues involved. The 20 naturally occurring amino acids can be grouped into the following six standard amino acid groups: (1) hydrophobic: Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr; Asn, Gln; (3) acidic: Asp, Glu; (4)
30 basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe.

As used herein, "conservative substitutions" are defined as exchanges of an amino acid by another amino acid listed within the same group of the six standard amino

acid groups shown above. For example, the exchange of Asp by Glu retains one negative charge in the so modified polypeptide. In addition, glycine and proline may be substituted for one another based on their ability to disrupt α -helices.

As used herein, "non-conservative substitutions" are defined as exchanges of an amino acid by another amino acid listed in a different group of the six standard amino acid groups (1) to (6) shown above.

The term "germline configured counterpart" as used herein refers to an antibody or antigen binding fragment thereof having a framework sequence which has been mutated to match its closest germline gene haplotype. For example, the sequences of variable regions of both heavy and light chains of antibody clones 2D8 and 7G7 were analyzed by the present inventors using IgBlast and their closest germline gene haplotypes were identified. For 2D8, while the heavy chain framework is identical to the closest germline framework sequence IGHV4-30-4*07, its kappa chain framework substantially deviates from the closest germline haplotype IGKV1-39*01 by 12 amino acid differences. For 7G7, the heavy chain framework only differs from the closest germline framework sequence IGHV1-18*01 by the first amino acid, however, the kappa chain framework has three amino acid differences from the closest germline haplotype IGKV2-28*01. With this information, the present inventors have reverse engineered the 2D8 and 7G7 antibodies by mutating their framework sequences to match their closest germline gene haplotypes and produced the germline configured counterparts which were named as "2D8-gl" and "7G7-gl", respectively (**Figure 7A**).

The term "affinity" refers to the strength of all noncovalent interactions between an antibody thereof and the target protein. Unless indicated otherwise, as used herein, the term "binding affinity" refers to intrinsic binding affinity which reflects a 1: 1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule for its binding partner can be generally represented by the dissociation constant (KD). Affinity can be measured by common methods known in the art, including those described herein.

The term " K_D " as used herein refers to the constant of dissociation which is obtained from the ratio of K_d to K_a (i.e. K_d / K_a) and is expressed as a molar concentration (M). K_d and K_a refer to the dissociation rate and association rate, respectively, of a particular antigen-antibody interaction. K_D values for antibodies can be determined using

methods well established in the art. As used herein, the term “low affinity” refers to K_D of 100 nM or more.

As used herein, the term “moderate affinity” refers to K_D ranging from 10nM to 100nM.

5 As used herein, the term “high affinity” refers to K_D of 1 to 10nM.

As used herein, the term “very high affinity” refers to K_D of 1nM or less.

The term “EC50,” as used herein, refers to the concentration of an antibody or an antigen-binding protein/portion thereof, which induces a response, either in an *in vivo* or an *in vitro* assay, which is 50% of the maximal response (i.e., halfway between the
10 maximal response and the baseline).

The term “multispecific” or “multi-specific antibody” as employed herein refers to an antibody as described herein which has at least two binding domains, i.e. two or more binding domains, for example two or three binding domains, wherein the at least two binding domains independently bind two different antigens or two different epitopes on
15 the same antigen. Multi-specific antibodies are generally monovalent for each specificity (antigen). Multi-specific antibodies described herein encompass monovalent and multivalent, e.g. bivalent, trivalent, tetravalent multi-specific antibodies.

The term “bispecific” or “bispecific antibody” as employed herein refers to an antibody with two antigen specificities or an antibody that has the ability to
20 simultaneously bind to two target antigens/sites.

As used herein, a bispecific T cell engager (BiTE) refers to a class of artificial bispecific monoclonal antibodies that direct a host's immune system, such as the T cells' cytotoxic activity against target cells (such as cancer cells). BiTEs are fusion proteins consisting of two single-chain variable fragments (scFvs) of different antibodies, or amino
25 acid sequences from four different genes, on a single peptide chain of about 55 kDa. One of the scFvs binds to an immune cell (such as a T cell via the CD3 receptor), and the other to target of interest (e.g., a tumor cell via a tumor specific molecule). Like other bispecific antibodies, BiTEs form a link between an immune cell (e.g., a T cell) and a target cell (such as a tumor cell). This causes the immune cell (e.g., T cell) to exert
30 cytotoxic activity on tumor cells. For example, if the immune cell is a T cell, the T cell would exert cytotoxic activity by producing proteins like perforin and granzymes that enter tumor cells and initiate the cell's apoptosis.

As used herein, a “nanobody with a heavy chain only” refers to nanobody-based heavy chain antibody. A heavy-chain antibody is an antibody which consists of two heavy chains and lacks the two light chains usually found in antibodies.

The term “antibody drug conjugate” or ADC as used herein refers to a class of therapeutic agents generally composed of three portions: an antibody or antigen-binding fragment, a drug portion (also known as the payload), and a linker used to conjugate the antibody/antigen-binding fragment to the drug. These molecules combine the advantages of the antibody’s highly specific targeting ability with the potent cancer killing properties of the drug to achieve accurate and efficient elimination of cancer cells.

The drug payloads used in ADCs are typically cytotoxic drugs which include but are not limited to: monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), mertansine (DM1), calicheamicin, SN-38 and exatecan.

Conjugation methods for linking the antibody to the drug are divided into two categories, the cleavable system and the noncleavable system, according to the linker. The former system allows the drug to be released from the antibody after entering the cell, but the latter cannot be separated. Commonly used linkers are cleavable valine-citrulline (VC) and noncleavable N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC).

The term “immune cell” refers to a type of specialized cell that plays a crucial role in the body’s defense against infections and foreign substances. They are a part of the immune system, which is responsible for identifying and eliminating harmful pathogens, such as bacteria, viruses, and parasites, as well as abnormal or cancerous cells. As used herein, an “immune cell” refers to any cell of the immune system, including but not limited to T-cells, helper T-cells, B-cells, natural killer (NK) cells, dendritic cells (DC), granulocytes (such as basophils, eosinophils, neutrophils), mast cells, monocytes, and macrophages.

As described herein, a “vector” is any molecule or composition that has the ability to carry a nucleic acid sequence into a suitable host cell where e.g., synthesis of the encoded polypeptide can take place. Typically, and preferably, a vector is a nucleic acid that has been engineered, using recombinant DNA techniques that are known in the art, to incorporate a desired nucleic acid sequence (e.g., a nucleic acid of the present disclosure). Expression vectors typically contain one or more of the following components (if they are not already provided by the nucleic acid molecules): a promoter,

one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a leader sequence for secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed,
5 and a selectable marker element.

Vectors are typically selected to be functional in the host cell in which the vector will be used (the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur. The vector as described herein may be an expression vector and/or a cloning vector.

10 The term "host cell," as used herein, is intended to refer to a cell into which an expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the
15 parent cell, but are still included within the scope of the term "host cell" as used herein.

The terms "treating", "treat" and "therapy", and synonyms thereof refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) a medical condition, which includes but is not limited to diseases, symptoms and disorders. A medical condition also includes a body's response
20 to a disease or disorder, e.g. inflammation. Those in need of such treatment include those already with a medical condition as well as those prone to getting the medical condition or those in whom a medical condition is to be prevented.

The term "subject" as used herein includes patients and non-patients. The term "patient" refers to individuals suffering or are likely to suffer from a medical condition,
25 while "non-patients" refer to individuals not suffering and are likely to not suffer from the medical condition. "Non-patients" include healthy individuals, non-diseased individuals and/or an individual free from the medical condition. The term "subject" includes humans and animals. Animals may include, but is not limited to, mammals (for example non-human primates, canine, murine and the like), and the like. "Murine" refers to any
30 mammal from the family Muridae and / or Leporidae, such as mouse, rat, rabbit, and the like.

The term "preventing" and/or "reducing the severity of symptoms" as used herein refers to process of delaying the onset, reducing the severity of symptoms, reducing

and/or preventing weight loss, preventing death, inhibiting deterioration, inhibiting further deterioration, and/or ameliorating at least one sign or symptom of a disease.

The term "and/or", e.g., "X and/or Y" is understood to mean either "X and Y" or "X or Y" and should be taken to provide explicit support for both meanings or for either
5 meaning.

Further, in the description herein, the word "substantially" whenever used is understood to include, but not restricted to, "entirely" or "completely" and the like. In addition, terms such as "comprising", "comprise", and the like whenever used, are intended to be non-restricting descriptive language in that they broadly include
10 elements/components recited after such terms, in addition to other components not explicitly recited. For example, when "comprising" is used, reference to a "one" feature is also intended to be a reference to "at least one" of that feature. Terms such as "consisting", "consist", and the like, may in the appropriate context, be considered as a subset of terms such as "comprising", "comprise", and the like. Therefore, in
15 embodiments disclosed herein using the terms such as "comprising", "comprise", and the like, it will be appreciated that these embodiments provide teaching for corresponding embodiments using terms such as "consisting", "consist", and the like. Further, terms such as "about", "approximately" and the like whenever used, typically means a reasonable variation, for example a variation of +/- 5% of the disclosed value, or a
20 variance of 4% of the disclosed value, or a variance of 3% of the disclosed value, a variance of 2% of the disclosed value or a variance of 1% of the disclosed value.

Furthermore, in the description herein, certain values may be disclosed in a range. The values showing the end points of a range are intended to illustrate a preferred range. Whenever a range has been described, it is intended that the range covers and
25 teaches all possible sub-ranges as well as individual numerical values within that range. That is, the end points of a range should not be interpreted as inflexible limitations. For example, a description of a range of 1% to 5% is intended to have specifically disclosed sub-ranges 1% to 2%, 1% to 3%, 1% to 4%, 2% to 3% etc., as well as individually, values within that range such as 1%, 2%, 3%, 4% and 5%. It is to be appreciated that the
30 individual numerical values within the range also include integers, fractions and decimals. Furthermore, whenever a range has been described, it is also intended that the range covers and teaches values of up to 2 additional decimal places or significant figures (where appropriate) from the shown numerical end points. For example, a

description of a range of 1% to 5% is intended to have specifically disclosed the ranges 1.00% to 5.00% and also 1.0% to 5.0% and all their intermediate values (such as 1.01%, 1.02% ... 4.98%, 4.99%, 5.00% and 1.1%, 1.2% ... 4.8%, 4.9%, 5.0% etc.) spanning the ranges. The intention of the above specific disclosure is applicable to any depth/breadth of a range.

“At least 95% identical” as employed herein is intended to refer to an amino acid sequence which over its full length is 95% identical or more to a reference sequence, such as 96, 97, 98 or 99% identical. Software programmes can be employed to calculate percentage identity.

Additionally, when describing some embodiments, the disclosure may have disclosed a method and/or process as a particular sequence of steps. However, unless otherwise required, it will be appreciated that the method or process should not be limited to the particular sequence of steps disclosed. Other sequences of steps may be possible. The particular order of the steps disclosed herein should not be construed as undue limitations. Unless otherwise required, a method and/or process disclosed herein should not be limited to the steps being carried out in the order written. The sequence of steps may be varied and still remain within the scope of the disclosure.

Furthermore, it will be appreciated that while the present disclosure provides embodiments having one or more of the features/characteristics discussed herein, one or more of these features/characteristics may also be disclaimed in other alternative embodiments and the present disclosure provides support for such disclaimers and these associated alternative embodiments.

DESCRIPTION OF EMBODIMENTS

It will be appreciated by a person skilled in the art that other variations and/or modifications may be made to the embodiments disclosed herein without departing from the spirit or scope of the disclosure as broadly described. For example, in the description herein, features of different exemplary embodiments may be mixed, combined, interchanged, incorporated, adopted, modified, included etc. or the like across different exemplary embodiments. The present embodiments are, therefore, to be considered in all respects to be illustrative and not restrictive.

In one aspect, there is provided an anti-Trop2 antibody or antigen-binding fragment thereof, comprising:

a CDRH1 sequence selected from the group comprising:

- i. GGSISSGGYY (SEQ ID NO: 1),
- ii. GYTFTSYG (SEQ ID NO: 2)
- iii. GFTFSSYS (SEQ ID NO: 3),
- iv. GFTFSSYW (SEQ ID NO: 4), and;

5

a CDRH2 sequence selected from the group comprising:

- i. IYYSGST (SEQ ID NO: 5),
- ii. ISAYNGNT (SEQ ID NO: 6)
- iii. ISSSSSYI (SEQ ID NO: 7),
- iv. IKQDGSEK (SEQ ID NO: 8), and;

10

a CDRH3 sequence selected from the group comprising:

- i. AREEGIAAAAFDI (SEQ ID NO: 9),
- ii. ARKYSGFDY (SEQ ID NO: 10)
- iii. ARDYDSSGYPYYYYGMDV (SEQ ID NO: 11),
- iv. ARDFVDWSATPFDY (SEQ ID NO: 12), and;

15

a CDRL1 sequence selected from the group comprising:

- i. QSVGSF (SEQ ID NO: 13),
- ii. QSLLHSNGYNY (SEQ ID NO: 14)
- iii. QSVSSSY (SEQ ID NO: 15),
- iv. RSLLHSNGYNY (SEQ ID NO: 16), and;

20

a CDRL2 sequence selected from the group comprising:

- i. GAS (SEQ ID NO: 17), and
- ii. LGS (SEQ ID NO: 18); and

a CDRL3 sequence selected from the group comprising:

- i. QQSDSSPFT (SEQ ID NO: 19),
- ii. MQNLQTPWT (SEQ ID NO: 20)
- iii. HQSGSSLRT (SEQ ID NO: 21),
- iv. MQALQIPKT (SEQ ID NO: 22).

25

30 In some embodiments, the antibody or antigen-binding fragment thereof is a monoclonal antibody.

In one embodiment, the antibody or antigen-binding fragment thereof is a full-length antibody.

Non-limiting examples of antigen-binding fragments includes one or more fragments or portions of an antibody that retain the ability to specifically bind to an antigen (e.g. HER2), or synthetic modifications of an antibody fragments that retain the desired binding ability to the antigen. In various embodiments, antigen-binding fragments include

5 single domain antibodies, further engineered molecules (such as, but is not limited to diabodies, triabodies, tetrabodies, minibodies, and the like), Fab fragments, Fab' fragments, F(ab')₂ fragments, Fd fragments, Fv fragments, single-chain Fv (scFv) molecules, seFv molecules, scFv dimer, BsFv molecules, dsFv molecules, (dsFv)₂ molecules, dsFv-dsFv' molecules, Fv fragments, dAb fragments, bispecific antibodies,

10 ds diabodies, nanobodies, domain antibodies, bivalent domain antibodies, and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g. an isolated complementarity determining region (CDR)).

In one embodiment, the antigen-binding fragment thereof is selected from the group comprising: an Fab, an modified Fab, an Fab', an modified Fab', an F(ab')₂, an

15 Fv, a single domain antibody, a VHH, an scFv, an Fv, an bivalent antibody, a trivalent antibody, a tetra-valent antibody, a Bis-scFv, a diabody, a triabody, a tetrabody, an epitope-binding fragment, and the like.

In one embodiment, the antibody or antigen-binding fragment thereof is an IgG, IgA, IgD, IgE, or IgM antibody. In one embodiment, the antibody or antigen-binding

20 fragment thereof is an IgG1 antibody.

In one embodiment, the antibody or antigen-binding fragment thereof is a chimeric antibody, a human antibody / humanized antibody, recombinant humanized antibody, an animal-derived antibody (such as llama antibody) or the like.

In some embodiments, the antibody or antigen-binding fragment thereof is

25 humanized. In one embodiment, the antibody or antigen-binding fragment thereof is fully human. In some embodiments, the antibody or antigen-binding fragment thereof is a chimeric antibody.

In some embodiments, an "antibody variant" refers to an antibody or antigen-binding fragment thereof comprising a VH and/or a VL wherein the non-CDR regions of

30 the antibody or antigen-binding fragment thereof has at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity, to an antibody described herein.

In some embodiments, the antibody or antigen-binding fragment thereof is an isolated antibody or antigen-binding fragment thereof.

In some embodiments, the antibody or antigen-binding fragment thereof comprises a sequence that is at least 60% identical to any one of the sequences disclosed herein. For example, the the antibody or antigen-binding fragment thereof may comprise a sequence that is at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, identical to any of the sequences disclosed herein (e.g. about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, about 99% or about 100% sequence identity to any one of the sequences disclosed herein. The percentage sequence identity can be determined using methods known in the art, for example using programs such as BLAST, NBLAST, XBLAST, and the like.

In some embodiments, the antibody or antigen-binding fragment thereof comprises a sequence or an amino acid region or is encoded by a nucleotide region that differs by about one, about two, about three, about four, about five, about six, about seven, about eight, about nine, about ten or more amino acids or nucleobase with the sequence as disclosed herein.

In some embodiments, the antibody or antigen-binding fragment thereof comprises an amino acid sequence having one or more amino acid mutations with respect to any one of the sequences disclosed herein. In some examples, the antigen binding protein comprises an amino acid sequence having one, or two, or three, or four, or five, or six, or seven, or eight, or nine, or ten, or fifteen, or twenty amino acid mutations

with respect to any one of the sequences disclosed herein. In some examples, the one or more amino acid mutations may be independently selected from substitutions, insertions, deletions, and truncations.

In some embodiments, the amino acid mutations are amino acid substitutions, and may include conservative and/or non-conservative substitutions.

In various embodiments, the substitution replaces the amino acids with natural amino acids, such as, but is not limited to, alanine, arginine, asparagine, aspartate, cysteine, glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. In various embodiments, the substitution does not include replacement with cysteine. Thus, in various embodiments, the substitution replaces the amino acids with natural amino acids, such as, but is not limited to, alanine, arginine, asparagine, aspartate, glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

In various embodiments, the substitution may be a conservative substitution that substitutes one residue for another of similar properties. For example, substitution of one amino acid with another from the same group. In various embodiments, the substitution may include substitution with an amino acid with different properties. In various embodiments, the substitution is made without affecting the biological activity of the antigen binding protein, variant or fragment thereof as described herein. In various embodiments, the substitution increases binding affinity to EpCAM.

In some embodiments, the substitutions may also include non-classical amino acids. Illustrative non-classical amino acids include, but are not limited to, selenocysteine, pyrrolysine, N-formylmethionine β -alanine, GABA and δ -Aminolevulinic acid, 4-aminobenzoic acid (PABA), D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β methyl amino acids, C α -methyl amino acids, N α -methyl amino acids, and amino acid analogs in general.

In some embodiments, the amino acid mutation may be in the CDRs of the antigen binding protein (e.g., the CDR1, CDR2 or CDR3 regions). In another example, amino acid alteration may be in the framework regions (FRs) of the antigen binding protein (e.g., the FR1, FR2, FR3, or FR4 regions).

- 5 Modification of the amino acid sequences may be achieved using any known technique in the art e.g., site-directed mutagenesis or PCR based mutagenesis.

In some embodiments, the mutations do not substantially reduce the antigen binding protein's capability to specifically bind to a target. In some examples, the mutations do not substantially reduce the antigen binding protein's capability to
10 specifically bind to a target and without functionally modulating (e.g., partially or fully neutralizing) the target.

In one embodiment, the antibody or antigen-binding fragment thereof binds to Trop2 with low affinity, moderate affinity or high affinity.

- In one embodiment, the antibody or antigen-binding fragment thereof binds to
15 Trop2 with low to moderate affinity (K_D ranging from 1-100nM). In one embodiment, the antibody or antigen-binding fragment thereof binds with moderate affinity K_D ranging from 10-100nM. In one embodiment, the antibody or antigen-binding fragment thereof binds to Trop2 with high affinity.

- Advantageously, the binding profiles of all four exemplified anti-Trop2 IgG
20 antibodies showed good binding specificity, with strong binding to the Trop2^{High} AGS cells (**Figure 3B**), around 40-50% binding to HT-29 cells (**Figure 3C**) and no binding to Trop2^{Negative} 293T cells (**Figure 3D**). This suggests that these antibodies bind to surface of the cells in a highly specific way dependent on Trop2 protein expression.

- In some embodiments, the binding affinity of the the antibody or antigen-binding
25 fragment thereof for the full-length and/or mature forms and/or isoforms and/or splice variants and/or fragments and/or monomeric and/or dimeric forms and/or any other naturally occurring or synthetic analogs, variants, or mutants (including monomeric and/or dimer forms) of antigen binding protein may be described by the equilibrium dissociation constant (K_D). In some examples, the antigen binding protein binds to the
30 full-length and/or mature forms and/or isoforms and/or splice variants and/or fragments and/or any other naturally occurring or synthetic analogs, variants, or mutants (including monomeric and/or dimeric forms) of antigen binding protein with a K_D of less than about 1 μ M, about 900 nM, about 800 nM, about 700 nM, about 600 nM, about 500 nM, about

400 nM, about 300 nM, about 200 nM, about 100 nM, about 90 nM, about 80 nM, about 70 nM, about 60 nM, about 50 nM, about 40 nM, about 30 nM, about 20 nM, about 10 nM, or about 5 nM, or about 1 nM, about 50 pM, about 40 pM, about 30 pM, about 20 pM, about 10 pM, about 5 pM or about 1 pM.

5 In one embodiment, the antibody or antigen-binding fragment thereof binds to an epitope that overlaps with the epitope bound by benchmark anti-Trop2 antibodies Sacituzumab and datopotamab. In one embodiment, the antibody or antigen-binding fragment thereof binds to a different epitope from the epitope bound by Sacituzumab and/or datopotamab.

10 Work by the present inventors showed that antibody clone 5A12 bound to the same construct (Construct 9) as Sacituzumab and Datopotamab, indicating that 5A12 shared the same epitope (amino acid 237 to 252) as the two reference antibodies (Ref - Sun et al. iScience 2011, 24:103190). As previously predicted by BLI analysis, 7G7-gl bound to a different construct (Construct 1), which corresponded to an epitope close to
15 the N-terminus of Trop2 protein (amino acid 45 to 63). Interestingly, different from what we predicted from the previous study by BLI analysis, 2D8-gl, in fact, bound to a different epitope (Construct 6, amino acid 181 to 198), which is far away from the linear binding epitope of Sacituzumab. The present inventors have further highlighted different antibody epitopes on the solved crystal structure (PDB: 7E5M) and found out that the
20 binding epitope of 2D8-gl is close to that of Sacituzumab after protein folding, hence it is possible that the binding of 2D8-gl IgG to the Trop2 protein will exclude the binding of Sacituzumab, and vice versa.

 Hence, antibody clones 7G7-gl and 2D8-gl (as well as their parental clones 7G7 and 2D8) may usefully target alternative epitopes compared to prior art antibodies
25 sacituzumab and datopotamab.

 In one embodiment, the antibody or antigen-binding fragment thereof is a germline configured counterpart of an antibody or antigen-binding fragment thereof as disclosed herein. In one embodiment, the antibody or antigen-binding fragment thereof is a germline counterpart of clone 2D8, 7G7, 5A12 and/or 6C10. In one embodiment, the
30 antibody or antigen-binding fragment thereof is a germline configured counterpart of clone 2D8 and/or 7G7. In one embodiment, the antibody or antigen-binding fragment thereof is a germline configured counterpart of clone 2D8, for example clone 2D8-gl as disclosed herein. In one embodiment, the antibody or antigen-binding fragment thereof

is a germline configured counterpart of clone 7G7, for example clone 7G7-gl as disclosed herein.

Advantageously, the disclosed germline configured counterparts, in particular 2D8-gl and 7G7-gl showed slightly increased binding affinity compared to the parental clones 2D8 and 7G7, respectively, with binding EC50 values close to 1nM. Importantly, the binding EC50 was still higher than the two benchmark clones Sacituzumab and Datopotamab, both of which are less than 1nM (**Figure 7B**). Furthermore, the binding potency of the germline configured counterparts were also improved in cell surface binding with the superior binding specificity unchanged (**Figure 7C and 7D**). Only Datopotamab stained Trop2^{Negative} 293T cells at high antibody concentration of 20nM, suggesting that the binding specificity of the disclosed germline counterpart anti-Trop2 antibodies are even better than the benchmark clone Datopotamab.

In some embodiments, the antigen-binding fragment thereof may be a nanobody.

In some embodiments, the antibody or antigen-binding fragment thereof binds to primate Trop2. In some embodiments, the antibody or antigen-binding fragment thereof binds to cynomolgus and/or chimpanzee Trop2. Thus, in some embodiments, the antibody or antigen-binding fragment thereof binds to cynomolgus Trop2. In some embodiments, the antibody or antigen-binding fragment thereof binds to chimpanzee Trop2.

In some embodiments, the antibody or antigen-binding fragment thereof does not bind to murine Trop2.

Advantageously, all four disclosed anti-Trop2 IgG clones exhibited cross-reactivity to cynomolgus Trop2 but not to mouse Trop2 (**Figure 3E**), indicating that these antibodies recognize common epitopes of Trop2 protein across human and cynomolgus antigens.

In some embodiments, the antibody or antigen-binding fragment thereof does not bind to renal cancer cells.

In some embodiments, the antibody or antigen-binding fragment thereof has a higher binding affinity to Trop2 expressing cells, such as AGS, compared to other prior art antibodies, for example sacituzumab and datopotomab.

In one embodiment, the antibody or antigen-binding fragment thereof induces antibody-dependent cell-mediated cytotoxicity (ADCC). Surprisingly, germline counterpart clone 2D8-gl showed an even higher killing potency in inducing ADCC of

AGS cells than its parental clone 2D8, which is similar to Sacituzumab and Datopotamab (Figure 8A). All IgG antibodies tested maintained good target specificity as they did not induce ADCC of Trop2^{Negative} 293T cells (Figure 8B).

5 In some embodiments, the multispecific antigen binding protein is a bispecific immune cell engager that is capable of engaging both an antigen and an immune cell. In some embodiments, the immune cell activation marker is selected from the group comprising CD3, NKG2D, CD4, CD8, CD16, CD64.

In some embodiments, the bispecific T cell engager (BiTE) is a nanobody with a heavy chain only (VHH).

10 In one embodiment, the bispecific T cell engager (BiTE) binds to two antigens; wherein the first antigen is Trop2; and wherein the second antigen is an immune cell marker.

In some embodiments, the second antigen is an immune cell marker that is involved in the activation of the immune cell.

15 In some embodiments, the antigen binding protein binds to Trop2 in solution and / or on the surface of the host cells.

In one embodiment, the second antigen targeted by the bispecific T cell engager (BiTE) is selected from the group comprising CD3, NKG2D, CD28, CD16, and the like.

20 In one embodiment, the bispecific T cell engager (BiTE) targets one or more antigens, wherein the first antigen is Trop2, and wherein the second antigen is CD3.

In some embodiments, the CD3 may include CD3 ζ , CD3 ϵ , CD3 γ , CD3 δ , and the like.

Thus, in one embodiment, the bispecific antibody further comprises an anti-CD3 binding domain.

25 In some embodiments, the anti-CD3 binding domain is an anti-CD3 scFv. In some embodiments, the anti-CD3 binding domain is Otk3.

30 In one embodiment, the antigen binding protein, variant or fragment thereof further comprises an immunoglobulin Fc fragment (region/domain), a protein capable of extending the half-life of the recombinant/fusion polypeptide (such as albumin, human albumin, and the like), linkers capable of enhancing binding valency (for example a rigid linker or a flexible linker, such as a GGGGS repeat), or combinations thereof, for example wherein the immunoglobulin Fc fragment is an IgG Fc fragment, such as a human IgG Fc fragment.

In various embodiments, the immunoglobulin fragment may include but is not limited to a Fc region/domain, one or more CH regions/domains, a Fab, a Fab', a F(ab')₂, a single chain Fv (ScFv) and/or Fv fragments, hinge regions/domain, as well as fragments or portions thereof.

- 5 In some embodiments, the antigen binding protein, variant or fragment thereof may also contain portions of immunoglobulin molecules or antibodies, such as including, but not limited to, all or portions of a constant heavy chain, a variable heavy chain, a constant light chain, a variable light chain, a hinge region, and/or an Fc domain of a Ig, as well as variants thereof. For example, a recombinant/fusion polypeptide as described
- 10 herein may be combined with portions of a human IgG. In various embodiments, the type of immunoglobulin may include one or more type such as, but is not limited to, IgG, IgE, IgM, IgD, IgA, and IgY. In various embodiments, the type of immunoglobulin may be an immunoglobulin of class IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or subclass thereof.

In one embodiment, the immunoglobulin fragment is an IgG Fc fragment.

- 15 In some embodiments, the polynucleotide sequence may be at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, identical to the sequences as disclosed herein. In some embodiments, the polynucleotide is an isolated polynucleotide.

- In some embodiments, the variant thereof has at least 80% identity thereto. In some embodiments, the variant thereof has at least 81% identity thereto. In some
- 20 embodiments, the variant thereof has at least 82% identity thereto. In some embodiments, the variant thereof has at least 83% identity thereto. In some embodiments, the variant thereof has at least 84% identity thereto. In some embodiments, the variant thereof has at least 85% identity thereto. In some embodiments, the variant thereof has at least 86% identity thereto. In some
- 25 embodiments, the variant thereof has at least 87% identity thereto. In some embodiments, the variant thereof has at least 88% identity thereto. In some embodiments, the variant thereof has at least 89% identity thereto. In some embodiments, the variant thereof has at least 90% identity thereto. In some embodiments, the variant thereof has at least 91% identity thereto. In some
- 30 embodiments, the variant thereof has at least 92% identity thereto. In some embodiments, the variant thereof has at least 93% identity thereto. In some embodiments, the variant thereof has at least 94% identity thereto. In some embodiments, the variant thereof has at least 95% identity thereto. In some

embodiments, the variant thereof has at least 96% identity thereto. In some embodiments, the variant thereof has at least 97% identity thereto. In some embodiments, the variant thereof has at least 98% identity thereto. In some embodiments, the variant thereof has at least 99% identity thereto.

5 In one embodiment, the vector is an expression vector.

In some embodiments, the vector is selected from the group consisting of a plasmid, a viral particle, a phage, a baculovirus, a yeast plasmid, a lipid based vehicle, a polymer microsphere, a liposome, and a cell based vehicle, a colloidal gold particle, lipopolysaccharide, polypeptide, polysaccharide, a viral vehicle, an adenovirus, a
10 retrovirus, a lentivirus, an adeno-associated viruses, a herpesvirus, a vaccinia virus, a foamy virus, a cytomegalovirus, a Semliki forest virus, a poxvirus, a pseudorabies virus, an RNA virus vector, a DNA virus vector and a vector derived from a combination of a plasmid and a phage DNA, further optionally wherein said polynucleotide is operatively linked to an expression control sequence(s) to direct peptide synthesis, even further
15 optionally wherein the vector comprises one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells.

In one embodiment, the vector is selected from the group comprising DNA vector, an RNA vector, a plasmid, a lentiviral vector, an adenoviral vector, an adeno-associated viral vector, and a retroviral vector.

20 In some embodiments, the vector is a lentiviral vector.

In some embodiments, the host cell comprises cloning or expression vectors as described above and/or nucleic acid sequences encoding for the antigen binding protein, antibodies and binding fragments thereof as described above.

In one aspect, there is provided a method of producing / generating the antibody
25 or antigen-binding fragment thereof as disclosed herein, comprising expressing the polynucleotide or vector as described herein in a host cell.

The host cell can be any type of cell capable of being transformed or transfected with the nucleic acid or vector so as to produce an antigen binding protein or binding fragment/protein thereof encoded thereby. The host cell comprising the nucleic acid or
30 vector can be used to produce the antigen binding protein or binding fragment/protein thereof, or a portion thereof (e.g., a heavy chain sequence, or a light chain sequence encoded by the nucleic acid or vector). After introducing the nucleic acid or vector into the cell, the cell is cultured under conditions suitable for expression of the encoded

sequence. The antibody, antigen binding protein, or fragment, or portion of the antibody then can be isolated from the cell.

The host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell, or a vertebrate cell). The host cell, when
5 cultured under appropriate conditions, expresses an antibody or binding fragment thereof which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). Selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity,
10 such as glycosylation or phosphorylation, and ease of folding into a biologically active molecule. Selection of the host cell will depend in part on whether the antibody or binding fragment thereof is to be post-transcriptionally modified (e.g., glycosylated and/or phosphorylated). The host cell may comprise a bacterial cell, a yeast cell, an animal cell e.g., a mammalian cell and/or a plant cell.

15 Suitable mammalian host cells include CHO, myeloma or hybridoma cells. Many are available from the American Type Culture Collection (ATCC), Manassas, Va. Examples include mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC No. CCL61), human embryonic kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), 3T3 cells (ATCC No. CCL92), or PER.C6 cells. Other cell types of use in expressing
20 antibodies include lymphocytic cell lines, e.g., NSO myeloma cells and SP2 cells, COS cells.

In one embodiment, the host cell expresses the antibody or antigen-binding fragment thereof as disclosed herein.

In some examples, the immune cell may include but is not limited to a
25 macrophage, a dendritic cell, a T cell, a B cell, an eosinophil, a basophil, a neutrophil, a mast cell, a natural killer T cell (NKT cell), natural killer cell (NK cell), a macrophage, a monocyte, and the like. In one embodiment, the immune cell is a NK cell. In one embodiment, the immune cell is a macrophage. In one embodiment, the immune cell is a dendritic cell. In one embodiment, the immune cell is a monocyte.

30 In one embodiment, the immune cell expresses an inducible bispecific T cell engager (BiTE) comprising the antibody or antigen-binding fragment as defined above.

In one embodiment, the immune cell expresses an inducible bispecific T cell engager (BiTE) that further binds to an immune cell activator.

In one embodiment, the immune cell expresses an inducible bispecific T cell engager (BiTE) that further binds to CD3.

In one embodiment, the disclosed anti-Trop2 clones are used to construct anti-Trop2 BiTE antibodies.

5 Advantageously, all the anti-Trop2 BiTE antibodies tested were able to kill 90-100% of Trop2^{High} MCF-7 cells within 72 hours at a concentration as low as 10pM (**Figure 10C**). These BiTE antibodies also efficiently killed Trop2^{Medium} HeyA8 cells, albeit with a relatively lower efficacy (**Figure 10D**). No killing of Trop2^{Negative} 293T cells were observed (**Figure 10E**), indicating superior specificity of these anti-Trop2 BiTE antibodies. In
10 addition, when co-cultured with Trop2^{High} MCF-7 cells and Trop2^{Medium} HeyA8 cells in the presence of anti-Trop2 BiTE antibodies, activated human T cells secreted remarkably high amount of interferon-gamma and IL-2. However, there was no secretion of interferon-gamma or IL-2 can be detected when the T cells were co-cultured with Trop2^{Negative} 293T cells in the presence of anti-Trop2 BiTE antibodies (**Figure 10F** and
15 **10G**). Hence, the presently disclosed anti-Trop2 BiTEs have high specificity and potency in inducing cell death of Trop2 expressing cells.

In one embodiment, the antibody or antigen-binding fragment is in the form of an antibody-drug conjugated (ADC), wherein the antibody or antigen-binding fragment is conjugated to a therapeutic agent. In one embodiment, the therapeutic agent is an anti-
20 cancer agent, for example a chemotherapeutic agent.

Advantageously, when compared to their parental clones (which were not in ADC forms), both 2D8-gl and 7G7-gl showed more potent cytotoxicity in inhibiting the growth of Trop2^{High} AGS cells with the use of MMAE. Interestingly, the potency of both 2D8 and 2D8-gl greatly exceeded that of Sacituzumab and Datopotamab, making 2D8 and 2D8-
25 gl very promising leads in further development into an ADC drug (**Figure 9A**). When used with the second payload DX-8951, 2D8-gl also showed slightly higher potency than the benchmark clones (**Figure 9B**). When used as ADC, all antibodies did not show growth inhibition of Trop2^{Negative} 293T cells (**Figure 9C** and **9D**).

In some embodiments, the therapeutic agent is selected from the group
30 comprising tubulin inhibitors, such as auristatins, maytansinoids and tubulysins; DNA damaging agents, such as calicheamicins, duocarmycins, exatecans and pyrrolobenzodiazepines; immunomodulators, such as TLR7/8 agonists and STING agonists; topoisomerase II inhibitors, such as doxorubicin, daunorubicin, idarubicin, and

PNU-159682; RNA polymerase II inhibitors, such as alpha-amanitin, alpha-amanitin, phalloidin, trichotecene, verrucarin A, roridin A; HDAC inhibitors such as ST7464AA1, vorinostat (SAHA) and dacinostat (VP-LAQ824); Bcl-XL inhibitors, such as clezutoclax; BET/BRD4 degraders such as GNE-987, MZ1 analogue and BRD4; NAMPT inhibitors
 5 such as FK-866 analogues; KSP inhibitors such as filanesib derivatives; carbonic anhydrase inhibitors, such as CA IX and XII peptides and DHFR inhibitors such as methotrexate; TK inhibitors such as genistein.

In some embodiments, the chemotherapeutic agent is selected from the group comprising: maytansine, MMAF, MMAD, DxD, DM2, DM3, DM4, PNU-159682, DMDM
 10 (duocarmycin DM), AAMT (alpha-amanitin), tubulysin D, tubulysin M, SG3199, eribulin mesylate, MMAE (vedotin), Dx-8951 (exatecan), AZ'0132, belotecan, KL610023, CPT, AMDCPT, ozogamicin, DM1, deruxtecan, SN-38, mcMMAF, PE38, PBD, topotecan, irinotecan, indotecan, indimitecan, genistein, ICRF193, amsacrine, etoposide, etoposide phosphate, teniposide, doxorubicin, fluoroquinolone, vinblastine, vincristine, vinorelbine,
 15 vinflunine, colchicine, combretastatin, halichondrins, 2-methoxyestradiol, duocarmycin, ecteinascidin 743, adozelesin, bizelesin, tallimustine, oligopyrrole, oligoimidazole carriers, such as netropsin and distamycin, bis-(benzimidazole) carriers, polybenzamide carriers, 9-anilinoacridine-4-carboxamide carriers, mitomycins such as mitomycin C, KW-2149 and proflomycin, carmethizole analogues, pyrrolobenzodiazepines,
 20 ecteinascidin analogues such as ecteinascidin 743, and duocarmycin and analogues .

In one embodiment, the therapeutic agent is a topoisomerase inhibitor.

In some embodiments, the topoisomerase inhibitor is a type I topoisomerase inhibitor. Type I inhibitors include DxD, SN-38, exatecan, topotecan, irinotecan, indotecan and indimitecan.

25 In some embodiments, the topoisomerase inhibitor is a type II topoisomerase inhibitor and/or type II topoisomerase poison. Type II inhibitors include genistein and ICRF 193. Type II poisons include amsacrine, etoposide, etoposide phosphate, teniposide and doxorubicin and fluoroquinolones.

In one embodiment, the topoisomerase inhibitor is DX-8951 (exatecan).

30 In one embodiment, the therapeutic agent is an inhibitor of tubulin polymerization.

In some embodiments, the inhibitor of tubulin polymerization is selected from the group comprising MMAF, MMAD, MMAE (vedotin), vinblastine, vincristine, vinorelbine, vinflunine, colchicine, combretastatin, halichondrins, and 2-Methoxyestradiol.

In one embodiment, the inhibitor of tubulin polymerization is MMAE (vedotin).

In one embodiment, the therapeutic agent is a DNA minor groove alkylator.

In some embodiments, the DNA minor groove alkylator is selected from the group comprising ecteinascidin 743, adozelesin, bizelesin, tallimustine, oligopyrrole, oligoimidazole carriers, such as netropsin and distamycin, bis-(benzimidazole) carriers, polybenzamide carriers, 9-anilinoacridine-4-carboxamide carriers, mitomycins such as mitomycin C, KW-2149 and proflomycin, carmethizole analogues, pyrrolobenzodiazepines, ecteinascidin analogues such as ecteinascidin 743, and duocarmycin and analogues.

10 In some embodiments, the therapeutic agent is MMAE. In some embodiments, the therapeutic agent is DX-8951. In some embodiments, the therapeutic agent is PBD. In some embodiments, the therapeutic agent is PNU-159682. In some embodiments, the therapeutic agent is DMDM. In some embodiments, the therapeutic agent is AAMT.

15 In some embodiments, the ADC exhibits a higher cytotoxicity for cancer cells compared to other prior art Trop2 antibodies, such as Sacituzumab and datopotamab.

Advantageously, clone 2D8 IgG showed superior potency in inhibiting the growth of Trop2^{High} AGS cells with the use of all three different payload toxins tested (i.e. MMAE, DX-8951 and PBD). 7G7 IgG also showed some cytotoxicity activity, albeit much weaker than 2D8 IgG (**Figure 5A, 5B, and 5C**). Further advantageously, when compared to their parental clones, both 2D8-gI and 7G7-gI showed more potent cytotoxicity in inhibiting the growth of Trop2^{High} AGS cells with the use of MMAE. Interestingly, the potency of both 2D8 and 2D8-gI greatly exceeded that of Sacituzumab and Datopotamab, making 2D8 and 2D8-gI very promising leads in further development into an ADC drug (**Figure 9A**). When used with the second payload DX-8951, 2D8-gI also showed slightly higher potency than the benchmark clones (**Figure 9B**). When used as ADC, all antibodies did not show growth inhibition of Trop2^{Negative} 293T cells (**Figure 9C and 9D**).

When clones 2D8-gI and 7G7-gI were conjugated to payloads PBD, PNU-159682 and DMDM, similarly impressive results were obtained. For example:

- The potency of both 2D8 and 2D8-gI greatly exceeded that of Sacituzumab and Datopotamab with PBD;
- In MCF-7 (**Figure 13A**), MDA-MB231 (**Figure 13B**) and HeyA8 (**Figure 13D**) cells, both 2D8 and 2D8-gI showed much higher cytotoxicity potency than that of Sacituzumab and Datopotamab, when used with PNU-159682; and

- Both 2D8 and 2D8-gl showed high cytotoxicity potency in all four cells tested (**Figure 14A to 14D**), regardless of Trop2 expression level, when used with DMDM. However, Sacituzumab and Datopotamab showed very weak cytotoxicity in MDA-MB23.

5 Thus, the presently disclosed anti-Trop2 ADCs have superior properties compared to ADCs formed with prior art benchmark antibodies Sacituzumab and datopotamab.

 In some embodiments, the CAR-T cell comprises one or more of the following: an IgH signal peptide, an IgH hinge, a CD28 transmembrane domain, a 4-1BB ICD
10 (intracellular signaling domain) and a CD3z ICD.

 In some embodiments, the CAR-T cell comprises an IgH signal peptide.

 In some embodiments, the CAR-T cell comprises an IgH hinge.

 In some embodiments, the CAR-T cell comprises a CD28 transmembrane domain.

15 In some embodiments, the CAR-T cell comprises a 4-1BB ICD. In some embodiments, the CAR-T cell comprises a CD3z ICD. In some embodiments, the CAR-T cell comprises a CD28 ICD.

 Advantageously, the presently disclosed CAR T cells are highly efficient in killing Trop2 expressing tumor cells. Within 96 hours of co-culture, clone 2D8, 2D8-gl, 7G7,
20 7G7-gl, 5A12, Sacituzumab, and Datopotamab based anti-Trop2 CAR T cells could lyse 80-100% of Trop2^{High} AGS cells (**Figure 11C**). The killing by these CAR T cells was highly specific to antigen expression, as the same CAR T cells only showed minimal background killing of Trop2^{Negative} 293T cells (10-40%, **Figure 11D**).

 In addition, when co-cultured with Trop2^{High} AGS cells, anti-Trop2 CAR T cells
25 based on all clones except clone 6C10 secreted a high level of interferon-gamma and IL-2. However, levels of interferon-gamma and IL-2 secretion were minimal when these anti-Trop2 CAR T cells were added to Trop2^{Negative} 293T cells, indicating the superior specificity of these anti-Trop2 CAR T cells in responding to and killing of Trop2 expressing cells (**Figure 11E and 11F**).

30 In one aspect, there is provided a pharmaceutical composition comprising the antigen binding protein, variant or fragment thereof as disclosed herein.

 In one embodiment, the composition is a prophylactic and/or therapeutic composition.

In some embodiments, the composition comprises one or more pharmaceutically acceptable agents. Pharmaceutically acceptable agents for use in the present pharmaceutical compositions include carriers, excipients, diluents, antioxidants, preservatives, colouring, flavouring and diluting agents, emulsifying agents, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, tonicity agents, cosolvents, wetting agents, complexing agents, buffering agents, antimicrobials, and surfactants.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient. A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

In one embodiment, the composition further comprises a pharmaceutically acceptable excipient, a buffer or carrier.

In one embodiment, the composition is for use in therapy/medicine/vaccine.

In one aspect, there is provided a method of preventing and/or reducing the severity of symptoms caused by a disease in a subject in need thereof, the method comprises administering to the subject an antigen binding protein, variant or fragment thereof, or composition as disclosed herein.

In one embodiment, the antigen binding protein, variant or fragment thereof or composition thereof is administered to the subject by mode of administrations known in the art, including but not limited to intramuscularly, subcutaneously, intravenously, intraarterially, intraarticularly, intraperitoneally, intranasally, parenterally, and the like.

In one embodiment, the subject or patient is a mammal, such as a monkey, rabbit, mouse, rat, pig or dog. In one embodiment, the subject or patient is a human.

In one embodiment, the cancer is Trop2+. Cancers which are Trop2+ are particularly good candidates for treatment with the disclosed anti-Trop2 antibodies and antigen binding fragments.

Hence, in one embodiment, the cancer is selected from the group comprising
5 breast, non-small cell lung cancer (NSCLC), cervical, colorectal, endometroid
endometrial (EEC), oesophageal, gastric, glioma, cholangiocarcinoma, chronic
lymphocytic lymphoma, extranodal NK/T-cell lymphoma (ENKTL), non-Hodgkin's
lymphoma (NHL), small-sized pulmonary adenocarcinoma, squamous cell carcinoma of
the oral cavity, ovarian, pancreatic, prostate, stomach, thyroid, bladder and uterine.

10 In some embodiments the cancer is gastric cancer, such as gastric
adenocarcinoma. In some embodiments the cancer is colon cancer. In some
embodiments the cancer is pancreatic cancer. In some embodiments the cancer is
breast cancer. In some embodiments the cancer is lung cancer.

In one aspect, there is provided an antibody, antigen-binding fragment thereof,
15 composition, polynucleotide, vector, method or use as described herein.

SEQUENCES

CDRs are in bold (CDR1);in bold and in italics (CDR2); or in bold, in italics and underlined
(CDR3).

20 1. **Amino-acid Sequences**

1.1. Heavy chain variable domain of human IgG1
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2D8, 2D8-g1

25 QVQLQESGPGLVKPSQTLSTCAVSG**GSISGGYY**WSWIRQPPGKGLEWIGY**IYSGSTYYNPS**
LKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC**AREEGIAAAAFDI**WGQGT

5A12

EVQLVQSGGGLVKPGGSLRLSCAAS**GFTFSSYS**MNWVRQAPGKGLEWVSS**ISSSSYIYY**ADSV
KGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC**ARDYYDSSGYPIYYYGMDV**WGQGT

30 **6C10**

EVQLVQSGGGLVQPGGSLRLSCAAS**GFTFSSYW**MSWVRQAPGKLEWVAN**IKQDGSEK**YYVDSV
KGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC**ARDFVDWSATPFDY**WGQGT

7G7
EVQLVQSGAEVKKPGASVKVSCKASGYTF~~TSYG~~ISWVRQAPGQGLEWMGW ~~ISAYNGNT~~NYAQKL
QGRVTMTTDTSTSTAYMELRSLRSDDTAVYYC ARKYSGFDYWGQGTL

7G7-gl
5 QVQLVQSGAEVKKPGASVKVSCKASGYTF~~TSYG~~ISWVRQAPGQGLEWMGW ~~ISAYNGNT~~NYAQKL
QGRVTMTTDTSTSTAYMELRSLRSDDTAVYYC ARKYSGFDYWGQGTL

1.2. Light chain variable domain of human IgG1

10 2D8
EIVLTQSPSSLSASVGDRVTITCRASQSVGSF~~LNWY~~Q~~Q~~KPGKAPQVLI~~F~~ ~~GAS~~NLESGVPSRFSG
RSGSGSEFTLTINSLQPEDFATYYC QQSDSSPFTFGGGTK

5A12
ETTLTQSPGTL~~SL~~SPGERATLSCRASQSVSSSY~~LAWY~~Q~~Q~~KPGQAPRLLIY ~~GAS~~SRATGIPDRFS
15 GSGSGTDFNLTISRLEPEDFAVYYC HQSGSSLRTFGQGT

6C10
DVVMTQSPLSLPVTPEPASISCRSSRSL~~LH~~SN~~GYNY~~LDWYVQKPGQSPQLLIY ~~LGS~~YRASGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYC MQALQIPKTFGQGTK

7G7
20 EIVLTQSPSLPVTPEPASISCRSSQSL~~LH~~SN~~GYNY~~LDWYLQKPGQSPQLLIY ~~LGS~~NRASGVP
DRFSGSGSGTDFTLKISRVEAEDVGIYYC MQNLQTPWTFGQGTK

2D8-gl
DIQMTQSPSSLSASVGDRVTITCRASQSVGSF~~LNWY~~Q~~Q~~KPGKAPKLLIY ~~GAS~~SLQSGVPSRFSG
SGSGTDFTLTISLQPEDFATYYC QQSDSSPFTFGGGTK

25 7G7-gl
DIVMTQSPLSLPVTPEPASISCRSSQSL~~LH~~SN~~GYNY~~LDWYLQKPGQSPQLLIY ~~LGS~~NRASGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYC MQNLQTPWTFGQGTK

2. Nucleotide Sequences

30 2.1. Heavy chain variable domain of human IgG1

2D8, 2D8-gl

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTCACCT
GCGCTGTCTCTGGTGGCTCCATCAGCAGTGGTGGTTACTACTGGAGTTGGATCCGCCAGCCCC
CGGGAAGGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTACTACAACCCGTCC
CTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTCTCCCTGAAGCTGAGCT
5 CTGTGACTGCCGCGGACACGGCCGTGTATTACTGT GCGAGAGAAGAGGGTATAGCAGCAGCTGC
TTTTGATATCTGGGGCCAAGGGACAATG

5A12

GAGGTCCAGCTGGTGCAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCCTGAGACTCTCCT
GTGCAGCCTCTGGATTACCTTCAGTAGCTATAGCATGAACTGGGTCCGCCAGGCTCCAGGGAA
10 GGGGCTGGAGTGGGTCTCATCCATTAGTAGTAGTAGTTACATACTACGCAGACTCAGTG
AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAAGTCACTGTATCTGCAAATGAACAGCC
TGAGAGCCGAGGACACGGCTGTTTATTACTGT GCGAGAGATTACTATGATAGTAGTGGCTATCC
CTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACG

6C10

GAGGTCCAGCTGGTACAGTCTGGGGGAGGCCTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCT
GTGCAGCCTCTGGATTACCTTTAGTAGCTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAA
15 AGGGCTGGAGTGGGTGGCCAACATAAAGCAAGATGGAAGTGAGAAATACTATGTGGACTCTGTG
AAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAAGTCACTGTATCTGCAAATGAACAGCC
TGAGAGCCGAGGACACGGCTGTGTATTACTGT GCGAGGGATTTTGTGGACTGGTCCGCCACACC
20 CTTTGACTACTTGGGGCCAGGGCACCCCTG

7G7

GAGGTCCAGCTGGTaCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCT
GCAAGGCTTCTGGTTACACCTTTACCAGCTACGGTATCAGCTGGGTGCGACAGGCCCCCTGGACA
AGGGCTTGAGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGCACAGAAGCTC
25 CAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCC
TGAGATCTGACGACACGGCCGTGTATTACTGT GCGAGAAAATACAGTGGCTTTGACTACTTGGGG
CCAGGGCACCCCTG

7G7-gl

CAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCT
30 GCAAGGCTTCTGGTTACACCTTTACCAGCTACGGTATCAGCTGGGTGCGACAGGCCCCCTGGACA
AGGGCTTGAGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGCACAGAAGCTC
CAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGGAGCTGAGGAGCC

TGAGATCTGACGACACGGCCGTGTATTACTGT GCGAGAAAATACAGTGGCTTTGACTAC TGGGG
CCAGGGCACCCCTG

2.2. Light chain variable domain of human IgG1

5

2D8

GAAATTGTGTTGACGCAGTCTCCATCCTCCCTGTCTGCTTCTGTGGGAGACAGAGTCACCATCA
CTTGCCGGGCAAGT **CAGAGCGTTGGCTCTTTT**TTAAATTGGTATCAGCAGAAACCAGGGAAAGC
CCCTCAGGTCTGATCTTT **GGTGCCCTCCA**ATTTAGAAAGTGGGGTCCCATCAAGGTTCAAGTGGC
10 AGAGGATCTGGGTCAGAATTCACCTCTCACCATCAACAGTCTGCAACCCGAAGATTTTGCAACTT
ACTACTGT CAACAGAGTGACAGTTCCCCCTTCACTTTCGGCGGAGGGACCAAG

5A12

GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCT
CCTGCAGGGCCAGT **CAGAGTGTTAGCAGCAGCTACTT**AGCCTGGTACCAGCAGAAACCTGGCCA
15 GGCTCCCAGGCTCCTCATCTAT **GGTGCATCC**AGCAGGGCCACTGGCATCCAGACAGGTTCACT
GGCAGTGGGTCTGGGACAGACTTCAATCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGACAG
TGTATTACTGT CACCAGTCTGGCAGTTCACTTCGGACGTTTCGGCCAAGGGACCACG

6C10

GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCCATCT
20 CCTGCAGGTCTAGT **CGGAGCCTCCTACATAGTAATGGATACAAC**TATTTGGATTGGTACGTGCA
GAAGCCAGGGCAGTCTCCACAGCTCCTGATCTAT **TTGGGTTCA**TATCGGGCCTCCGGGGTCCCT
GACAGGTTTAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAGATCAGCAGGTTGGAGGCTG
AGGATGTTGGAGTTTATTACTGC ATGCAAGCTTTACAAATTCCGAAGACGTTTCGGCCAAGGGAC
CAAG

25 7G7

GAAATTGTGCTGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCCATCT
CCTGCAGGTCTAGT **CAGAGTCTCCTGCATAGTAATGGATACAAC**TATTTGGATTGGTACCTGCA
GAAGCCAGGGCAGTCTCCACAGCTCCTGATCTAT **TTGGGTTCT**AATCGGGCCTCCGGGGTCCCT
GACAGGTTCACTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATCAGCAGAGTGGAGGCTG
30 AGGATGTTGGGATTTATTACTGC ATGCAAAATCTTCAAACTCCGTGGACGTTTCGGCCAAGGGAC
CAAG

2D8-ql

GACATTCAGATGACGCAGTCTCCATCCTCCCTGTCTGCTTCTGTGGGAGACAGAGTCACCATCA
 CTTGCCCGGGCAAGT**CAGAGCGTTGGCTCTTTTTTAAATTGGTATCAGCAGAAACCAGGGAAAGC**
 CCCTAAGCTCCTGATCTAT**GGTGCCCTCC**AGTTTACAGAGTGGGGTCCCATCAAGGTTCAAGTGGC
 AGTGGATCTGGGACAGATTTCACCTCTCACCATCAGCAGTCTGCAACCCGAAGATTTGCAACTT
 5 ACTACTGT**CAACAGAGTGACAGTTCCCCCTTCACTT**TCGGCGGAGGGACCAAG
7G7-gl
 GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCCATCT
 CCTGCAGGTCTAGT**CAGAGTCTCCTGCATAGTAATGGATACAACATATTTGGATTGGTACCTGCA**
 GAAGCCAGGGCAGTCTCCACAGCTCCTGATCTAT**TTGGGTTCTAATCGGGCCTCCGGGTTCCCT**
 10 GACAGGTTCAAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATCAGCAGAGTGGAGGCTG
 AGGATGTTGGGGTTTATTACTGC**ATGCAAAATCTTCAAACCTCCGTGGACGTT**CGGCCAAGGGAC
 CAAG

BRIEF DESCRIPTION OF FIGURES

15

Figure 1 shows the flow cytometry analysis of the surface expression of human Trop2 protein on multiple cell lines including AGS, Colo205, HT-29, BxPC3, Capan-1, MCF-7, MDA-MB231, MDA-MB468, HCC1937, HeyA8, 293T, 786-O, and OVISe. A commercially available mouse anti-human Trop2 monoclonal antibody directly
 20 conjugated to PE fluorescent dye was used.

Figure 2A shows a graph of the ELISA assay results for the four anti-Trop2 human Fab clones tested using crude culture supernatants in a binding ELISA assay to assess their antigen binding to biotinylated human Trop2 protein with a poly-His tag (hTrop2-His), and detected by goat-anti-human Fab-HRP.

25 **Figure 2B** shows the flow cytometry analysis of the four Fab clones in their binding to cell surface expressed Trop 2. Using crude culture supernatants, the 4 Fab clones were tested on their binding to Trop2^{High} AGS and Trop2^{Negative} 293T cells.

Figure 3A shows binding results for the four anti-Trop2 Fab clones that were constructed to full-length human IgG antibodies and were tested for their binding to
 30 biotinylated human Trop2 protein antigen using binding ELISA.

Figure 3B shows the flow cytometry analysis for the four anti-Trop2 human IgG antibodies at different concentration in their binding to Trop2^{High} AGS cells. **Figure 3C** shows the flow cytometry analysis for the four anti-Trop2 human IgG antibodies at

different concentration in their binding to Trop2^{Positive/Negative} HT-29 cells. **Figure 3D** shows the flow cytometry analysis for the four anti-Trop2 human IgG antibodies at different concentration in their binding to Trop2^{Negative} 293T cells. The percentages of cells positively stained by anti-Trop2 IgG antibodies were plotted.

- 5 **Figure 3E** shows the cross-reactivity binding ELISA results of four anti-Trop2 IgG antibodies to human Trop2 (hTrop2-His), cynomolgus Trop2 (cynoTrop2-His) as well as mouse Trop2 (mTrop2-His) protein, coated at 100nM to the ELISA plate overnight and antibodies were tested at 10nM.

- Figure 4A** shows the NK cell mediated cytotoxicity killing assay results for
10 Trop2^{High} AGS cells. **Figure 4B** shows the NK cell mediated cytotoxicity killing assay results for Trop2^{Negative} 293T cells. The cells were subjected to NK cell mediated cytotoxicity killing assay (E:T ratio = 3:1) in the presence of different concentrations of anti-Trop2 antibodies 2D8 (circle), 5A12 (inverted triangle), 6C10 (square) and 7G7 (triangle). Cell viability was monitored using real-time xCELLigence RTCA system.
15 Percentages of specific cytolysis at 72 hours were calculated and plotted by normalizing to wells of target cells incubated only with effector NK cells in the absence of anti-Trop2 antibodies.

- Figure 5A** shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) in targeting Trop2^{High} AGS cells evaluated using anti-human IgG Fc specific secondary antibodies conjugated to MMAE (20nM, Fab fragment). **Figure 5B**
20 shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) in targeting Trop2^{High} AGS cells evaluated using anti-human IgG Fc specific secondary antibodies conjugated to DX-8951 (20nM, Fab fragment). **Figure 5C** shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) in targeting Trop2^{High} AGS
25 cells evaluated using anti-human IgG Fc specific secondary antibodies conjugated to PBD (0.1nM, full IgG). **Figure 5D** shows the evaluation of the anti-Trop2 ADCs in their killing of Trop2^{Negative} 293T cells using anti-human IgG Fc specific secondary antibodies conjugated to MMAE (20nM, Fab fragment). **Figure 5E** shows the evaluation of the anti-Trop2 ADCs in their killing of Trop2^{Negative} 293T cells using anti-human IgG Fc specific secondary antibodies conjugated to DX-8951 (20nM, Fab fragment). **Figure 5F** shows
30 the evaluation of the anti-Trop2 ADCs in their killing of Trop2^{Negative} 293T cells using anti-human IgG Fc specific secondary antibodies conjugated to PBD (0.1nM, full IgG). Circle = ADCs using clone 2D8, inverted triangle = ADCs using clone 5A12, square = ADCs

using clone 6C10, and triangle = ADCs using clone 7G7. An antibody not targeting Trop2 was included as a negative control (Neg Ctrl, grey diamond). After 5 days of incubation with primary anti-Trop2 antibodies and payload conjugated secondary antibodies, the cell viability of each well was measured using CellTiter-Glo® Luminescent Cell Viability Assay and percentages of cytotoxicity were calculated and plotted by normalizing to the readings of wells only incubated with secondary antibodies in the absence of anti-Trop2 primary antibodies.

Figure 6A shows the epitope binning of anti-Trop2 IgG (clone 2D8) by BLI analysis, wherein clone 2D8 was immobilized as the ligand. Protein antigen of human Trop2 (hTrop2-His) was introduced as the first analyte. The second antibody (clone 7G7, Sacituzumab or Datopotamab) was introduced as the second analyte. As controls, buffer alone, an isotype IgG, and 2D8 IgG were also included as the second analyte. **Figure 6B** shows the epitope binning of anti-Trop2 IgG (clone 7G7) by BLI analysis, wherein clone 7G7 was immobilized as the ligand. Protein antigen of human Trop2 (hTrop2-His) is introduced as the first analyte. The second antibody (clone 2D8, Sacituzumab or Datopotamab) was introduced as the second analyte. As controls, buffer alone, an isotype IgG, and 7G7 IgG were also included as the second analyte. For each sub-panel of figures, the second analyte was labelled and shown.

Figure 7A shows the heavy and light chain variable region framework sequences (FR1, FR2 and FR3) of 2D8, 7D7 and their germline configured counterparts 2D8-gl and 7G7-gl.

Figure 7B shows the binding results of Anti-Trop2 IgG antibodies (2D8 and 7G7) and their germline configured counterparts (2D8-gl and 7G7-gl) when tested for their binding to biotinylated human Trop2 protein antigen (hTrop2-His) using binding ELISA and compared to the two benchmark antibody clones Sacituzumab and Datopotamab. Binding EC50 values of these anti-Trop2 IgG antibodies to the antigen protein were calculated by PRISM and are shown in the table.

Figure 7C shows the flow cytometry analysis results for the anti-Trop2 IgG antibodies in their binding to Trop2^{High} AGS cells. Cell binding EC50 values of these anti-Trop2 IgG antibodies to the Trop2^{High} AGS cells were calculated by PRISM and shown in the table. **Figure 7D** shows the flow cytometry analysis results for the anti-Trop2 IgG antibodies in their binding to Trop2^{Negative} 293T cells. The percentages of cells positively stained by anti-Trop2 IgG antibodies were plotted.

Figure 8A shows the results of an experiment to assess the ADCC killing of Trop2^{High} AGS cells using anti-Trop 2 IgG antibodies 2D8, 7G7 and their germline counterparts. **Figure 8B** shows the results of an experiment to assess the ADCC killing of Trop2^{Negative} 293T using anti-Trop 2 IgG antibodies 2D8, 7G7 and their germline counterparts. The cells were subjected to killing by fresh human NK cells (E:T ratio = 3:1) mediated by different concentrations of anti-Trop2 antibodies 2D8 (filled circle), 7G7 (filled triangle), as well as their germline configured counterparts 2D8-gl (open circle) and 7G7-gl (open triangle). Two benchmark antibody clones Sacituzumab and Datopotamab were included as reference controls. Cell viability was monitored using real-time xCELLigence RTCA system. Percentages of specific cytolysis at 72 hours were calculated and plotted by normalizing to wells of target cells incubated only with effector NK cells in the absence of anti-Trop2 antibodies. The EC50 values for ADCC killing of the Trop2^{High} AGS cells were calculated by PRISM and shown in the table.

Figure 9A shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) derived from clones 2D8 and 7G7 in targeting Trop2^{High} AGS cells evaluated using anti-human IgG Fc specific secondary antibodies conjugated to MMAE (20nM, Fab fragment). **Figure 9B** shows the cytotoxic potency of the anti-Trop2 ADCs derived from clones 2D8 and 7G7 in targeting Trop2^{High} AGS cells evaluated using anti-human IgG Fc specific secondary antibodies conjugated to DX-8951 (20nM, Fab fragment). **Figure 9C** shows the cytotoxic potency of the anti-Trop2 ADCs in targeting Trop2^{Negative} 293T cells evaluated using anti-human IgG Fc specific secondary antibodies conjugated to MMAE (20nM, Fab fragment). **Figure 9C** shows the cytotoxic potency of the anti-Trop2 ADCs in targeting Trop2^{Negative} 293T cells evaluated using anti-human IgG Fc specific secondary antibodies conjugated to DX-8951 (20 nM, Fab fragment). After 5 days of ADC antibody incubation, cell viability was measured using CellTiter-Glo® Luminescent Cell Viability Assay and percentages of cytotoxicity was calculated and plotted by normalizing to the readings of wells only incubated with secondary antibody in the absence of anti-Trop2 primary antibodies. The EC50 values for ADC killing of the Trop2^{High} AGS cells were calculated by PRISM and shown in the tables. filled circle = 2D8, filled triangle = 7G7, open circle = 2D8-gl, open triangle = 7G7-gl, grey diamond = an antibody not targeting Trop2 as negative control. Benchmark antibodies Sacituzumab and Datopotamab were also included.

Figure 10A shows the Structural format of anti-Trop2 bi-specific T cell-engaging antibodies. Clone Okt3 was used as the anti-CD3 arm in the format of single chain variable fragment (scFv).

Figure 10B shows the ELISA binding results of anti-Trop2 BiTE antibodies for binding to biotinylated human Trop2 protein antigen (hTrop2-His) when compared to anti-Trop2 BiTE antibodies constructed from two benchmark clones Sacituzumab and Datopotamab. Binding EC50 values of these anti-Trop2 BiTE antibodies to the antigen protein were calculated by PRISM and shown in the table.

Figure 10C shows the results of a T-cell mediated killing assay for the anti-Trop2 BiTE antibodies for Trop2^{High} MCF-7 cells. **Figure 10D** shows the results of a T-cell mediated killing assay for the anti-Trop2 BiTE antibodies for Trop2^{Medium} HeyA8 cells. **Figure 10E** shows the results of a T-cell mediated killing assay for the anti-Trop2 BiTE antibodies for Trop2^{Negative} 293T cells. Anti-Trop2 BiTE antibodies were subjected to T cell mediated killing assay with time-course measurement using real-time xCELLigence RTCA system up to 72 hours. Percentage of specific cytolysis mediated by activated human T cells (E:T ratio = 4:1) upon treatment with different anti-Trop2 BiTE antibodies (clone 2D8, 2D8-gl, 7G7, 7G7-gl, 5A12, 6C10, Sacituzumab and Datopotamab) at 72 hours are shown.

Figure 10F shows the ELISA measurement of interferon-gamma secretion from activated human T cells. **Figure 10G** shows the ELISA measurement of IL-2 secretion from activated human T cells. Results show ELISA measurements from activated human T cells after 48 hours of co-culture with Trop2^{High} MCF-7 cells (black bar), Trop2^{Medium} HeyA8 cells (grey bar), and Trop2^{Negative} 293T cells (white bar) with addition of different anti-Trop2 BiTE antibodies at concentration of 1nM.

Figure 11A shows the structural format of anti-Trop2 CAR constructs.

Figure 11B shows the percentages of CAR expression on anti-Trop2 CAR T cells after lentivirus transduction were detected by flow cytometry analysis.

Figure 11C shows the percentage of cytolysis of Trop2^{High} AGS cells mediated by anti-Trop2 CAR T cells with time-course measurement using real-time xCELLigence RTCA system (40% CAR, E:T ratio = 3:1).

Figure 11D shows the percentage of cytolysis of Trop2^{Negative} 293T cells mediated by anti-Trop2 CAR T cells with time-course measurement using real-time xCELLigence RTCA system (40% CAR, E:T ratio = 3:1).

Figure 11E shows the ELISA measurement of interferon-gamma secretion from anti-Trop2 CAR T cells. **Figure 11G** shows the ELISA measurement of IL-2 secretion from anti-Trop2 CAR T cells. Results show ELISA measurements from anti-Trop2 CAR T cells after 24 hours of co-culture with either Trop2^{High} AGS cells (black bar) or Trop2^{Negative} 293T cells (white bar). Anti-Trop2 CAR T cells generated from scFv sequences using the benchmark clones Sacituzumab and Datopotamab were used as reference controls.

Figure 12A shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{High} AGS cells. **Figure 12B** shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{High} Colo-205 cells. **Figure 12C** shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{Negative} 293T cells. The cytotoxic potency was evaluated using anti-human IgG Fc specific secondary antibodies conjugated to PBD (0.25nM). After 5 days of ADC antibody incubation, cell viability was measured using CellTitre-Glo Luminescent Cell Viability Assay and percentages of cytotoxicity was calculated by normalizing to the readings of wells only incubated with secondary antibody alone. filled circle = 2D8, filled triangle = 7G7, open circle = 2D8-gl, open triangle = 7G7-gl, grey diamond = an antibody not targeting Trop2 as negative control. Benchmark antibodies Sacituzumab and Datopotamab were also included.

Figure 13A shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{High} MCF-7 cells. **Figure 13B** shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{High} MDA-MB231 cells. **Figure 13C** shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{Medium} MDA-MB468 cells. **Figure 13D** shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{Medium} HeyA8. The cytotoxic potency was evaluated using anti-human IgG Fc specific secondary antibodies

conjugated to PNU-159682 (2.5nM or 5nM or 10nM). After 5 days of ADC antibody incubation, cell viability was measured using CellTitre-Glo Luminescent Cell Viability Assay and percentages of cytotoxicity was calculated by normalizing to the readings of wells only incubated with secondary antibody alone. filled circle = 2D8, filled triangle = 7G7, open circle = 2D8-gl, open triangle = 7G7-gl, grey diamond = an antibody not targeting Trop2 as negative control. Benchmark antibodies Sacituzumab and Datopotamab were also included.

Figure 14A shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{High} MCF-7 cells. **Figure 14B** shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{High} MDA-MB231 cells. **Figure 14C** shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{Medium} MDA-MB468 cells. **Figure 14D** shows the cytotoxic potency of the anti-Trop2 antibody-drug conjugates (ADCs) using clones 2D8, 7G7 and their germline counterparts 2D8-gl and 7G7-gl when targeting Trop2^{Medium} HeyA8. The cytotoxic potency was evaluated using anti-human IgG Fc specific secondary antibodies conjugated to DMDM (20nM). After 5 days of ADC antibody incubation, cell viability was measured using CellTitre-Glo Luminescent Cell Viability Assay and percentages of cytotoxicity was calculated by normalizing to the readings of wells only incubated with secondary antibody alone. filled circle = 2D8, filled triangle = 7G7, open circle = 2D8-gl, open triangle = 7G7-gl, grey diamond = an antibody not targeting Trop2 as negative control. Benchmark antibodies Sacituzumab and Datopotamab were also included.

Figure 15A shows a structural map of 10 constructs used the epitope mapping of anti-Trop2 IgGs. These Trop2 chimeric proteins were constructed based on the mouse Trop2 protein sequences including signal peptide, ECD (extracellular domain), TM (transmembrane domain) and ICD (intracellular domain). In each of the 10 constructs, a small peptide sequence of the mouse Trop2 (grey boxes with numbers 1-10) was replaced by the sequence of its human Trop2 counterpart. The replaced human Trop2 peptide sequences were shown in the table and those amino acids that were different from the mouse Trop2 sequence was highlighted (bold and underlined). An HA-tag

sequence was inserted between the signal peptide and the ECD for the purpose of monitoring the expression efficiency.

Figure 15B shows the flow cytometry analysis results for the 10 constructs when transfected into Trop2 negative 293T cells and stained with different anti-Trop2 IgGs.

- 5 Each of the 10 constructs were individually transfected into the Trop2 negative 293T cells by transient transfection system and 48 hours post transfection, the cells were harvested and stained with different anti-Trop2 IgGs (2D8-gl, 5A12, 7G7-gl, Sacituzumab, Datopotamab) for flow cytometry analysis and histograms of mean fluorescence intensity were shown. An anti-HA antibody staining was included as
- 10 positive control to show efficient surface expressions of the Trop2 chimeric proteins on 293T cells.

EXAMPLES

- Example embodiments of the disclosure will be better understood and readily
- 15 apparent to one of ordinary skill in the art from the following discussions and if applicable, in conjunction with the figures. It should be appreciated that other modifications may be made without deviating from the scope of the invention. Example embodiments are not necessarily mutually exclusive as some may be combined with one or more
- embodiments to form new exemplary embodiments. The example embodiments should
- 20 not be construed as limiting the scope of the disclosure.

Example 1 - Discovery of fully human anti-Trop2 antibodies from naïve human Fab phage display library

- To identify cell lines that can be used for characterizing the properties of anti-
- 25 Trop2 antibodies involved in this invention, the present inventors analysed the surface Trop2 antigen expression on a panel of human cell lines derived from different origins of cancers, using a commercially available anti-Trop2 antibody. Trop2 had been detected with moderate to high level of surface expression on most cells tested including a gastric adenocarcinoma cell line AGS, a colon cancer cell line Colo205, two pancreatic cancer
- 30 cell lines BxPC3 and Capan-1, three breast cancer cell lines MCF-7, MDA-MB231, and HCC1937, as well as an ovarian cancer cell line HeyA8. However, Trop2 expression cannot be detected on 293T, a renal cell carcinoma cell line 786-O, and an ovarian clear

cell carcinoma cell line OVISE. Interestingly, Trop2 was only expressed on half of the HT-29 cells and its expression was relatively low on MDA-MB468 cells (**Figure 1**).

Next, biotinylated recombinant human Trop2 protein with a poly-His tag (hTrop2-His) was used to isolate Trop2 binders from a naïve library of Fab sequences constructed in SIgN, using phage display technology. Sixteen clones showing the positive Fab supernatant binding signals to biotinylated hTrop2-His protein as well as specific binding to Trop2 expressing cells were sequenced and 4 unique sequences were identified: 2D8, 5A12, 6C10 and 7G7 (Annex). The results of binding ELISA and flow cytometry analysis of cell binding to Trop2^{High} AGS and Trop2^{Negative} 293T cells using crude Fab supernatants for the 4 unique clones were shown in **Figure 2**.

Example 2 - Antigen binding profiles of anti-Trop2 IgG antibodies

The 4 Fab antibody clones were then sub-cloned and expressed as human IgG1 format and tested in ELISA against recombinant human Trop2 protein to assess their binding affinity for the target, with an irrelevant IgG used as negative control antibody (**Figure 3A**).

Next, the ability of anti-Trop2 antibodies to specifically recognise and bind Trop2 on the surface of cells was measured using AGS, HT-29 and 293T cells. AGS cells constitutively express Trop2, only half of HT-29 cells express Trop2, while 293T cells do not express Trop2.

In this assay, the binding profiles of all four tested anti-Trop2 IgG antibodies showed good binding specificity, with strong binding to the Trop2^{High} AGS cells (**Figure 3B**), around 40-50% binding to HT-29 cells (**Figure 3C**) and no binding to Trop2^{Negative} 293T cells (**Figure 3D**). This suggests that these antibodies bind to surface of the cells in a highly specific way dependent on Trop2 protein expression.

As Trop2 is a highly conserved gene, the present inventors performed cross-reactivity ELISA to assess the cross species reactivity of our anti-Trop2 antibodies, where recombinant human, cynomolgus and mouse Trop2 proteins were each used in a binding ELISA to test their binding to each of the anti-Trop2 IgG clones.

All four anti-Trop2 IgG clones exhibited cross-reactivity to cynomolgus Trop2 but not to mouse Trop2 (**Figure 3E**), indicating that these antibodies recognize common epitopes of Trop2 protein across human and cynomolgus antigens.

Example 3 - Functional characterization for lead clone selection

To assess the ability of these anti-Trop2 antibodies to induce ADCC, Trop2^{High} AGS cells or Trop2^{Negative} 293T cells were mixed with naïve natural killer (NK) cells freshly isolated from human PBMCs at an E:T ratio of 4:1 in the absence or presence of each anti-Trop2 IgG clones at different concentrations. The cell index values were then measured continuously using xCELLigence RTCA system at an interval of 1 hour for a total of 72 hours. The end-point cytotoxicity at different IgG concentrations was plotted and shown in **Figure 4**. 2D8 IgG showed the highest potency in inducing NK cell mediated ADCC of Trop2^{High} AGS cells, followed by 7G7, 5A12 and 6C10. All four IgG antibodies did not induce ADCC of Trop2^{Negative} 293T cells, further indicating the superior target specificity of these anti-Trop2 antibodies.

Next, the *in vitro* anti-cancer potency of four anti-Trop2 IgG antibodies was evaluated in the format of antibody-drug conjugate (ADC) using anti-human IgG Fc specific secondary antibodies conjugated to different payload toxins, including MMAE (blocking tubulin polymerisation), DX-8951 (topoisomerase inhibitor), and PBD (DNA minor groove alkylator). Cell viability was measured after 5 days of antibody incubation using CellTitre-Glo Luminescent Cell Viability Assay. To reflect the effect in inhibiting target cell growth brought by the ADCs, the percentages of cytotoxicity were calculated by normalizing to the luminescence readings of wells where the target cells were only incubated with secondary antibody in the absence of any anti-Trop2 antibodies. 2D8 IgG showed superior potency in inhibiting the growth of Trop2^{High} AGS cells with the use of all three different payload toxins. 7G7 IgG also showed some cytotoxicity activity, albeit much weaker than 2D8 IgG (**Figure 5A, 5B, and 5C**). Both 2D8 and 7G7 IgGs could not inhibit growth of Trop2^{Negative} 293T cells, when they were used with any of the three payload toxins (**Figure 5D, 5E, and 5F**).

Example 4 - Epitope mapping of 2D8 and 7G7

Since 2D8 and 7G7 were identified as the two lead clones with highest potency in ADCC and ADC assays, the present inventors were interested to find out if 2D8 and 7G7 bind to the same epitope on Trop2 protein. Hence, the present inventors performed stepwise binding BLI assays. First, 2D8 IgG was immobilized as the ligand and the Trop2 protein antigen was introduced as the first analyte. When the second antibody 7G7 IgG

was introduced as the second analyte, a second binding signal was detected, suggesting that 2D8 and 7G7 bind to different epitopes on Trop2 protein. Interestingly, 2D8 shared overlapping epitopes with two benchmark antibody clones Sacituzumab (the parental antibody of "Trodelvy") and Datopotamab (the parental antibody of the DS-1062 ADC) (Figure 6A). Conversely, when 7G7 IgG was immobilized as the ligand and Trop2 protein as the first analyte, followed by other antibody clones 2D8, Sacituzumab and Datopotamab as the second analyte, a second binding signal was detected in all cases (Figure 6B), suggesting that 7G7 bound to a different non-overlapping epitope from 2D8, Sacituzumab or Datopotamab, all of which share the same or overlapping epitope.

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Example 5 - Construction of germline configured counterparts of 2D8 and 7G7

To further improve the performance of 2D8 and 7G7, the present inventors generated the germline configured counterparts of these two antibodies. The present inventors analysed the sequences of variable regions of both heavy and light chains of 2D8 and 7G7 using IgBlast and identified their closest germline gene haplotypes. For 2D8, while the heavy chain framework is identical to the closest germline framework sequence IGHV4-30-4*07, its kappa chain framework substantially deviates from the closest germline haplotype IGKV1-39*01 by 12 amino acid differences. For 7G7, the heavy chain framework only differs from the closest germline framework sequence IGHV1-18*01 by the first amino acid, however, the kappa chain framework has three amino acid differences from the closest germline haplotype IGKV2-28*01. With this information, we have reverse engineered the 2D8 and 7G7 antibodies by mutating their framework sequences to match their closest germline gene haplotypes and produced the germline configured counterparts and they were named as "2D8-gl" and "7G7-gl", respectively (Figure 7A).

The antigen binding of the germline configured counterparts were assessed by binding ELISA and showed slightly increased binding affinity compared to the parental clones 2D8 and 7G7, respectively, with binding EC50 values close to 1nM. However, the binding EC50 was still higher than the two benchmark clones Sacituzumab and Datopotamab, both of which are less than 1nM (Figure 7B).

Next, the ability of these anti-Trop2 antibodies to specifically bind to Trop2 on the surface of cells was measured using Trop2^{High} AGS and Trop2^{Negative} 293T cells. Similar to the ELISA result, the binding potency of the germline configured counterparts were

also improved in cell surface binding with the superior binding specificity unchanged (Figure 7C and 7D). Only Datopotamab stained Trop2^{Negative} 293T cells at high antibody concentration of 20nM, suggesting that the binding specificity of our anti-Trop2 antibodies are even better than the benchmark clone Datopotamab.

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Example 6 - Use in antibody-dependent cell-mediated cytotoxicity (ADCC)

With germline configured counterparts of 2D8 and 7G7 constructed, the present inventors extended the antibody functional characterization to the two new antibodies for their use in ADCC and compared the potency with their parental clones as well as the benchmark clones Sacituzumab and Datopotamab. Similarly, Trop2^{High} AGS cells and Trop2^{Negative} 293T cells were used as target cells. Surprisingly, 2D8-gl showed an even higher killing potency in inducing ADCC of AGS cells than its parental clone 2D8, which is similar to Sacituzumab and Datopotamab (Figure 8A). All IgG antibodies tested maintained good target specificity as they did not induce ADCC of Trop2^{Negative} 293T cells (Figure 8B).

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Example 7 - Use as antibody-drug conjugate (ADC)

The present inventors also compared 2D8-gl and 7G7-gl with their parental counterparts and the benchmark antibodies for their use as antibody-drug conjugate (ADC). Similarly, anti-human IgG Fc specific secondary antibodies conjugated to either MMAE (blocking tubulin polymerisation) or DX-8951 (topoisomerase inhibitor), were used. Compared to their parental clones, both 2D8-gl and 7G7-gl showed more potent cytotoxicity in inhibiting the growth of Trop2^{High} AGS cells with the use of MMAE. Interestingly, the potency of both 2D8 and 2D8-gl greatly exceeded that of Sacituzumab and Datopotamab, making 2D8 and 2D8-gl very promising leads in further development into an ADC drug (Figure 9A). When used with the second payload DX-8951, 2D8-gl also showed slightly higher potency than the benchmark clones (Figure 9B). When used as ADC, all antibodies did not show growth inhibition of Trop2^{Negative} 293T cells (Figure 9C and 9D).

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Example 8 - Use as anti-Trop2 Bi-specific T cell-Engaging (BiTE) antibodies

The anti-Trop2 antibodies were also used to construct anti-Trop2 bi-specific T cell-engaging (BiTE) antibodies. In these constructs, the Fab region of one arm from

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each of the anti-Trop2 antibody clones (2D8, 2D8-gl, 7G7, 7G7-gl, 5A12, 6C10) was replaced by the anti-CD3 scFv fragment (clone Okt3). Knob-in-hole mutations were introduced to facilitate correct heavy-chain pairing and LALA mutations to remove Fcγ receptor binding (**Figure 10A**).

- 5 The ability of these anti-Trop2 BiTE antibodies to recognize and bind to Trop2 protein was measured by binding ELISA (**Figure 10B**). The cytotoxic function of these anti-Trop2 BiTE antibodies in killing Trop2 expressing tumor cells was examined by subjecting them to the antibody dependent T cell mediated cytotoxicity assay. Briefly, T cells from healthy donors were isolated and incubated with either MCF-7 (Trop2^{High}),
10 HeyA8 (Trop2^{Medium}) or 293T (Trop2^{Negative}) cells at an E:T ratio of 4:1, in the presence of anti-Trop2 BiTE antibodies at 7 different concentrations, and the cell viability was monitored by xCELLigence RTCA system for up to 72 hours. All anti-Trop2 BiTE antibodies tested were able to kill 90-100% of Trop2^{High} MCF-7 cells within 72 hours at a concentration as low as 10pM (**Figure 10C**). These BiTE antibodies also efficiently killed
15 Trop2^{Medium} HeyA8 cells, albeit with a relatively lower efficacy (**Figure 10D**). No killing of Trop2^{Negative} 293T cells were observed (**Figure 10E**), indicating superior specificity of these anti-Trop2 BiTE antibodies.

- In addition, cell culture supernatants were collected and subjected to ELISA to assess the cytokine release of interferon-gamma and IL-2 from anti-Trop2 BiTE treated
20 T cells. When co-cultured with Trop2^{High} MCF-7 cells and Trop2^{Medium} HeyA8 cells in the presence of anti-Trop2 BiTE antibodies, activated human T cells secreted remarkably high amount of interferon-gamma and IL-2. However, there was no secretion of interferon-gamma or IL-2 can be detected when the T cells were co-cultured with Trop2^{Negative} 293T cells in the presence of anti-Trop2 BiTE antibodies (**Figure 10F** and
25 **10G**). Results from the above assays showcased the use of our anti-Trop2 antibodies in the format of BiTE with high specificity and potency in inducing cell death of Trop2 expressing cells.

Example 9 - Use in a Chimeric Antigen Receptor (CAR) T cell construct

- The sequences of these anti-Trop2 antibodies can also be used to construct anti-
30 Trop2 CAR T cells. All anti-Trop2 clones characterized in this invention were expressed as CAR in a second-generation CAR format (CD3zeta plus 4-1BB intracellular domain, **Figure 11A**). To assess the function of these anti-Trop2 CAR T cells, T cells from healthy

donors were activated by TransAct™ (CD3/CD28 agonists) in the presence of IL-2 (50 unit/ml) for 72 hours and the CAR genes were introduced by lentiviral based transduction process. Six days post viral transduction, the transduction rate was assessed by flow cytometry analysis and expressed as percentages of CAR gene expression on the surface of the CAR T cells (**Figure 11B**). Interestingly, the transduction rates for different CAR T cells varied. Most CAR T cells showed high transduction rates of 40-55% (2D8-g1, 7G7, 5A12, Sacituzumab, Datopotamab). However, CAR T cells using clone 2D8 had only 28.6% CAR expression, and those constructed from clone 7G7-g1 and 6C10 showed the lowest (<20% CAR). We have applied a process of puromycin selection for 5 days to enrich the CAR positive populations and the CAR expression for all constructs reached more than 40% (ranging 44.2% to 95.1%). After removing puromycin, the potency of these anti-Trop2 CAR T cells were subjected to a T cell-mediated cytotoxicity assay using xCELLigence RTCA system. The CAR T cells proved to be highly efficient in killing Trop2 expressing tumor cells. Within 96 hours of co-culture, clone 2D8, 2D8-g1, 7G7, 7G7-g1, 5A12, Sacituzumab, and Datopotamab based anti-Trop2 CAR T cells could lyse 80-100% of Trop2^{High} AGS cells (**Figure 11C**). The killing by these CAR T cells was highly specific to antigen expression, as the same CAR T cells only showed minimal background killing of Trop2^{Negative} 293T cells (10-40%, **Figure 11D**).

In addition, 24 hours post co-culture of anti-Trop2 CAR T cells with Trop2^{High} AGS cells or Trop2^{Negative} 293T cells, cell culture supernatants were collected and subjected to ELISA to assess the cytokine release of interferon-gamma and IL-2 from anti-Trop2 CAR T cells. When co-cultured with Trop2^{High} AGS cells, anti-Trop2 CAR T cells based on all clones except clone 6C10 secreted a high level of interferon-gamma and IL-2. However, levels of interferon-gamma and IL-2 secretion were minimal when these anti-Trop2 CAR T cells were added to Trop2^{Negative} 293T cells, indicating the superior specificity of these anti-Trop2 CAR T cells in responding to and killing of Trop2 expressing cells (**Figure 11E and 11F**).

Example 10 - Use as antibody-drug conjugate (ADC) with payload PBD

The present inventors compared 2D8-g1 and 7G7-g1 with their parental counterparts and two benchmark antibodies Sacituzumab and Datopotamab for their use as antibody-drug conjugate (ADC) with the third type of cytotoxic payload pyrrolobenzodiazepine (PBD), which exerts its cytotoxic function by alkylating DNA and

inhibiting DNA replication. As PBD has a much more potent cytotoxicity than MMAE or DX-8951, a much lower concentration of 0.25nM of PBD conjugated secondary antibodies was used in the assay. Compared to their parental clones, both 2D8-g1 and 7G7-g1 showed slightly more potent cytotoxicity than their parental clones in inhibiting the growth of Trop2^{High} AGS and Trop2^{High} Colo-205 cells with the use of PBD. The potency of both 2D8 and 2D8-g1 also greatly exceeded that of Sacituzumab and Datopotamab (**Figure 12A and Figure 12B**). In addition, all antibodies did not show growth inhibition of Trop2^{Negative} 293T cells at all tested doses (**Figure 12C**).

10 **Example 11 - Use as antibody-drug conjugate (ADC) with payload PNU-159682**

Next, the present inventors compared 2D8-g1 and 7G7-g1 with their parental counterparts and benchmark antibodies Sacituzumab and Datopotamab for their use as antibody-drug conjugate (ADC) with the fourth type of cytotoxic payload PNU-159682, which exerts its cytotoxic function by intercalating DNA and inhibiting DNA replication.

15 The present inventors included four cells in this assay to test the ADC effects on cells with different surface expression levels of Trop2, including MCF-7 and MDA-MB231 cells expressing very high level of Trop2, as well as MDA-MB468 and HeyA8 cells expressing relatively lower level of Trop2. Similar to PBD, PNU-159682 also showed very potent cytotoxicity and hence the concentrations ranging from 2.5nM, 5nM to 10nM of PNU-159682 conjugated secondary antibodies were used, depending on cell types. In MCF-7 (**Figure 13A**), MDA-MB231 (**Figure 13B**) and HeyA8 (**Figure 13D**) cells, both 2D8 and 2D8-g1 showed much higher cytotoxicity potency than that of Sacituzumab and Datopotamab, when used with PNU-159682. Interestingly, in MDA-MB468 cells showing the lowest surface expression among the four tested cells (**Figure 1**), 2D8-g1 showed the similar potency of cytotoxicity as Sacituzumab and Datopotamab when used with PNU-159682 (**Figure 13C**).

Example 12 - Use as antibody-drug conjugate (ADC) with payload DMDM

Next, the present inventors compared 2D8-g1 and 7G7-g1 with their parental counterparts and the benchmark antibodies Sacituzumab and Datopotamab for their use as antibody-drug conjugate (ADC) with the fifth type of cytotoxic payload DMDM, which is a DNA minor groove alkylator of the adenine. Similarly, the present inventors used four different cells as used in **Figure 13** to compare the ADC effects on cells with different

surface expression levels of Trop2. Both 2D8 and 2D8-gl showed high cytotoxicity potency in all four cells tested (**Figure 14A to 14D**), regardless of Trop2 expression level, when used with DMDM. However, Sacituzumab and Datopotamab showed very weak cytotoxicity in MDA-MB231 (**Figure 14B**) and HeyA8 cells (**Figure 14D**).

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Example 13- Epitope mapping of anti-Trop2 antibodies

The present inventors previously performed stepwise binding BLI assays to find out if 2D8 or 7G7 IgGs bind to the same epitope on the Trop2 protein (**Figure 6**). From that experiment, the present inventors concluded that 2D8 and 7G7 bind to a different epitope and 2D8 may share a similar or overlapping epitope as Sacituzumab and Datopotamab.

To precisely locate the binding epitopes of our anti-Trop2 antibodies, the present inventors designed a series of constructs expressing 10 different human/mouse Trop2 chimeric proteins (**Figure 15A**). As none of the anti-Trop2 antibodies cross-react with the mouse Trop2 (**Figure 3E**), by performing a sequence alignment of human and mouse Trop2 protein sequences, the present inventors identified 10 potential binding regions containing short peptides of 12 to 21 amino acids spanning the extracellular domain (ECD) of human Trop2 protein, with at least 3 amino acids which are different from the mouse counterpart. The present inventors transiently transfected these expressing constructs into the Trop2 negative 293T cells and after 48 hours of transfection, harvested each transfectants for a flow cytometry analysis for antibody staining with different anti-Trop2 antibody clones, including 2D8-gl, 5A12, 7G7-gl, Sacituzumab and Datopotamab.

The results showed that 5A12 bound to the same construct (Construct 9) as Sacituzumab and Datopotamab, indicating that 5A12 shared the same epitope (amino acid 237 to 252) as the two reference antibodies (Ref - Sun et al. iScience 2011, 24:103190). As expected, 7G7-gl bound to a different construct (Construct 1), which corresponded to an epitope close to the N-terminus of Trop2 protein (amino acid 45 to 63). Interestingly, different from what we predicted from the previous study by BLI analysis, 2D8-gl, in fact, bound to a different epitope (Construct 6, amino acid 181 to 198), which is far away from the linear binding epitope of Sacituzumab. The present inventors highlighted different antibody epitopes on the solved crystal structure (PDB: 7E5M) and found out that the binding epitope of 2D8-gl is close to that of Sacituzumab

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after protein folding, hence it is possible that the binding of 2D8-gl IgG to the Trop2 protein will exclude the binding of Sacituzumab, and vice versa.

Example 14 - Binding affinity of anti-Trop2 human IgG₁ antibodies

5 Binding affinities of 4 anti-Trop2 IgG₁ antibodies 2D8, 5A12, 6C10 and 7G7 were measured via Bio-Layer Interferometry (BLI) analysis using Octet RED96 system and shown in **Table 1**. 2D8 and 7G7 showed much higher affinities than 5A12 and 6C10. Next, binding affinities of 2 germline configured counterparts of anti-Trop2 IgG₁ antibodies 2D8-gl and 7G7-gl were measured via Bio-Layer Interferometry (BLI) analysis
10 using Octet RED96 system and shown in Table 2. Both antibody clones exhibited binding affinities to the recombinant Trop2 protein at the nanomolar scales. Compared to the 2 benchmark reference antibodies Sacituzumab and Datopotamab, our anti-Trop2 IgG₁ antibodies had a similar off-rates (k_d) as Datopotamab, but slower than that of Sacituzumab. Hence, the binding affinities of 2D8-gl and 7G7-gl are slightly lower than
15 Sacituzumab but are comparable to Datopotamab.

Table 1. Binding affinity (K_D), association rate (k_a), and dissociation rate (k_d) of anti-Trop2 human IgG₁ antibodies to human Trop2 protein measured by Octet BLI analysis.

Clone	K_D (nM)	k_a (1/Ms)	k_d (1/s)
2D8	1.78	2.79×10^5	5.28×10^{-4}
5A12	15.4	8.87×10^4	1.37×10^{-3}
6C10	22.3	9.49×10^4	2.11×10^{-3}
7G7	3.42	2.86×10^5	9.79×10^{-4}

20 **Table 2.** Binding affinity (K_D), association rate (k_a), and dissociation rate (k_d) of the germline configured anti-Trop2 human IgG₁ antibodies and benchmark reference antibodies to human Trop2 protein measured by Octet BLI analysis.

Clone	K_D (nM)	k_a (1/Ms)	k_d (1/s)
2D8-gl	2.444	5.548×10^5	1.356×10^{-3}
7G7-gl	4.610	3.641×10^5	1.679×10^{-3}
Sacituzumab	0.856	1.873×10^5	1.603×10^{-4}
Datopotamab	9.454	1.652×10^5	1.562×10^{-3}

APPLICATIONS

The presently disclosed antibodies and antigen fragments thereof may be useful for one or more of the following:

- 5 • As diagnostic antibodies for detecting solid tumors with high Trop2 expression;
- Anti-Trop2 BiTE antibodies as disclosed herein can be used in treating Trop2 expressing solid tumors;
- Anti-Trop2 ADC as disclosed herein can be used in treating Trop2 expressing solid tumors;
- 10 • Anti-Trop2 CAR T cells as disclosed herein can be used for treating Trop2 expressing solid tumors.

CLAIMS

1. An anti-Trop2 antibody or antigen-binding fragment thereof, comprising the complementarity determining region (CDR) sequences selected from the group comprising:
- 5 (i) a CDRH1 sequence of GGSISSGGYY (SEQ ID NO: 1),
a CDRH2 sequence of IYYSGST (SEQ ID NO: 5),
a CDRH3 sequence of AREEGIAAAFDI (SEQ ID NO: 9),
a CDRL1 sequence of QSVGSF (SEQ ID NO: 13),
a CDRL2 sequence of GAS (SEQ ID NO: 17), and
10 a CDRL3 sequence of QQSDSSPFT (SEQ ID NO: 19);
- (ii) a CDRH1 sequence of GYTFTSYG (SEQ ID NO: 2),
a CDRH2 sequence of ISAYNGNT (SEQ ID NO: 6),
a CDRH3 sequence of ARKYSGFDY (SEQ ID NO: 10)
a CDRL1 sequence of QSLLHSNGYNY (SEQ ID NO: 14)
15 a CDRL2 sequence of LGS (SEQ ID NO: 18); and
a CDRL3 sequence of MQNLQTPWT (SEQ ID NO: 20)
- (iii) a CDRH1 sequence of GFTFSSYS (SEQ ID NO: 3),
a CDRH2 sequence of ISSSSSYI (SEQ ID NO: 7),
a CDRH3 sequence of ARDYDSSGYPYYYYGMDV (SEQ ID NO: 11),
20 a CDRL1 sequence of QSVSSSY (SEQ ID NO: 15),
a CDRL2 sequence of GAS (SEQ ID NO: 17), and
a CDRL3 sequence of HQSGSSLRT (SEQ ID NO: 21); and
- (iv) a CDRH1 sequence of GFTFSSYW (SEQ ID NO: 4),
a CDRH2 sequence of IKQDGSEK (SEQ ID NO: 8),
25 a CDRH3 sequence of ARDFVDWSATPFDY (SEQ ID NO: 12),
a CDRL1 sequence of RSLHHSNGYNY (SEQ ID NO: 16),
a CDRL2 sequence of LGS (SEQ ID NO: 18), and
a CDRL3 sequence of MQALQIPKT (SEQ ID NO: 22).
- 30 2. The antibody or antigen-binding fragment thereof according to claim 1, wherein the antibody or antigen-binding fragment thereof is a germline configured counterpart thereof.

3. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, comprising a VH domain sequence selected from the group comprising:
- 5 QVQLQESGPGLVKPSQTLSTCAVSGGSISSGGYYWSWIRQPPGKGLEWIGY
IYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREEGIAAA
AFDIWGQGTM (SEQ ID NO: 23),
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWI
SAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARKYSGF
10 DYWGQGTL (SEQ ID NO: 24),
EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWI
SAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARKYSGF
DYWGQGTL (SEQ ID NO: 25), and
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSI
15 SSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDYYDSS
GYPIYYYGMDVWGQGT (SEQ ID NO: 26),
EVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVAN
IKQDGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDFVD
WSATPFDYWGQGTL (SEQ ID NO: 27),
20 or a variant thereof at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%
identical thereto.
4. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, comprising a VL domain sequence selected from the group comprising:
- 25 DIQMTQSPSSLSASVGDRVTITCRASQSVGSFLNWFYQQKPGKAPKLLIYGASS
LQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSDSPFTFGGGTK
(SEQ ID NO: 28),
EIVLTQSPSSLSASVGDRVTITCRASQSVGSFLNWFYQQKPGKAPQVLIFGASN
30 LESGVPSRFSGRSGSEFTLTINSLQPEDFATYYCQQSDSPFTFGGGTK
(SEQ ID NO: 29),

- DIVMTQSPLSLPVTGPGEPAISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLI
 YLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQNLQTPWTFG
 QGTK (SEQ ID NO: 30),
 EIVLTQSPLSLPVTGPGEPAISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIY
 5 LGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQNLQTPWTFGQG
 TK (SEQ ID NO: 31),
 ETTLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGA
 SSRATGIPDRFSGSGSGTDFTLISRLEPEDFAVYYCHQSGSSLRTFGQGT
 (SEQ ID NO: 32), and
 10 DVVMTQSPLSLPVTGPGEPAISCRSSRSLHNSNGYNYLDWYVQKPGQSPQLLI
 YLGSYRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQIPKTFGQ
 GTK (SEQ ID NO: 33),
 or a variant thereof at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%
 identical thereto.
- 15
5. The antibody or antigen-binding fragment thereof according to any one of the
 preceding claims, comprising a VH domain and a VL domain selected from the
 group comprising:
- (i) a VH domain sequence of:
 20 QVQLQESGPGLVKPSQTLSTCAVSGGSISSGGYYWSWIRQPPGKGL
 EWIGYIYYSGSTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYY
 CAREEGIAAAAFDIWGQGT (SEQ ID NO: 23) or a variant thereof at
 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto,
 and
 25 a VL domain sequence of:
 DIQMTQSPSSLSASVGDRVTITCRASQSVGSFLNWWYQQKPGKAPKLLI
 YGASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSDSSPF
 TFGGGTK (SEQ ID NO: 28) or a variant thereof at least 80%, 85%, 90%,
 95%, 96%, 97%, 98%, or 99% identical thereto;
- 30 (ii) a VH domain sequence of:
 QVQLQESGPGLVKPSQTLSTCAVSGGSISSGGYYWSWIRQPPGKGL
 EWIGYIYYSGSTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYY
 CAREEGIAAAAFDIWGQGT (SEQ ID NO: 23) or a variant thereof at

least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto,
and

a VL domain sequence of:

5 EIVLTQSPSSLSASVGDRVITICRASQSVGSFLNWWYQQKPGKAPQVLI
FGASNLESGVPSRFSGRGSGSEFTLTINSLQPEDFATYYCQQSDSSP
FTFGGGTK **(SEQ ID NO: 29)** or a variant thereof at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identical thereto;

(iii) a VH domain sequence of:

10 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLE
WMGWISAYNGNTNYAQLQGRVTMTTDTSTSTAYMELRSLRSDDTA
VYYCARKYSGFDYWGQGT **(SEQ ID NO: 24)** or a variant thereof at
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto,
and

a VL domain sequence of:

15 DIVMTQSPLSLPVTGPGEPAISCRSSQSLHNSGYNLYDWYLQKPGQS
PQLLIYLGSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQ
NLQTPWTFGQGTK **(SEQ ID NO: 30)** or a variant thereof at least 80%,
85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto;

(iv) a VH domain sequence of:

20 EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLE
WMGWISAYNGNTNYAQLQGRVTMTTDTSTSTAYMELRSLRSDDTA
VYYCARKYSGFDYWGQGT **(SEQ ID NO: 25)** or a variant thereof at
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto,
and

a VL domain sequence of:

25 EIVLTQSPLSLPVTGPGEPAISCRSSQSLHNSGYNLYDWYLQKPGQS
PQLLIYLGSNRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGIYYCMQN
LQTPWTFGQGTK **(SEQ ID NO: 31)** or a variant thereof at least 80%,
85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto;

(v) a VH domain sequence of:

30 EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLE
WVSSISSSSYIYYADSVKGRFTISRDAKNSLYLQMNSLRAEDTAVYY
CARDYYDSSGYPYYYYGMDVWGQGT **(SEQ ID NO: 26)** or a variant

- thereof at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and
a VL domain sequence of:
ETTLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRL
LIYGASSRATGIPDRFSGSGSGTDFNLTISRLEPEDFAVYYCHQSGSSL
RTFGQGTT (SEQ ID NO: 32) or a variant thereof at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identical thereto; and
(vi) a VH domain sequence of:
EVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLE
WVANIKQDGSEKYYVDSVKGRFTISRDNAKNSLYQMNSLRAEDTAV
YYCARDFVDWSATPFDYWGQGTL (SEQ ID NO: 27) or a variant thereof
at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto,
and
a VL domain sequence of:
DVVMTQSPLSLPVTPGEPASISCRSSRSLHSGNYLDWYVQKPGQ
SPQLLIYLGSYRASGVPDRFSGSGSGTDFTLKISRVEADVGVYYCMQ
ALQIPKTFGQGTK (SEQ ID NO: 33) or a variant thereof at least 80%,
85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.
6. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, wherein the antibody is a full-length antibody.
7. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, wherein the antibody is an IgG antibody, such as an IgG1 antibody.
8. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, wherein the antibody or antigen-binding fragment thereof is a fully human antibody or antigen-binding fragment thereof.
9. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, wherein the antibody or antigen-binding fragment thereof is specific to human Trop2.

10. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, wherein the antibody or antigen-binding fragment thereof exhibits cross-reactivity with more two or more different species.
- 5 11. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, wherein the antibody or antigen-binding fragment thereof binds to human Trop2 and also cynomolgus Trop2.
- 10 12. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, wherein the antibody or antigen-binding fragment thereof is conjugated to a therapeutic agent.
- 15 13. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, wherein the therapeutic agent is an anti-cancer agent, for example a chemotherapeutic agent.
- 20 14. The antibody or antigen-binding fragment thereof according to any one of the preceding claims, wherein the therapeutic agent is selected from the group comprising MMAE (vedotin), PNU-159682, DX-8951 (exatecan), PBD (pyrrolobenzodiazepine) and DMDM.
- 25 15. An antibody-drug conjugate (ADC) comprising the antibody or antigen binding fragment thereof according to any one of the preceding claims.
- 30 16. A multi-specific antibody comprising an anti-Trop2 binding domain, wherein the anti-Trop2 binding domain is an antibody or antigen-binding fragment thereof according to any one of the preceding claims.
17. The multi-specific antibody according to claim 16, wherein the multi-specific antibody is an immune cell engager.
18. The multi-specific antibody according to claim 17, wherein the immune cell engager binds to an immune marker selected from the group consisting of CD3, NKG2D, CD4, CD8, CD16 and CD64.

19. The multi-specific antibody according to claims 17 or 18, wherein the immune cell engager is selected from the group comprising a T cell engager, an NK cell engager, a monocyte engager and a macrophage engager.
- 5 20. The multi-specific antibody according to claim 19, wherein the immune engager is a bispecific T cell engager (BiTE), such as an inducible BiTE, a non-inducible BiTE or a constitutive expression BiTE.
- 10 21. An immune cell comprising an anti-Trop2 antibody or antigen-binding fragment thereof according to any one of claims 1 to 15 or multi-specific antibody according to any one of claims 16 to 20.
- 15 22. The immune cell according to claim 21, wherein the immune cell is selected from the group comprising a T-cell, a CAR T-cell, a natural killer (NK) cell, a monocyte and a macrophage, in particular a CAR T-cell.
- 20 23. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof, the antibody drug conjugate, the multi-specific antibody, or the immune cell according to any one of the preceding claims, wherein the composition optionally comprises a pharmaceutically acceptable excipient, buffer and/or additive.
- 25 24. A polynucleotide encoding an anti-Trop2 antibody or antigen-binding fragment thereof according to any one of claims 1 to 14, an antibody drug conjugate according to claim 15, or a multi-specific antibody according to any one of claims 16 to 20.
- 30 25. A polynucleotide according to claim 24, comprising a sequence selected from the group comprising:
- i. CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTC
ACAGACCCTGTCCCTCACCTGCGCTGTCTCTGGTGGCTCCATCAG
CAGTGGTGGTTACTACTGGAGTTGGATCCGCCAGCCCCCGGGAA
GGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTA

- CTACAACCCGTCCTCAAGAGTCGAGTTACCATATCAGTAGACACG
TCTAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACTGCCGCG
GACACGGCCGTGTATTACTGTGCGAGAGAAGAGGGTATAGCAGCA
GCTGCTTTTGATATCTGGGGCCAAGGGACAATG (**SEQ ID NO: 34**) or
5 a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,
or 99% identity thereto,
- ii. CAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGG
GCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCA
GCTACGGTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTG
10 AGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGC
ACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCAC
GAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC
GGCCGTGTATTACTGTGCGAGAAAATACAGTGGCTTTGACTACTGG
GGCCAGGGCACCCCTG (**SEQ ID NO: 35**) or a variant thereof having at
15 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
- iii. GAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGG
GCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCA
GCTACGGTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTG
20 AGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGC
ACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCAC
GAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC
GGCCGTGTATTACTGTGCGAGAAAATACAGTGGCTTTGACTACTGG
GGCCAGGGCACCCCTG (**SEQ ID NO: 36**) or a variant thereof having at
25 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
- iv. GAGGTCCAGCTGGTGACAGTCTGGGGGAGGCCTGGTCAAGCCTGG
GGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGT
AGCTATAGCATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTG
GAGTGGGTCTCATCCATTAGTAGTAGTAGTAGTTACATATACTACG
CAGACTCAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCA
30 AGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
CGGCTGTTTATTACTGTGCGAGAGATTACTATGATAGTAGTGGCTA
TCCCTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC

- G (SEQ ID NO: 37) or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
- v. GAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTCCAGCCTGG
GGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTTAGT
5 AGCTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAAGGGCTG
GAGTGGGTGGCCAACATAAAGCAAGATGGAAGTGAGAAATACTAT
GTGGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAACGCC
AAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
CGGCTGTGTATTACTGTGCGAGGGATTTTGTGGACTGGTCCGCCA
10 CACCCTTTGACTACTGGGGCCAGGGCACCCTG (SEQ ID NO: 38) or
a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,
or 99% identity thereto,
- vi. GACATTCAGATGACGCAGTCTCCATCCTCCCTGTCTGCTTCTGTGG
GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCGTTGGCT
15 CTTTTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCT
CCTGATCTATGGTGCCTCCAGTTTACAGAGTGGGGTCCCATCAAGG
TTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCA
GTCTGCAACCCGAAGATTTTGCAACTTACTACTGTCAACAGAGTGA
CAGTTCCCCCTTCACTTTCCGGCGAGGGACCAAG (SEQ ID NO: 39)
20 or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%,
98%, or 99% identity thereto,
- vii. GAAATTGTGTTGACGCAGTCTCCATCCTCCCTGTCTGCTTCTGTGG
GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCGTTGGCT
CTTTTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTCAGGT
25 CCTGATCTTTGGTGCCTCCAATTTAGAAAGTGGGGTCCCATCAAGG
TTCAGTGGCAGAGGATCTGGGTGAGAATTCATCTCACCATCAACA
GTCTGCAACCCGAAGATTTTGCAACTTACTACTGTCAACAGAGTGA
CAGTTCCCCCTTCACTTTCCGGCGAGGGACCAAG (SEQ ID NO: 40)
30 or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%,
98%, or 99% identity thereto,
- viii. GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGTCTCCTGC
ATAGTAATGGATACAACTATTTGGATTGGTACCTGCAGAAGCCAGG

- GCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCC
GGGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTT
ACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATT
ACTGCATGCAAAATCTTCAAACCTCCGTGGACGTTTCGGCCAAGGGAC
5 CAAG (**SEQ ID NO: 41**) or a variant thereof having at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
- ix. GAAATTGTGCTGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGTCTCCTGC
ATAGTAATGGATACAACCTATTTGGATTGGTACCTGCAGAAGCCAGG
10 GCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCC
GGGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTT
ACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGATTTATT
ACTGCATGCAAAATCTTCAAACCTCCGTGGACGTTTCGGCCAAGGGAC
CAAG (**SEQ ID NO: 42**) or a variant thereof having at least 80%, 85%,
15 90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
- x. GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG
GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCA
GCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCA
GGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAG
20 ACAGGTTCACTGGCAGTGGGTCTGGGACAGACTTCAATCTCACCAT
CAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCACCAG
TCTGGCAGTTCACTTCGGACGTTTCGGCCAAGGGACCACG (**SEQ ID
NO: 43**) or a variant thereof having at least 80%, 85%, 90%, 95%, 96%,
97%, 98%, or 99% identity thereto, and
- xi. GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCGGAGCCTCCTAC
ATAGTAATGGATACAACCTATTTGGATTGGTACGTGCAGAAGCCAGG
GCAGTCTCCACAGCTCCTGATCTATTTGGGTTTCATATCGGGCCTCC
GGGGTCCCTGACAGGTTTACTGGCAGTGGATCAGGCACAGATTTT
30 ACACTGAAGATCAGCAGGGTGGAGGCTGAGGATGTTGGAGTTTAT
TACTGCATGCAAGCTTTACAAATTCCGAAGACGTTTCGGCCAAGGGA
CCAAG (**SEQ ID NO: 44**) or a variant thereof having at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

26. A vector comprising one or more polynucleotides according to any one of claims 24 or 25.
- 5 27. The vector according to claim 26, comprising a polynucleotide selected from the group comprising:
- (i) a polynucleotide having the sequence:
- 10 CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTC
ACAGACCCTGTCCCTCACCTGCGCTGTCTCTGGTGGCTCCATCAG
CAGTGGTGGTTACTACTGGAGTTGGATCCGCCAGCCCCCGGGAA
GGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTA
CTACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACG
TCTAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACTGCCGCG
GACACGGCCGTGTATTACTGTGCGAGAGAAGAGGGTATAGCAGCA
15 GCTGCTTTTGATATCTGGGGCCAAGGGACAATG (SEQ ID NO: 34) or
a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,
or 99% identity thereto, and
a polynucleotide having the sequence:
- 20 GACATTCAGATGACGCAGTCTCCATCCTCCCTGTCTGCTTCTGTGG
GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCGTTGGCT
CTTTTTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCT
CCTGATCTATGGTGCCTCCAGTTTACAGAGTGGGGTCCCATCAAGG
TTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCA
GTCTGCAACCCGAAGATTTTGCACTTACTACTGTCAACAGAGTGA
25 CAGTTCCTCCCTTCACTTTTCGGCGGAGGGACCAAG (SEQ ID NO: 39)
or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%,
98%, or 99% identity thereto;
- (ii) a polynucleotide having the sequence:
- 30 CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTC
ACAGACCCTGTCCCTCACCTGCGCTGTCTCTGGTGGCTCCATCAG
CAGTGGTGGTTACTACTGGAGTTGGATCCGCCAGCCCCCGGGAA
GGGCCTGGAGTGGATTGGGTACATCTATTACAGTGGGAGCACCTA
CTACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACG

TCTAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACTGCCGCG
GACACGGCCGTGTATTACTGTGCGAGAGAAGAGGGTATAGCAGCA
GCTGCTTTTGATATCTGGGGCCAAGGGACAATG (SEQ ID NO: 34) or
a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,
or 99% identity thereto, and
a polynucleotide having the sequence:
GAAATTGTGTTGACGCAGTCTCCATCCTCCCTGTCTGCTTCTGTGG
GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCGTTGGCT
CTTTTTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCCTCAGGT
CCTGATCTTTGGTGCCTCCAATTTAGAAAGTGGGGTCCCATCAAGG
TTCAGTGGCAGAGGATCTGGGTCAGAATTCACTCTCACCATCAACA
GTCTGCAACCCGAAGATTTTGCAACTTACTACTGTCAACAGAGTGA
CAGTTCCCCCTTCACTTTCGGCGGAGGGACCAAG (SEQ ID NO: 40)
or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%,
98%, or 99% identity thereto;
(iii) a polynucleotide having the sequence:
CAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGG
GCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCA
GCTACGGTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTG
AGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGC
ACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCAC
GAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC
GGCCGTGTATTACTGTGCGAGAAAATACAGTGGCTTTGACTACTGG
GGCCAGGGCACCCCTG (SEQ ID NO: 35) or a variant thereof having at
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
and
a polynucleotide having the sequence:
GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTACCCCTG
GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGTCTCCTGC
ATAGTAATGGATACAACATTTGGATTGGTACCTGCAGAAGCCAGG
GCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCC
GGGGTCCCTGACAGGTTCACTGGCAGTGGATCAGGCACAGATTTT
ACACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATT

- ACTGCATGCAAAATCTTCAAACCTCCGTGGACGTTTCGGCCAAGGGAC
CAAG (SEQ ID NO: 41) or a variant thereof having at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identity thereto;
- (iv) a polynucleotide having the sequence:
- 5 GAGGTCCAGCTGGTACAGTCTGGAGCTGAGGTGAAGAAGCCTGGG
GCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTTACCA
GCTACGGTATCAGCTGGGTGCGACAGGCCCTGGACAAGGGCTTG
AGTGGATGGGATGGATCAGCGCTTACAATGGTAACACAACTATGC
ACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCAC
10 GAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACAC
GGCCGTGTATTACTGTGCGAGAAAATACAGTGGCTTTGACTACTGG
GGCCAGGGCACCCTG (SEQ ID NO: 36) or a variant thereof having at
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto,
and
- 15 a polynucleotide having the sequence:
- GAAATTGTGCTGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGTCTCCTGC
ATAGTAATGGATACAACATTTGGATTGGTACCTGCAGAAGCCAGG
GCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCC
20 GGGGTCCCTGACAGGTTCAAGTGGCAGTGGATCAGGCACAGATTTT
AACTGAAAATCAGCAGAGTGGAGGCTGAGGATGTTGGGATTTATT
ACTGCATGCAAAATCTTCAAACCTCCGTGGACGTTTCGGCCAAGGGAC
CAAG (SEQ ID NO: 42) or a variant thereof having at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identity thereto;
- 25 (v) a polynucleotide having the sequence:
- GAGGTCCAGCTGGTGCAGTCTGGGGGAGGCCTGGTCAAGCCTGG
GGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGT
AGCTATAGCATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTG
GAGTGGGTCTCATCCATTAGTAGTAGTAGTTACATATACTACG
30 CAGACTCAGTGAAGGGCCGATTACCATCTCCAGAGACAACGCCA
AGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
CGGCTGTTTATTACTGTGCGAGAGATTACTATGATAGTAGTGGCTA
TCCCTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCAC

G (**SEQ ID NO: 37**) or a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, and

a polynucleotide having the sequence:

5 GAAACGACACTCACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAG
GGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCA
GCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCA
GGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAG
ACAGGTTCACTGGCAGTGGGTCTGGGACAGACTTCAATCTCACCAT
CAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCACCAG
10 TCTGGCAGTTCACCTTCGGACGTTCCGCCAAGGGACCACG (**SEQ ID
NO: 43**) or a variant thereof having at least 80%, 85%, 90%, 95%, 96%,
97%, 98%, or 99% identity thereto; and

(vi) a polynucleotide having the sequence:

15 GAGGTCCAGCTGGTACAGTCTGGGGGAGGCTTGGTCCAGCCTGG
GGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTTAGT
AGCTATTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAAGGGCTG
GAGTGGGTGGCCAACATAAAGCAAGATGGAAGTGAGAAATACTAT
GTGGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAACGCC
AAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACA
20 CGGCTGTGTATTACTGTGCGAGGGATTTTGTGGACTGGTCCGCCA
CACCTTTGACTACTGGGGCCAGGGCACCCCTG (**SEQ ID NO: 38**) or
a variant thereof having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%,
or 99% identity thereto, and

a polynucleotide having the sequence:

25 GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTG
GAGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCGGAGCCTCCTAC
ATAGTAATGGATACAACATTTTGGATTGGTACGTGCAGAAGCCAGG
GCAGTCTCCACAGCTCCTGATCTATTTGGGTTTCATATCGGGCCTCC
GGGGTCCCTGACAGGTTTGTAGTGGCAGTGGATCAGGCACAGATTTT
30 ACACTGAAGATCAGCAGGGTGGAGGCTGAGGATGTTGGAGTTTATT
ACTGCATGCAAGCTTTACAAATCCGAAGACGTTCCGCCAAGGGAC
CAAG (**SEQ ID NO: 44**) or a variant thereof having at least 80%, 85%,
90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

28. A host cell comprising a vector according to any one of claims 26 or 27.
- 5 29. A method of treating a patient in need thereof, comprising administering the antibody or antigen-binding fragment thereof, the antibody drug conjugate, the multi-specific antibody, the immune cell or the pharmaceutical composition according to any one of claims 1 to 23.
- 10 30. An antibody or antigen-binding fragment thereof, an antibody drug conjugate, a multi-specific antibody, an immune cell or a pharmaceutical composition according to any one of claims 1 to 23 for use in the treatment of a patient.
- 15 31. Use of an antibody or antigen-binding fragment thereof, an antibody drug conjugate, a multi-specific antibody, an immune cell or a pharmaceutical composition according to any one of claims 1 to 23 in the manufacture of a medicament for the treatment of a patient, for example for the treatment of cancer.
- 20 32. The method, antibody or antigen-binding fragment thereof, antibody drug conjugate, multi-specific antibody, immune cell or a pharmaceutical composition for use or use according to any one of the claims 29 to 31, wherein the patient has cancer.
- 25 33. The method, antibody or antigen-binding fragment thereof, antibody drug conjugate, multi-specific antibody, immune cell or pharmaceutical composition for use, or use according to claims 31 or 32, wherein the cancer is Trop2+.
- 30 34. A method of diagnosing a tumor expressing Trop2 comprising contacting the tumor with an antibody or antigen-binding fragment thereof according to any one of claims 1 to 14, a multi-specific antibody according to any one of claims 16 to 20 or a composition according to claim 23.

35. The method, antibody or antigen-binding fragment thereof, an antibody drug conjugate, multi-specific antibody, immune cell or pharmaceutical composition for use, or use according to any one of claims 31 to 34, wherein the cancer is selected from the group comprising gastric adenocarcinoma, colon cancer, pancreatic cancer and breast cancer.
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