

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2017233121 B2**

(54) Title
Multispecific Fab fusion proteins and use thereof

(51) International Patent Classification(s)
C07K 16/30 (2006.01) **A61P 35/00** (2006.01)
A61K 39/395 (2006.01) **C07K 16/46** (2006.01)

(21) Application No: **2017233121** (22) Date of Filing: **2017.03.15**

(87) WIPO No: **WO17/157305**

(30) Priority Data

(31)	Number	(32)	Date	(33)	Country
	201610147227.8		2016.03.15		CN

(43) Publication Date: **2017.09.21**

(44) Accepted Journal Date: **2023.12.21**

(71) Applicant(s)
ITabMed (HK) Limited

(72) Inventor(s)
Cui, Yumin;Huang, Zhihua;Chen, Hanyang;Zhang, Xinfeng;Qi, Bo;Yan, Xiaoqiang

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
WO 2012/158818 A2



(51) International Patent Classification:

C07K 16/30 (2006.01) *A61K 39/395* (2006.01)
C07K 16/46 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:

PCT/CN2017/076816

(22) International Filing Date:

15 March 2017 (15.03.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

201610147227.8 15 March 2016 (15.03.2016) CN

(71) Applicant: **GENERON (SHANGHAI) CORPORATION LTD.** [CN/CN]; Suite 307, 1011 Ha Lei Road, China (Shanghai) Pilot Free Trade Zone, Shanghai 201203 (CN).

(72) Inventors: **CUI, Yumin**; Suite 307, 1011 Ha Lei Road, China (Shanghai) Pilot Free Trade Zone, Shanghai 201203 (CN). **HUANG, Zhihua**; Suite 307, 1011 Ha Lei Road, China (Shanghai) Pilot Free Trade Zone, Shanghai 201203 (CN). **CHEN, Hanyang**; Suite 307, 1011 Ha Lei Road, China (Shanghai) Pilot Free Trade Zone, Shanghai 201203 (CN). **ZHANG, Xinfeng**; Suite 307, 1011 Ha Lei Road, China (Shanghai) Pilot Free Trade Zone, Shanghai 201203 (CN). **QI, Bo**; Suite 307, 1011 Ha Lei Road, China (Shanghai) Pilot Free Trade Zone, Shanghai 201203 (CN). **LIU, Heng**; Suite 307, 1011 Ha Lei Road, China (Shanghai) Pilot Free Trade Zone, Shanghai 201203 (CN). **YAN,**

Xiaoqiang; Suite 307, 1011 Ha Lei Road, China (Shanghai) Pilot Free Trade Zone, Shanghai 201203 (CN).

(74) Agent: **WU, FENG & ZHANG**; Sinosteel Plaza, 21st Floor A/D, 8 Haidian st., Haidian, Beijing 100080 (CN).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: MULTISPECIFIC FAB FUSION PROTEINS AND USE THEREOF

(57) Abstract: The present invention provides multispecific Fab fusion proteins (MSFP) that specifically bind to CD3 and EpCAM. The present invention further provides uses of the MSFPs for the preparation of pharmaceutical compositions, methods of treating cancer, and kits comprising the MSFPs. Also provided are anti-EpCAM antibodies or antigen-binding fragments thereof.



MULTISPECIFIC FAB FUSION PROTEINS AND USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[1] This application claims priority benefit of Chinese Patent Application No. 201610147227.8 filed March 15, 2016, the contents of which are incorporated herein by reference in their entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[2] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 720622000841SEQLIST.txt, date recorded: March 13, 2017, size: 48 KB).

FIELD OF THE INVENTION

[3] The present invention relates to multi-specific Fab fusion proteins (MSFP) that specifically bind to CD3 and EpCAM. Further provided herein are pharmaceutical compositions comprising the MSFPs, methods of treating cancer using the MSFPs, and kits comprising the MSFPs.

BACKGROUND OF THE INVENTION

[4] Some antigens are over-expressed, mutagenized, or selectively mutagenized in tumor tissues. Therefore, antibodies targeting specific antigens on the surface of cancer cells can be used as cancer therapeutics. Epithelial cell adhesion molecule (EpCAM, CD326), also known as 17-1A, ESA, AUA1, EGP40, *etc.*, is a 40 kD transmembrane glycoprotein composed of 314 amino acid. EpCAM is specifically expressed in various types of epithelial cells, and major types of human malignancies. For example, EpCAM is highly expressed in colon cancer, lung cancer, prostate cancer, liver cancer, pancreatic cancer, breast cancer and ovarian cancer. Thus, EpCAM has become a hot target in cancer therapy, including vaccines, murine or human monoclonal antibodies, and antibodies conjugated to bacterial toxins or chemotherapy drugs, such as EpCAM specific antibodies ING-1, adecatumumab, edrecolomab, *etc.*

[5] CD3, comprising three different polypeptide chains (ϵ , δ and γ chains), is an antigen expressed by T cells. The three CD3 polypeptide chains associate with the T-cell receptor (TCR) and the ζ -chain to form the TCR complex, which has the function of activating signaling cascades in T cells. Currently, many therapeutic strategies target the TCR signal transduction to treat diseases using anti-human CD3 monoclonal antibodies. The CD3 specific antibody OKT3 is the first monoclonal antibody approved for human therapeutic use, and is clinically used as an immunomodulator for the treatment of allogenic transplant rejections.

[6] During the past twenty years, efforts in the field of bispecific antibodies have gradually yielded clinical success. In 2009, Catumaxomab (anti-CD3, anti-EpCAM trifunctional antibody) was approved in the European Union for the treatment of symptomatic malignant ascites. However, although bispecific antibodies have been shown to have potential in effectively killing cancer cells, severe adverse effects, including systemic immune activation, immunogenicity (anti-drug antibody effect), and the generally poor manufacturability of these molecules, have greatly limited the widespread application of this type of drugs. Another drawback of CD19 \times CD3 bispecific scFv-scFv (single-chain variable fragment) fusion protein (Blinatumomab) is that this drug needs to be administered intravenously (i.v.) on a daily basis due to its short half-life and incompatibility with subcutaneous administration; yet, neurological effects such as disorientation, confusion, speech and language impairment, tremor or convulsion still occurred during clinical trials (*Science* 321:974-977, 2008). To better control these unwanted adverse effects, bispecific single-chain antibodies (BiTE) were administered intravenously with continuous infusion over a longer period of time. US20120244161 disclosed the stage I clinical trial of EpCAM \times CD3 bispecific scFv-scFv fusion protein (MT110), in which low dosage (1-12 μ g/kg/24hr) continuous intravenous infusion was applied over a long period of time, with glucocorticoid administered prior to MT110 infusion or dosage increment. Furthermore, scFv-scFv fusion protein has a tendency for aggregation.

[7] The drawbacks of current formats of bispecific antibodies remain great challenges for their widespread application in the treatment of cancer patients with good efficacy and safety. Therefore, there is an urgent need in the field for the development of new bispecific antibodies or treatment regimen with improved efficacy, stability, safety and manufacturability.

[7a] Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of the common general knowledge in the field.

[7b] Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

BRIEF SUMMARY OF THE INVENTION

[7c] In a first aspect, the present invention provides an anti-EpCAM antibody or antigen-binding fragment thereof, comprising a heavy chain variable region (VH) and a light chain variable region (VL),

wherein the VH comprises: (1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13; (2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; and (3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15;

wherein the VL comprises: (1) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16; (2) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 17; and (3) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 18.

[7d] In a second aspect, the present invention provides an antigen-binding fragment of the anti-EpCAM antibody of the first aspect, wherein the antigen-binding fragment is a single-chain Fv (scFv).

[7e] In a third aspect, the present invention provides a multispecific Fab fusion protein comprising the anti-EpCAM antigen-binding fragment of the first aspect.

[7f] In a fourth aspect, the present invention provides a composition comprising the anti-EpCAM antibody or antigen-binding fragment thereof of the first aspect or the multispecific Fab fusion protein of the second aspect, and optionally a pharmaceutically acceptable carrier.

[7g] In a fifth aspect, the present invention provides a method of treating an EpCAM-positive cancer in an individual, comprising administering to the individual an effective amount of the anti-EpCAM antibody or antigen-binding fragment thereof of the first aspect, the multispecific Fab fusion protein of the second aspect, or the composition of the third aspect, optionally wherein the EpCAM-positive cancer is a carcinoma or an adenocarcinoma.

[7h] In a sixth aspect, the present invention provides use of an effective amount of the anti-EpCAM antibody or antigen-binding fragment thereof of the first aspect, the multispecific Fab fusion protein of the second aspect, or the composition of the third aspect in the preparation of a medicament for treating an EpCAM-positive cancer in an individual, optionally wherein the EpCAM-positive cancer is a carcinoma or an adenocarcinoma.

[7i] In a seventh aspect, the present invention provides a method of treating an EpCAM-positive cancer in an individual, comprising administering to the individual an effective amount of a multispecific Fab fusion protein comprising: (i) an anti-CD3 Fab fragment, and (ii) a binding domain that specifically binds to EpCAM (“anti-EpCAM binding domain”), wherein the anti-EpCAM binding domain comprises the anti-EpCAM antigen-binding fragment of the first or second aspects; wherein the anti-EpCAM binding domain is fused to an N-terminus of the anti-CD3 Fab fragment; optionally wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg; optionally wherein the EpCAM-positive cancer is a carcinoma or an adenocarcinoma.

[7j] In an eighth aspect, the present invention provides use of an effective amount of a multispecific Fab fusion protein in the preparation of a medicament for treating an EpCAM-positive cancer in an individual, wherein the multispecific Fab fusion protein comprises (i) an anti-CD3 Fab fragment and (ii) an anti-EpCAM binding domain, wherein the anti-EpCAM binding domain comprises the anti-EpCAM antigen-binding fragment of the first or second aspects; wherein the anti-EpCAM binding domain is fused to an N-terminus of the anti-CD3 Fab fragment; optionally wherein the medicament is administered at a dose of about 0.01 µg/kg to about 250 µg/kg; optionally wherein the EpCAM-positive cancer is a carcinoma or an adenocarcinoma.

[7k] In a ninth aspect, the present invention provides an isolated nucleic acid encoding the anti-EpCAM antibody or antigen-binding fragment thereof of the first aspect or the multispecific Fab fusion protein of the second aspect.

[7l] In a tenth aspect, the present invention provides a vector comprising the isolated nucleic acid of the ninth aspect.

[7m] In an eleventh aspect, the present invention provides an isolated host cell comprising the nucleic acid of the ninth aspect, or the vector of the tenth aspect.

[7n] In a twelfth aspect, the present invention/disclosure provides a method of producing an anti-EpCAM antibody or antigen-binding fragment thereof or a multispecific Fab fusion protein, comprising

(i) culturing a host cell comprising the nucleic acid of the ninth aspect, a vector of the tenth aspect, or the isolated host cell of the eleventh aspect, under a condition suitable for the expression of the anti-EpCAM antibody or antigen-binding fragment thereof or the multispecific Fab fusion protein; and

(ii) recovering the expressed anti-EpCAM antibody or antigen-binding fragment thereof or the multispecific Fab fusion protein from the cell culture.

[8] The present invention provides multi-specific Fab fusion proteins (such as bispecific Fab fusion proteins, or BSFP) that specifically bind to CD3 and EpCAM, and methods of treating cancer using the MSFPs.

[9] In one aspect of the present invention, there is provided a method of treating a cancer in an individual (such as a human individual), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the binding domain is an scFv. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence.

[10] In some embodiments according to any of the methods described above, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency (such as any of less than about once per 1, 2, 3, 4, 6, 9, or 12 months, such as a single dose). In some embodiments, the multispecific Fab fusion protein is administered at a dose equivalent to about 0.1 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$ (such as about 0.3 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, or about 5 $\mu\text{g/kg}$ to about 20 $\mu\text{g/kg}$) for a cynomolgus monkey. In some embodiments, the multispecific Fab fusion protein is administered

at a dose that does not induce cytokine storm. In some embodiments, the multispecific Fab fusion protein is administered at a dose equivalent to no higher than about 30 $\mu\text{g/kg}$ (such as no higher than about 20 $\mu\text{g/kg}$, 10 $\mu\text{g/kg}$, or 1 $\mu\text{g/kg}$) for a cynomolgus monkey. In some embodiments, the individual is a human individual.

[11] In some embodiments according to any of the methods described above, the multispecific Fab fusion protein is administered at a first dose for a first period of time to the

individual, and subsequently, the multispecific Fab fusion protein is administered at a second dose for a second period of time to the individual, and wherein the second dose exceeds the first dose. In some embodiments, the second period of time exceeds the first period of time. In some embodiments, the first period of time is at least about 7 days. In some embodiments, the second period of time is at least about 2 weeks. In some embodiments, the first dose is no more than about 1 µg/kg. In some embodiments, the second dose is about 0.1 µg/kg to about 10 µg/kg.

[12] In some embodiments according to any of the methods described above, the method further comprises administering a glucocorticoid to the individual. In some embodiments, the glucocorticoid is dexamethasone. In some embodiments, the glucocorticoid is administered prior to the first dose of the multispecific Fab fusion protein. In some embodiments, the glucocorticoid is administered at a dose of about 0.1 mg/kg to about 5 mg/kg.

[13] In some embodiments according to any of the methods described above, the Fab fragment specifically binds to the N-terminus of CD3 epsilon, such as an epitope within amino acids 1-27 of CD3 epsilon. In some embodiments, the VH of the Fab fragment comprises: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3. In some embodiments, the VL of the Fab fragment comprises a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the VH of the Fab fragment comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to an amino acid sequence selected from SEQ ID NOs: 7 and 39-43.. In some embodiments, the VL of the Fab fragment comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to an amino acid sequence selected from SEQ ID NOs: 8 and 44-47. In some embodiments, the Fab fragment comprises a human immunoglobulin heavy chain constant region 1 (CH1) comprising the amino acid sequence of SEQ ID NO:9. In some embodiments, the Fab fragment comprises a human lambda light chain constant region comprising the amino acid sequence of SEQ ID NO:10. In some embodiments, the CH1 and the CL of the Fab fragment are connected by one or more disulfide bonds. In some embodiments, the Fab fragment comprises a first polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO: 11. In some embodiments, the Fab fragment

comprises a second polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO: 12.

[14] In some embodiments according to any of the methods described above, the cancer is an EpCAM-positive solid cancer. In some embodiments, the EpCAM-positive solid cancer is a carcinoma or adenocarcinoma. In some embodiments, the EpCAM-positive solid cancer is selected from the group consisting of small intestine cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, endometrial cancer, breast cancer, bile duct cancer, and head and neck cancer. In one embodiment, the cancer is colorectal adenocarcinoma. In one embodiment, the cancer is lung adenocarcinoma.

[15] In some embodiments according to any of the methods described above, the binding domain (such as scFv) comprises an N-VH-VL-C fusion polypeptide. In some embodiments, the VH of the scFv comprises a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15. In some embodiments, the VL of the scFv comprises a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the VH of the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:19. In some embodiments, the VL of the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:20. In some embodiments, the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:21.

[16] In some embodiments according to any of the methods described above, the multispecific Fab fusion protein comprises a first polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:22. In some embodiments, the multispecific Fab fusion protein comprises a second polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:23.

[17] The present invention further provides an anti-EpCAM antibody. In some embodiments, there is provided an anti-EpCAM antibody or antigen-binding fragment thereof, comprising a heavy chain variable region comprising: (1) a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; (2) a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and (3) a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and a light chain variable region comprising: (1) a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; (2) a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and (3) a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the heavy chain variable domain sequence comprises a VH comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:19. In some embodiments, the light chain variable domain sequence comprises a VL comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:20.

[18] In some embodiments according to any one of the anti-EpCAM antibodies described above, the anti-EpCAM antibody comprises an Fc sequence of a human IgG. In some embodiments, the anti-EpCAM antibody is a multispecific antibody, such as a bispecific antibody.

[19] In some embodiments according to any one of the anti-EpCAM antigen-binding fragments described above, the antigen-binding fragment is a single-chain Fv (scFv). In some embodiments, the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO: 21.

[20] In some embodiments, there is provided a multispecific (such as bispecific) Fab fusion protein comprising any one of the anti-EpCAM antigen-binding fragments described above. In some embodiments, the multispecific Fab fusion protein comprises a Fab fragment that specifically binds to CD3, a first copy of the anti-EpCAM antigen-binding fragment, and a second copy of the anti-EpCAM antigen binding fragment; wherein the first copy of the anti-EpCAM antigen-binding fragment is fused to the N-terminus of the VH of the Fab fragment; and wherein the second copy of the anti-EpCAM antigen binding fragment is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the Fab fragment specifically binds to the N-terminus of CD3 epsilon, such as an epitope within amino acids 1-27 of CD3 epsilon. In some

embodiments, the VH of the Fab fragment comprises a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3. In some embodiments, the VL of the Fab fragment comprises: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the VH of the Fab fragment comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs:7 and 39-43. In some embodiments, the VL of the Fab fragment comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 44-47. In some embodiments, the Fab fragment comprises a human immunoglobulin heavy chain constant region 1 (CH1) comprising the amino acid sequence of SEQ ID NO:9. In some embodiments, the Fab fragment comprises a human lambda light chain constant region comprising the amino acid sequence of SEQ ID NO:10. In some embodiments, the CH1 and the CL of the Fab fragment are connected by one or more disulfide bonds. In some embodiments, the Fab fragment comprises a first polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:11. In some embodiments, the Fab fragment comprises a second polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:12. In some embodiments, the multispecific (such as bispecific) Fab fusion protein comprising a first polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:22, and a second polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:23.

[21] In some embodiments, there is provided a composition comprising any of the anti-EpCAM antibodies or antigen-binding fragments thereof or the multispecific Fab fusion proteins described above and a pharmaceutically acceptable carrier. In some embodiments, there is provided a method of treating cancer in an individual, comprising administering an effective amount of the composition to the individual.

[22] Further provided are isolated nucleic acid molecules that encode the MSFPs, anti-EpCAM antibodies or antigen-binding fragments thereof described above, expression vectors encoding the isolated nucleic acid molecules, isolated host cells comprising the expression vectors, and methods of producing the MSFPs, anti-EpCAM antibodies or antigen-binding fragments thereof, comprising culturing the isolated host cells and recovering the MSFPs, anti-EpCAM antibodies or antigen-binding fragments thereof from the cell culture.

[23] Also provided herein are uses, compositions (such as pharmaceutical compositions), kits and articles of manufactures comprising any of the multispecific Fab fusion proteins or the anti-EpCAM antibodies or antigen-binding fragments thereof described above.

[24] Use of any of the multispecific Fab fusion proteins described above or any of the anti-EpCAM antibodies or antigen-binding fragments thereof described above in the preparation of a medicament for treating a cancer is further provided herein.

[25] These and other aspects and advantages of the present invention will become apparent from the subsequent detailed description and the appended claims. It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention.

[26] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

[27] FIG. 1 depicts the structure of an exemplary EpCAM×CD3 Fab fusion protein.

[28] FIG. 2A depicts an SDS-PAGE gel of a purified exemplary EpCAM×CD3 Fab fusion protein (referred herein after as ITAB1002) under non-reducing conditions. The non-reducing SDS-PAGE shows a molecular weight of about 100 kD for the purified protein, similar to the theoretical molecular weight of the EpCAM×CD3 Fab fusion protein.

[29] FIG. 2B depicts an SDS-PAGE gel of purified ITAB1002 under reducing conditions. The reducing SDS-PAGE shows that the apparent molecular weight of the purified protein is between about 45 kD and about 66 kD.

[30] FIG. 3A depicts CE-SDS analysis results of purified ITAB1002 under non-reducing conditions. The non-reducing CE-SDS analysis shows a single protein peak at a migration time of about 21.59 min.

[31] FIG. 3B depicts CE-SDS analysis results of purified ITAB1002 under reducing conditions. The reducing CE-SDS analysis shows two single protein peaks at migration times of about 18.37 min and about 18.84 min, corresponding to the light chain and the heavy chain of the Fab fusion protein, respectively.

[32] FIG. 4A depicts the binding affinity of ITAB1002 to human PBMC expressing cell surface antigen CD3.

[33] FIG. 4B depicts the binding affinity of ITAB1002 to cynomolgus monkey PBMC expressing cell surface antigen CD3.

[34] FIG. 5 depicts the binding affinity of ITAB1002 to SW480 cells and CyEpCAM-CHO cells expressing cell surface antigen EpCAM.

[35] FIG. 6A shows the ability of ITAB1002 and OKT3 in activating CD4⁺ human PBMC under various conditions.

[36] FIG. 6B shows the ability of ITAB1002 and OKT3 in activating CD8⁺ human PBMC under various conditions.

[37] FIG. 6C shows the ability of ITAB1002 in stimulating proliferation of CD4⁺ human PBMC under various conditions.

[38] FIG. 6D shows the ability of ITAB1002 in stimulating proliferation of CD8⁺ human PBMC under various conditions.

[39] FIG. 7 depicts ITAB1002-mediated human or cynomolgus monkey PBMC cytotoxicity against SW480 tumor cells.

[40] FIG. 8 depicts ITAB1002-mediated human PBMC cytotoxicity against various representative cancer cell lines.

[41] FIG. 9A depicts growth inhibitory effects of various dosages of ITAB1002 against subcutaneous SW480 xenograft in mice that were co-inoculated with human PBMC.

[42] FIG. 9B shows pictures of tumors from mice in various treatment groups at the end of the experiment.

[43] FIG. 10A depicts growth inhibitory effects of various dosages of ITAB1002 against SW480 xenograft in immunodeficient mice having their immune system reconstructed with human PBMC.

[44] FIG. 10B shows pictures of tumors from mice in various treatment groups at the end of the experiment.

[45] FIG. 10C show percentages of human CD3⁺ cells in white blood cell samples of mice at the end of the experiment.

[46] FIG. 11 depicts growth inhibitory effects of ITAB1002 against NCI-H1975 xenograft in immunodeficient mice having their immune system reconstructed with human PBMC.

[47] FIG. 12A depicts change of the CD4⁺ T cell number in the blood of cynomolgus monkeys over time after intravenous administration of ITAB1002 at various dosages. X-axis: H=hours; D=days.

[48] FIG. 12B depicts change of the CD8⁺ T cell number in the blood of cynomolgus monkeys over time after intravenous administration of ITAB1002 at various dosages. X-axis: H=hours; D=days.

[49] FIG. 13A depicts change of the concentration of IL-2 in the serum of cynomolgus monkeys over time after intravenous administration of ITAB1002 at various dosages.

[50] FIG. 13B depicts change of the concentration of IL-4 in the serum of cynomolgus monkeys over time after intravenous administration of ITAB1002 at various dosages.

[51] FIG. 13C depicts change of the concentration of IL-5 in the serum of cynomolgus monkeys over time after intravenous administration of ITAB1002 at various dosages.

[52] FIG. 13D depicts change of the concentration of IL-6 in the serum of cynomolgus monkeys over time after intravenous administration of ITAB1002 at various dosages.

[53] FIG. 13E depicts change of the concentration of TNF in the serum of cynomolgus monkeys over time after intravenous administration of ITAB1002 at various dosages.

[54] FIG. 13F depicts change of the concentration of IFN- γ in the serum of cynomolgus monkeys over time after intravenous administration of ITAB1002 at various dosages.

[55] FIG. 14 depicts change of the concentration of ITAB1002 in the serum of cynomolgus monkeys after a single intravenous administration of ITAB1002 at various dosages.

[56] FIG. 15 compares ITAB1002 vs. ITAB1012-mediated human PBMC cytotoxicity against SW480 tumor cells.

[57] FIG. 16 depicts MSFP-mediated SW480 cell killing activity in the presence or absence of dexamethasone (DXM).

[58] FIG. 17 depicts MSFP-mediated IL-6 release by human T cells in the presence or absence of dexamethasone (DXM).

[59] FIG. 18A shows serum levels of alanine aminotransferase (ALT) in monkeys treated with ITAB1002 only.

[60] FIG. 18B shows serum levels of alanine aminotransferase (ALT) in monkeys treated with ITAB1002 with dexamethasone (DXM) pre-treatment.

[61] FIG. 19A shows serum levels of total bilirubin(TBil) in monkeys treated with ITAB1002 only.

[62] FIG. 19B shows serum levels of total bilirubin(TBil) in monkeys treated with ITAB1002 with dexamethasone (DXM) pre-treatment.

[63] FIG. 20A shows serum levels of alkaline phosphatase (ALP) in monkeys treated with ITAB1002 only.

[64] FIG. 20B shows serum levels of alkaline phosphatase (ALP) in monkeys treated with ITAB1002 with dexamethasone (DXM) pre-treatment.

[65] FIG. 21A shows IL-6 levels in monkeys treated with ITAB1002 only.

[66] FIG. 21B shows IL-6 levels in monkeys treated with ITAB1002 with dexamethasone (DXM) pre-treatment.

DETAILED DESCRIPTION OF THE INVENTION

[67] The present invention provides methods of treating cancer using a multispecific Fab fusion protein (MSFP) comprising a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM. In some embodiments, the MSFP comprises an anti-CD3 Fab fragment having the N-termini of its heavy chain and light chain polypeptides each fused to an anti-EpCAM scFv. Unlike current anti-cancer bispecific antibodies in the art, which suffer from poor manufacturability, aggregation, short half-life, severe adverse effects, and long infusion time, the multispecific Fab fusion proteins described herein have improved stability and safety profiles, which enabled methods of treatment using lower dosage and decreased dosing frequency, avoiding undesirable adverse effects, such as induction of cytokine storm. Decreased dosing frequency and shortened infusion time facilitate the treatment of patients, conducive to improving the quality of life of patients. For example, it was surprisingly found that the MSFPs described herein can be administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$, such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$. Further provided are novel anti-EpCAM antibodies or antigen-binding fragments thereof.

[68] The MSFPs described herein have the following advantages compared to other multispecific Fab fusion proteins known in the art. The MSFPs have higher stability, and enhanced efficacy in killing cancer cells. The extended half-life of the present MSFPs enable lower dosing frequency and shorter infusion time, providing more convenience to the patients. The cross-reactivity of the present MSFPs with primates, such as cynomolgus monkeys, facilitates toxicology studies. The present MSFPs have fewer adverse effects, including diminished neural effects, good safety and tolerance in cynomolgus monkeys. .

[69] Accordingly, in one aspect, the present invention provides a method of treating a cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM, wherein the binding domain is fused to an N-terminus of the Fab fragment, and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as

about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg).

[70] In another aspect, the present invention provides an anti-EpCAM antibody or antigen-binding fragment thereof, comprising a heavy chain variable region comprising (1) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13; (2) a HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; and (3) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15; and a light chain variable region comprising (1) a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; (2) a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and (3) a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18.

[71] Also provided are kits and articles manufacture useful for the methods described herein.

I. Definitions

[72] The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, Current Protocols in Molecular Biology or Current Protocols in Immunology, John Wiley & Sons, New York, N.Y.(2009); Ausubel et al, Short Protocols in Molecular Biology, 3rd ed., Wiley & Sons, 1995; Sambrook and Russell, Molecular Cloning: A Laboratory Manual (3rd Edition, 2001); Maniatis *et al.* Molecular Cloning: A Laboratory Manual (1982); DNA Cloning: A Practical Approach, vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., 1984); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., 1985); Transcription and Translation (B. Hames & S. Higgins, eds., 1984); Animal Cell Culture (R. Freshney, ed., 1986); Perbal, A Practical Guide to Molecular Cloning (1984) and other like references.

[73] As used herein, the term “treatment” refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully “treated” if one or more symptoms associated with cancer are mitigated or eliminated, including, but are not limited to, reducing the proliferation of (or destroying) cancerous cells, decreasing symptoms resulting from the disease, increasing the quality of life of

those suffering from the disease, decreasing the dose of other medications required to treat the disease, and/or prolonging survival of individuals.

[74] As used herein, an “effective amount” refers to an amount of an agent or drug effective to treat a disease or disorder in a subject. In the case of cancer, the effective amount of the agent may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective amount” may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[75] As used herein, an “individual” or a “subject” refers to a mammal, including, but not limited to, human, bovine, horse, feline, canine, rodent, or primate. In some embodiments, the individual is a human.

[76] The term “antibody” is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity or function. As used herein, the terms “immunoglobulin” (Ig) and “antibody” are used interchangeably.

[77] The terms “native antibody”, “full length antibody,” “intact antibody” and “whole antibody” are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain an Fc region. Native antibodies are usually heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains.

Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[78] The term “constant domain” refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The constant domain contains the C_{H1} , C_{H2} and C_{H3} domains (collectively, CH) of the heavy chain and the CHL (or CL) domain of the light chain.

[79] The “variable region” or “variable domain” of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as “VH.” The variable domain of the light chain may be referred to as “VL.” These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

[80] The term “variable” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions (HVRs, also referred to as CDRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[81] The “light chains” of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa (“κ”) and lambda (“λ”), based on the amino acid sequences of their constant domains.

[82] The term IgG “isotype” or “subclass” as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions.

[83] Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: 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 α, γ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas *et al.* Cellular and Mol. Immunology, 4th ed. (W.B. Saunders, Co., 2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

[84] “Antibody fragments” comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. In some embodiments, the antibody fragment described herein is an antigen-binding fragment. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[85] Papain digestion of antibodies produces two identical antigen-binding fragments, called “Fab” fragments, each with a single antigen-binding site, and a residual “Fc” fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[86] “Fv” is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a “dimeric” structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain

interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[87] The Fab fragment has two polypeptide chains, containing the heavy- and light-chain variable domains, and also containing the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[88] “Single-chain Fv” or “scFv” antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, *e.g.*, Pluckthün, *The Pharmacology of Monoclonal Antibodies*. Springer Berlin Heidelberg, 1994. 269-315.

[89] The “Fc” fragment comprises the carboxy-terminal portions of both H chains held together by di-sulfides. The effector functions of antibodies are determined by sequences in the Fc region, which region is also the part recognized by Fc receptors (FcR) found on certain types of cells.

[90] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, the individual antibodies comprising the population are identical except for possible mutations, *e.g.*, naturally occurring mutations, that may be present in minor amounts. Thus, the modifier “monoclonal” indicates the character of the antibody as not being a mixture of discrete antibodies. In some embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of

polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

[91] The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein, *Nature* 256:495-97 (1975); Hongo *et al.*, *Hybridoma* 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567), phage-display technologies (see, *e.g.*, Clackson *et al.*, *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004)), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger,

Nature Biotechnol. 14: 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13: 65-93 (1995)).

[92] The monoclonal antibodies herein specifically include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see, *e.g.*, U.S. Pat. No. 4,816,567; and Morrison *et al.*, Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)). Chimeric antibodies include PRIMATTZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with the antigen of interest.

[93] “Humanized” forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a HVR of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, Nature 321:522-525 (1986); Riechmann *et al.*, Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also, *e.g.*, Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[94] A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human 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. Hoogenboom and Winter, *J. Mol. Biol.* 227:381 (1991); Marks *et al.*, *J. Mol. Biol.* 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, 77 (1985); Boerner *et al.*, *J. Immunol.* 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001). Human antibodies can be prepared by 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 (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA* 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[95] The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Xu *et al.*, *Immunity* 13:37-45 (2000); Johnson and Wu, in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, N.J., 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, *e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993); Sheriff *et al.*, *Nature Struct. Biol.* 3:733-736 (1996). HVR is also referred to as “CDR” or “complementarity determining region”.

[96] The structures and locations of immunoglobulin variable regions may be determined by reference to Kabat, E. A. *et al.*, *Sequences of Proteins of Immunological Interest*. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on the Internet (immuno.bme.nwu.edu).

[97] “Framework” or “FR” residues are those variable domain residues other than the HVR residues as herein defined.

[98] As use herein, the term “binds”, “specifically binds to” or is “specific for” refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that binds to or specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, an antibody that specifically binds to a target has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, or $\leq 0.1\text{ nM}$. In some embodiments, an antibody specifically binds to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

[99] As used herein, “Percent (%) amino acid sequence identity” and “homology” with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGNTM (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[100] An amino acid substitution may include but are not limited to the replacement of one amino acid in a polypeptide with another amino acid. Exemplary substitutions are shown in Table A. Amino acid substitutions may be introduced into an antibody of interest and the

products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE A

Original Residue	Exemplary Substitutions
Ala (A)	Val; Leu; Ile
Arg (R)	Lys; Gln; Asn
Asn (N)	Gln; His; Asp, Lys; Arg
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn; Glu
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln; Lys; Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gln; Asn
Met (M)	Leu; Phe; Ile
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Val; Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine

[101] Amino acids may be grouped according to common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[102] As used herein, a “Fab fusion protein” refers to a protein having a Fab fragment covalently linked to one or more binding domains that have different characteristics compared to the Fab fragment. The characteristics may be biological characteristics, such as *in vitro* or *in vivo* activity. The characteristics may also be simple chemical or physical properties, such as binding to a target molecule, catalytic reactions, and the like. The Fab fragment and the one or more binding domains may be directly connected by a single peptide bond, or connected via a peptide linker, but to each other in an in-frame manner.

[103] The term “multispecific” as used in conjunction with an antibody (such as a Fab fusion protein) refers to an antibody (such as a Fab fusion protein) having polyepitopic specificity (*i.e.*, is capable of specifically binding to two, three, or more, different epitopes on one biological molecule or is capable of specifically binding to epitopes on two, three, or more, different biological molecules).

[104] The term “bispecific” as used in conjunction with an antibody (such as a Fab fusion protein) refers to an antibody (such as a Fab fusion protein) capable of specifically binding to two different epitopes on one biological molecule, or capable of specifically binding to epitopes on two different biological molecules. Unless otherwise indicated, the order in which the antigens bound by a bispecific antibody are listed in a bispecific antibody or Fab fusion protein name is arbitrary. That is, the terms “anti-CD3/EpCAM,” “anti-EpCAM/CD3,” “EpCAM×CD3” and “CD3×EpCAM” may be used interchangeably to refer to bispecific antibodies (such as bispecific Fab fusion proteins) that specifically bind to both CD3 and EpCAM.

[105] The terms “multispecific Fab fusion protein” and “MSFP” are used herein interchangeably to refer to a Fab fusion protein that has polyepitopic specificity.

[106] As used herein, the “C terminus” of a polypeptide refers to the last amino acid residue of the polypeptide which donates its amine group to form a peptide bond with the carboxyl group of its adjacent amino acid residue. “N terminus” of a polypeptide as used herein refers to the first amino acid of the polypeptide which donates its carboxyl group to form a peptide bond with the amine group of its adjacent amino acid residue.

[107] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

[108] The term “cell” includes the primary subject cell and its progeny.

[109] The term “cytokine storm,” also known as a “cytokine cascade” or “hypercytokinemia,” is a potentially fatal immune reaction typically consisting of a positive feedback loop between

cytokines and immune cells, with highly elevated levels of various cytokines (*e.g.* INF- γ , IL-10, IL-6, CCL2, etc.).

[110] It is understood that embodiments of the invention described herein include “consisting” and/or “consisting essentially of” embodiments.

[111] Reference to “about” a value or parameter herein includes (and describes) variations that are directed to that value or parameter *per se*. For example, description referring to “about X” includes description of “X”.

[112] As used herein, reference to “not” a value or parameter generally means and describes “other than” a value or parameter. For example, the method is not used to treat cancer of type X means the method is used to treat cancer of types other than X.

[113] The term “about X-Y” used herein has the same meaning as “about X to about Y.”

[114] As used herein and in the appended claims, the singular forms “a,” “or,” and “the” include plural referents unless the context clearly dictates otherwise.

II. Methods of treating cancer

[115] One aspect of the present application provides a method of treating a cancer in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain (such as scFv) is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method does not induce cytokine storm.

[116] In some embodiments, there is provided a method of killing cancer cells in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that

specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain (such as scFv) is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the tumor cell death rate mediated by the MSFP is at least about any of 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method does not induce cytokine storm. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[117] In some embodiments, there is provided a method of inhibiting proliferation of cancer cells in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain (such as scFv) is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method does not induce cytokine storm. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[118] In some embodiments, there is provided a method of inducing redistribution of peripheral T cells in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the multispecific Fab fusion

protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method does not induce cytokine storm. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[119] In some embodiments, the MSFP is a bispecific Fab fusion protein that specifically binds to CD3 and EpCAM.

[120] In some embodiments, the MSFP specifically binds to more than two (such as 3 or more) epitopes. In some embodiments, the MSFP is a trispecific Fab fusion protein further comprising a binding domain (such as an scFv) that specifically binds to a cell surface protein, such as a tumor antigen, that is not EpCAM. In some embodiments, the MSFP comprises a multispecific (such as bispecific) binding domain.

[121] In some embodiments, the MSFP comprise a single binding domain that specifically binds to EpCAM. In some embodiments, the binding domain comprises a single polypeptide chain. In some embodiments, the binding domain is an scFv. In some embodiments, the single binding domain is fused to the N-terminus of the heavy chain polypeptide of the Fab fragment. In some embodiments, the single binding domain is fused to the N-terminus of the light chain polypeptide of the Fab fragment.

[122] In some embodiments, the MSFP comprises two binding domains that specifically bind to EpCAM. In some embodiments, the two binding domains target the same epitope in EpCAM. In some embodiments, the two binding domains have the same amino acid sequence. In some embodiments, the two binding domains have different amino acid sequences. In some embodiments, the two binding domains target different epitopes in EpCAM. In some embodiments, each of the two binding domains comprises a single polypeptide chain. In some embodiments, each of the two binding domains is an scFv. In some embodiments, one binding domain is fused to the N-terminus of the heavy chain polypeptide of the Fab fragment, and the other binding domain is fused to the N-terminus of the light chain polypeptide of the Fab fragment.

[123] Thus, in some embodiments, there is provided a method of treating a cancer in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: (1) a Fab fragment that

specifically binds to CD3; (2) a first binding domain (such as an scFv) that specifically binds to EpCAM; (3) and a second binding domain (such as an scFv) that specifically binds to EpCAM, wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment, wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment, and wherein the multispecific (such as bispecific) Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the first binding domain (such as scFv) and the second binding domain (such as scFv) have the same sequence. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[124] In some embodiments, there is provided a method of killing cancer cells in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: (1) a Fab fragment that specifically binds to CD3; (2) a first binding domain (such as an scFv) that specifically binds to EpCAM; (3) and a second binding domain (such as an scFv) that specifically binds to EpCAM, wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment, wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment, and wherein the multispecific (such as bispecific) Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the first binding domain (such as scFv) and the second binding domain (such as scFv) have the same sequence. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[125] In some embodiments, there is provided a method of inhibiting proliferation of cancer cells in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: (1) a Fab fragment that specifically binds to CD3; (2) a first binding domain (such as an scFv) that specifically binds

to EpCAM; (3) and a second binding domain (such as an scFv) that specifically binds to EpCAM, wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment, wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment, and wherein the multispecific (such as bispecific) Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the first binding domain (such as scFv) and the second binding domain (such as scFv) have the same sequence. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[126] In some embodiments, there is provided a method of inducing redistribution of peripheral T cells in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: (1) a Fab fragment that specifically binds to CD3; (2) a first binding domain (such as an scFv) that specifically binds to EpCAM; (3) and a second binding domain (such as an scFv) that specifically binds to EpCAM, wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment, wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment, and wherein the multispecific (such as bispecific) Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the first binding domain (such as scFv) and the second binding domain (such as scFv) have the same sequence. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[127] The Fab fragment may be derived from any suitable anti-CD3 antibodies known in the art. In some embodiments, the Fab fragment specifically binds to the N-terminus of CD3 epsilon. In some embodiments, the Fab fragment specifically binds to an epitope within amino acids 1-27 of CD3 epsilon. In some embodiments, the Fab fragment is derived from SP34. In some embodiments, the Fab fragment comprises any one, two, or three HVRs (or CDRs) of the heavy

chain variable region of SP34, such as HVRs comprising the amino acid sequences of SEQ ID NOs: 1-3. In some embodiments, the Fab fragment comprises any one, two, or three HVRs (or CDRs) of the light chain variable region of SP34, such as HVRs comprising the amino acid sequences of SEQ ID NOs: 4-6. In some embodiments, the Fab fragment comprises the VH of SP34, such as the VH comprising an amino acid sequence selected from SEQ ID NOs: 7 and 39-43. In some embodiments, the Fab fragment comprises the VL of SP34, such as the VL comprising an amino acid sequence selected from SEQ ID NOs: 8 and 44-47. In some embodiments, the Fab fragment comprises a human immunoglobulin heavy chain constant region 1 (CH1), such as the CH1 comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the CH1 and the CL of the Fab fragment are connected by one or more disulfide bonds. In some embodiments, the Fab fragment comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 11. In some embodiments, the Fab fragment comprises a second polypeptide comprising the amino acid sequence of SEQ ID NO: 12.

[128] Thus, in some embodiments, there is provided a method of treating a cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain (such as scFv) is fused to an N-terminus of the Fab fragment comprising a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence selected from SEQ ID NOs: 7 and 39-43, and/or a light chain variable region (VL) comprising an amino acid sequence selected from SEQ ID NOs: 8 and 44-47. In some embodiments, the Fab fragment comprises a heavy chain polypeptide comprising the amino acid sequence of SEQ ID NO: 11, and/or a light chain polypeptide comprising the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[129] In some embodiments, there is provided a method of treating a cancer in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: (1) a Fab fragment that specifically binds to CD3; wherein the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; (2) a first binding domain (such as an scFv) that specifically binds to EpCAM; (3) and a second binding domain (such as an scFv) that specifically binds to EpCAM, wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment, wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment, and wherein the multispecific (such as bispecific) Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the first binding domain (such as scFv) and the second binding domain (such as scFv) have the same sequence. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence selected from SEQ ID NOs: 7 and 39-43, and/or a light chain variable region (VL) comprising an amino acid sequence selected from SEQ ID NOs: 8 and 44-47. In some embodiments, the Fab fragment comprises a heavy chain polypeptide comprising the amino acid sequence of SEQ ID NO: 11, and/or a light chain polypeptide comprising the amino acid sequence of SEQ ID NO: 12. In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some

embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[130] In some embodiments, the binding domain that specifically binds to EpCAM (also referred herein as EpCAM binding domain) is an antigen-binding fragment of an anti-EpCAM antibody. In some embodiments, the EpCAM binding domain is a single chain antigen-binding fragment of an anti-EpCAM antibody. In some embodiments, the EpCAM binding domain is a scFv. In some embodiments, the scFv comprises an N-VH-VL-C fusion polypeptide. In some embodiments, the EpCAM binding domain is derived from the EpCAM antibody of the present invention, comprising any one, two, or three of the HVRs of the heavy chain variable region comprising the amino acid sequences of SEQ ID NOs: 13-15. In some embodiments, the EpCAM binding domain comprises any one, two, or three of the HVRs of the light chain variable region comprising the amino acid sequences of SEQ ID NOs: 16-18. In some embodiments, the EpCAM binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 19. In some embodiments, the EpCAM binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the EpCAM binding domain is an scFv comprising the amino acid sequence of SEQ ID NO: 21. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[131] Thus, in some embodiments, there is provided a method of treating a cancer in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: (1) a Fab fragment that specifically binds to CD3 (such as the N-terminal 1-27 amino acids of CD3 epsilon); (2) a first scFv that specifically binds to EpCAM; (3) and a second scFv that specifically binds to EpCAM, wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment, wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment, wherein the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID

NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18; and wherein the multispecific (such as bispecific) Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the anti-EpCAM scFv comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 19, and/or a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the anti-EpCAM scFv comprises the amino acid sequence of SEQ ID NO: 21. In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[132] In some embodiments, there is provided a method of treating a cancer in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: (1) a Fab fragment that specifically binds to CD3; wherein the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; (2) a first scFv that specifically binds to EpCAM; (3) and a second scFv that specifically binds to EpCAM, wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment, wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment, wherein the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ

ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18; and wherein the multispecific (such as bispecific) Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence selected from SEQ ID NOs: 7 and 39-43, and/or a light chain variable region (VL) comprising an amino acid sequence selected from SEQ ID NOs: 8 and 44-47. In some embodiments, the Fab fragment comprises a heavy chain polypeptide comprising the amino acid sequence of SEQ ID NO: 11, and/or a light chain polypeptide comprising the amino acid sequence of SEQ ID NO: 12. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the anti-EpCAM scFv comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 19, and/or a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the anti-EpCAM scFv comprises the amino acid sequence of SEQ ID NO: 21. In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[133] The EpCAM binding domain may be fused to the N-terminus of the heavy chain polypeptide and/or the N-terminus of the light chain polypeptide of the Fab fragment via a linker, such as a flexible peptide linker, for example, a peptide linker comprising glycines and serines. In some embodiments, the multispecific (such as bispecific) Fab fusion protein comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the multispecific (such as bispecific) Fab fusion protein comprises a second polypeptide comprising the amino acid sequence of SEQ ID NO: 23.

[134] Thus, in some embodiments, there is provided a method of treating a cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 22 and a second polypeptide comprising the amino acid sequence of

SEQ ID NO:23, wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[135] The methods provided herein may be practiced in an adjuvant setting. In some embodiments, the method is practiced in a neoadjuvant setting, i.e., the method may be carried out before the primary/definitive therapy. In some embodiments, the method is used to treat an individual who has previously been treated. Any of the methods of treatment provided herein may be used to treat an individual who has not previously been treated. In some embodiments, the method is used as a first line therapy. In some embodiments, the method is used as a second line therapy. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[136] The methods described herein are useful for various aspects of cancer treatment. In some embodiments, there is provided a method of inhibiting cell proliferation (such as tumor growth) in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, at least about 10% (including for example at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%) cell proliferation is inhibited. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the

second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[137] In some embodiments, there is provided a method of inhibiting tumor metastasis in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, at least about 10% (including for example at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%) metastasis is inhibited. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have

the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[138] In some embodiments, there is provided a method of reducing (such as eradicating) pre-existing tumor metastasis (such as metastasis to the lymph node) in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, at least about 10% (including for example at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%) metastasis is reduced. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In

some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[139] In some embodiments, there is provided a method of reducing incidence or burden of preexisting tumor metastasis (such as metastasis to the lymph node) in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy

chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[140] In some embodiments, there is provided a method of reducing tumor size in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the tumor size is reduced at least about 10% (including for example at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%). In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid

sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[141] In some embodiments, there is provided a method of prolonging time to disease progression of cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the method prolongs the time to disease progression by at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid

sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[142] In some embodiments, there is provided a method of prolonging survival of an individual having cancer, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method prolongs the survival of the individual by at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, or 24 month. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ

ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[143] In some embodiments, there is provided a method of alleviating one or more symptoms in an individual having cancer, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a

HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[144] The methods described herein are suitable for treating a variety of cancers, including both solid cancer and liquid cancer. The methods are applicable to cancers of all stages, including early stage cancer, non-metastatic cancer, primary cancer, advanced cancer, locally advanced cancer, metastatic cancer, or cancer in remission. The methods described herein may be used as a first therapy, second therapy, third therapy, or combination therapy with other types of cancer therapies known in the art, such as chemotherapy, surgery, radiation, gene therapy, immunotherapy, bone marrow transplantation, stem cell transplantation, targeted therapy, cryotherapy, ultrasound therapy, photodynamic therapy, radio-frequency ablation or the like, in an adjuvant setting or a neoadjuvant setting. In some embodiments, the cancer has been refractory to prior therapy.

[145] Examples of cancers that may be treated by the methods of the invention include, but are not limited to, adenocortical carcinoma, AIDS-related cancers (*e.g.*, AIDS-related lymphoma), anal cancer, appendix cancer, astrocytoma (*e.g.*, cerebellar and cerebral), basal cell carcinoma, bile duct cancer (*e.g.*, extrahepatic), bladder cancer, bone cancer, (osteosarcoma and malignant fibrous histiocytoma), brain tumor (*e.g.*, glioma, brain stem glioma, cerebellar or cerebral astrocytoma (*e.g.*, pilocytic astrocytoma, diffuse astrocytoma, anaplastic (malignant) astrocytoma), malignant glioma, ependymoma, oligodendglioma, meningioma, craniopharyngioma, haemangioblastomas, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, and glioblastoma), breast

cancer, bronchial adenomas/carcinoids, carcinoid tumor (*e.g.*, gastrointestinal carcinoid tumor), carcinoma of unknown primary, central nervous system lymphoma, cervical cancer, colon cancer, colorectal cancer, chronic myeloproliferative disorders, endometrial cancer (*e.g.*, uterine cancer), ependymoma, esophageal cancer, Ewing's family of tumors, eye cancer (*e.g.*, intraocular melanoma and retinoblastoma), gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), germ cell tumor, (*e.g.*, extracranial, extragonadal, ovarian), gestational trophoblastic tumor, head and neck cancer, hepatocellular (liver) cancer (*e.g.*, hepatic carcinoma and hepatoma), hypopharyngeal cancer, islet cell carcinoma (endocrine pancreas), laryngeal cancer, leukemia, lip and oral cavity cancer, oral cancer, liver cancer, lung cancer (*e.g.*, small cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), lymphoid neoplasm (*e.g.*, lymphoma), medulloblastoma, melanoma, mesothelioma, metastatic squamous neck cancer, mouth cancer, multiple endocrine neoplasia syndrome, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, oropharyngeal cancer, ovarian cancer (*e.g.*, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor), pancreatic cancer, parathyroid cancer, penile cancer, cancer of the peritoneal, pharyngeal cancer, pheochromocytoma, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary tumor, pleuropulmonary blastoma, lymphoma, primary central nervous system lymphoma (microglioma), rectal cancer, renal cancer, renal pelvis and ureter cancer (transitional cell cancer), rhabdomyosarcoma, salivary gland cancer, skin cancer (*e.g.*, non-melanoma (*e.g.*, squamous cell carcinoma), melanoma, and Merkel cell carcinoma), small intestine cancer, squamous cell cancer, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, urethral cancer, vaginal cancer, vulvar cancer, Wilms' tumor, and post-transplant lymphoproliferative disorder (PTLD), abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome.

[146] In some embodiments, the method is suitable for treating cancers that overexpress EpCAM on the surface of the cancer cells, such as EpCAM-positive solid cancers. In some embodiments, the cancer cells in the individual express at least about any of more than 2, 5, 10, 20, 50, 100, 200, 500, 1000 or more fold of EpCAM compared to normal cells. In some embodiments, the EpCAM-positive solid cancer is a carcinoma or adenocarcinoma. In some

embodiments, the EpCAM-positive solid cancer is selected from the group consisting of small intestine cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, breast cancer, bile duct cancer, and head and neck cancer.

[147] Thus, in some embodiments, there is provided a method of treating an EpCAM-positive solid cancer (such as carcinoma or adenocarcinoma) in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the EpCAM-positive solid cancer is selected from the group consisting of small intestine cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, breast cancer, bile duct cancer, and head and neck cancer.

[148] In some embodiments, there is provided a method of treating an EpCAM-positive solid cancer (such as carcinoma or adenocarcinoma) in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: (1) a Fab fragment that specifically binds to CD3; (2) a first binding domain (such as an scFv) that specifically binds to EpCAM; (3) and a second binding domain (such as an scFv) that specifically binds to EpCAM, wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment, wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment, and wherein the multispecific (such as bispecific) Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the first binding domain (such as scFv) and the second binding domain (such as scFv) have the same

sequence. In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the EpCAM-positive solid cancer is selected from the group consisting of small intestine cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, breast cancer, bile duct cancer, and head and neck cancer.

[149] In some embodiments, there is provided a method of treating an EpCAM-positive solid cancer (such as carcinoma or adenocarcinoma) in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain (such as scFv) is fused to an N-terminus of the Fab fragment comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence selected from SEQ ID NOs: 7 and 39-43, and/or a light chain variable region (VL) comprising an amino acid sequence selected from SEQ ID NOs: 8 and 44-47. In some embodiments, the Fab fragment comprises a heavy chain polypeptide comprising the amino acid sequence of SEQ ID NO: 11, and/or a light chain polypeptide comprising the amino acid sequence of SEQ ID NO: 12. In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein is administered

intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the EpCAM-positive solid cancer is selected from the group consisting of small intestine cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, breast cancer, bile duct cancer, and head and neck cancer.

[150] In some embodiments, there is provided a method of treating an EpCAM-positive solid cancer (such as carcinoma or adenocarcinoma) in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: (1) a Fab fragment that specifically binds to CD3 (such as the N-terminal 1-27 amino acids of CD3 epsilon); (2) a first scFv that specifically binds to EpCAM; (3) and a second scFv that specifically binds to EpCAM, wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment, wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment, wherein the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18; and wherein the multispecific (such as bispecific) Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the anti-EpCAM scFv comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 19, and/or a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the anti-EpCAM scFv comprises the amino acid sequence of SEQ ID NO: 21. In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some

embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the EpCAM-positive solid cancer is selected from the group consisting of small intestine cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, breast cancer, bile duct cancer, and head and neck cancer.

[151] In some embodiments, there is provided a method of treating an EpCAM-positive solid cancer (such as carcinoma or adenocarcinoma) in an individual (such as a human), comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: (1) a Fab fragment that specifically binds to CD3; wherein the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; (2) a first scFv that specifically binds to EpCAM; (3) and a second scFv that specifically binds to EpCAM, wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment, wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment, wherein the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18; and wherein the multispecific (such as bispecific) Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence selected from SEQ ID NOs: 7 and 39-43, and/or a light chain variable region (VL) comprising an amino acid sequence selected from SEQ ID NOs: 8

and 44-47. In some embodiments, the Fab fragment comprises a heavy chain polypeptide comprising the amino acid sequence of SEQ ID NO: 11, and/or a light chain polypeptide comprising the amino acid sequence of SEQ ID NO: 12. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the anti-EpCAM scFv comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 19, and/or a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the anti-EpCAM scFv comprises the amino acid sequence of SEQ ID NO: 21. In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the EpCAM-positive solid cancer is selected from the group consisting of small intestine cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, breast cancer, bile duct cancer, and head and neck cancer.

[152] In some embodiments, there is provided a method of treating an EpCAM-positive solid cancer (such as carcinoma or adenocarcinoma) in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 22 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 23, wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the EpCAM-positive solid cancer is selected from the group consisting of small intestine cancer,

colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, breast cancer, bile duct cancer, and head and neck cancer.

[153] In some embodiments, there is provided a method of treating small intestine cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously.

In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[154] In some embodiments, there is provided a method of treating colorectal cancer (such as colorectal adenocarcinoma) in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of

SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the colorectal cancer is adenocarcinoma, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, leiomyosarcoma, melanoma, or squamous cell carcinoma.

[155] In some embodiments, there is provided a method of treating lung cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain

variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the lung cancer is a non-small cell lung cancer (NSCLC). Examples of NSCLC include, but are not limited to, large-cell carcinoma, adenocarcinoma, neuroendocrine lung tumors, and squamous cell carcinoma.

[156] In some embodiments, there is provided a method of treating cervical cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv

comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual.

[157] In some embodiments, there is provided a method of treating liver cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv

comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the liver cancer is liver cell carcinoma, fibrolamellar variants of hepatocellular carcinoma, or mixed hepatocellular cholangiocarcinoma.

[158] In some embodiments, there is provided a method of treating gastric cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2

comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the gastric cancer is an adenocarcinoma, lymphoma, gastrointestinal stromal tumor (GIST), carcinoid tumor, squamous cell carcinoma, small cell carcinoma, or leiomyosarcoma.

[159] In some embodiments, there is provided a method of treating pancreatic cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of

SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the pancreatic cancer is a serous cystic neoplasm, mucinous cystic neoplasm, intraductal papillary mucinous neoplasm, pancreatic adenocarcinoma, adenosquamous carcinoma, squamous cell carcinoma, signet ring cell carcinoma, undifferentiated carcinoma, undifferentiated carcinoma with giant cells, solid pseudopapillary neoplasm, ampullary cancer, or pancreatic neuroendocrine tumor. In some embodiments, the pancreatic cancer is a pancreatic adenocarcinoma.

[160] In some embodiments, there is provided a method of treating skin cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM;

wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the skin cancer is melanoma. In some embodiments, the melanoma is superficial spreading melanoma, lentigo maligna melanoma, nodular melanoma, mucosal melanoma, polypoid melanoma, desmoplastic melanoma, amelanotic melanoma, soft-tissue melanoma, or acral lentiginous melanoma.

[161] In some embodiments, there is provided a method of treating renal cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100

μg/kg). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the cancer is renal cell carcinoma. In some embodiments, the renal cell carcinoma is an adenocarcinoma. In some embodiments, the renal cell carcinoma is a clear cell renal cell carcinoma, papillary renal cell carcinoma (also called chromophilic renal cell carcinoma), chromophobe renal cell carcinoma, collecting duct renal cell carcinoma, granular renal cell carcinoma, mixed granular renal cell carcinoma, renal angiomyolipomas, or spindle renal cell carcinoma.

[162] In some embodiments, there is provided a method of treating bladder cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some

embodiments, the bladder cancer is a low grade bladder cancer. In some embodiments, the bladder cancer is a high grade bladder cancer. In some embodiments, the bladder cancer is muscle invasive (*e.g.*, T2, T3 or T4). In some embodiments, the bladder cancer is non-invasive (*e.g.*, Ta, T1 Cis, Cis with Ta and/or T1). In some embodiments, the bladder cancer is transitional cell carcinoma or urothelial carcinoma (such as metastatic urothelial carcinoma), including, but not limited to, papillary tumors and flat carcinomas. In some embodiments, the bladder cancer is squamous cell carcinoma, non-squamous cell carcinoma, adenocarcinoma, or small cell carcinoma.

[163] In some embodiments, there is provided a method of treating thyroid cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid

sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the thyroid cancer is papillary carcinoma, follicular carcinoma, Hurthle cell carcinoma, medullary thyroid carcinoma, anaplastic carcinoma, thyroid lymphoma, thyroid sarcoma, or parathyroid cancer.

[164] In some embodiments, there is provided a method of treating prostate cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2

comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the prostate cancer is an adenocarcinoma. In some embodiments, the prostate cancer is a sarcoma, neuroendocrine tumor, small cell cancer, ductal cancer, or a lymphoma.

[165] In some embodiments, there is provided a method of treating ovarian cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3

comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method does not induce cytokine storm. In some embodiments, the ovarian cancer is ovarian epithelial cancer.

[166] In some embodiments, there is provided a method of treating endometrial cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3

comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the endometrial cancer is adenocarcinoma, carcinosarcoma, squamous cell carcinoma, undifferentiated carcinoma, small cell carcinoma, or transitional carcinoma.

[167] In some embodiments, there is provided a method of treating breast cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$ (such as about 0.01 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 2.5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a

heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the breast cancer is early stage breast cancer, non-metastatic breast cancer, advanced breast cancer, stage IV breast cancer, locally advanced breast cancer, metastatic breast cancer, breast cancer in remission, breast cancer in an adjuvant setting, or breast cancer in a neoadjuvant setting. In some embodiments, the breast cancer is fibroadenoma, or intraductal papilloma. In some embodiments, the breast cancer is HER2 positive or HER2 negative. In some embodiments, the breast cancer is a triple negative breast cancer.

[168] In some embodiments, there is provided a method of treating bile duct cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100 µg/kg). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first

scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the bile duct cancer is an intrahepatic bile duct cancer, a perihilar bile duct cancer, a distal bile duct cancer. In some embodiments, the bile duct cancer is a cholangiocarcinoma, sarcoma, lymphoma, or small cell cancer.

[169] In some embodiments, there is provided a method of treating head and neck cancer in an individual, comprising administering to the individual an effective amount of a multispecific (such as bispecific) Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain (such as scFv) that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg (such as about 0.01 µg/kg to about 5 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 2.5 µg/kg to about 100

μg/kg). In some embodiments, the method has one or more of the following biological activities: (1) killing cancer cells; (2) inhibits proliferation of cancer cells; and (3) induces redistribution of peripheral T cells. In some embodiments, the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first binding domain (such as scFv) is fused to the N-terminus of the VH of the Fab fragment; and wherein the second binding domain (such as scFv) is fused to the N-terminus of the VL of the Fab fragment. In some embodiments, the first scFv and the second scFv have the same sequence. In some embodiments, the Fab fragment binds to the N-terminus (such as N-terminal 1-27 amino acids) of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the first scFv and/or the second scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein is administered intravenously. In some embodiments, the multispecific Fab fusion protein is administered at a low frequency. In some embodiments, the method further comprises administering a glucocorticoid (such as dexamethasone) to the individual. In some embodiments, the head and neck cancer is a squamous cell carcinoma in the head and neck. In some embodiments, the head and neck cancer is a hypopharyngeal cancer, laryngeal cancer, lip and oral cavity cancer, metastatic squamous neck cancer with occult primary, nasopharyngeal cancer, oropharyngeal cancer, paranasal sinus and nasal cavity cancer, or salivary gland cancer.

[170] Exemplary routes of administration of the multispecific Fab fusion protein (MSFP) include, but are not limited to, oral, intravenous, intracavitary, intratumoral, intraarterial, intramuscular, subcutaneous, parenteral, transmucosal, transdermal, ocular, topical,

intraperitoneal, intracranial, intrapleural, and epidermal routes, or be delivered into lymph glands, body spaces, organs or tissues known to contain cancer cells. In some embodiments, the MSFP is administered intravenously. In some embodiments, the MSFP is administered by infusion. In some embodiments, the MSFP is administered subcutaneously. In some embodiments, the MSFP is administered by injection.

[171] In some embodiments, the MSFP is administered by intravenous infusion. In some embodiments, the MSFP is infused to the individual over a period of time no more than about any of 24 hours, 20 hours, 15 hours, 10 hours, 8 hours, 6 hours, 3 hours, 2 hours, 1 hours, 30 minutes, or less. In some embodiments, the MSFP is infused to the individual over a period of time of any one of about 30 minutes to about 1 hour, about 1 hour to about 2 hours, about 2 hours to about 4 hours, about 4 hours to about 6 hours, about 6 hours to about 8 hours, about 8 hours to about 10 hours, about 10 hours to about 12 hours, about 12 hours to about 18 hours, about 18 hours to about 24 hours, about 30 minutes to about 2 hours, about 2 hours to about 5 hours, about 5 hours to about 10 hours, about 10 hours to about 20 hours, about 30 minutes to about 10 hours, or about 30 minutes to about 20 hours. The MSFP may be infused to the individual at any suitable rate. In some embodiments, the MSFP may be infused at a rate more than about any of 0.01 $\mu\text{g/kg/hr}$, 0.02 $\mu\text{g/kg/hr}$, 0.05 $\mu\text{g/kg/hr}$, 0.1 $\mu\text{g/kg/hr}$, 0.2 $\mu\text{g/kg/hr}$, 0.5 $\mu\text{g/kg/hr}$, 0.6 $\mu\text{g/kg/hr}$, 0.7 $\mu\text{g/kg/hr}$, 0.8 $\mu\text{g/kg/hr}$, 0.9 $\mu\text{g/kg/hr}$, 1 $\mu\text{g/kg/hr}$, 1.5 $\mu\text{g/kg/hr}$, 2 $\mu\text{g/kg/hr}$, 3 $\mu\text{g/kg/hr}$, 4 $\mu\text{g/kg/hr}$, 5 $\mu\text{g/kg/hr}$, 10 $\mu\text{g/kg/hr}$, 15 $\mu\text{g/kg/hr}$, 20 $\mu\text{g/kg/hr}$, 25 $\mu\text{g/kg/hr}$, 50 $\mu\text{g/kg/hr}$, 75 $\mu\text{g/kg/hr}$, 100 $\mu\text{g/kg/hr}$ or more.

[172] The dosing regimen of the MSFPs administered to the individual may vary with the particular MSFP composition, the method of administration, and the particular type and stage of cancer being treated. In some embodiments, that effective amount of the MSFP is below the level that induces a toxicological effect (*i.e.*, an effect above a clinically acceptable level of toxicity) or is at a level where a potential side effect can be controlled or tolerated when the composition is administered to the individual.

[173] In some embodiments, the effective amount of the MSFP is below the level that induces an adverse effect in the central nervous system. For example, an adverse effect observed in antibody therapy is the occurrence of infusion-related side effects, such as the cytokine release syndrome ("CRS"), the severe cases of which are known as "cytokine storms". When a "cytokine

storm” is induced, the healthy individual's immune system is activated and releases large amounts of the pro-inflammatory cytokines, such as INF- γ , CCL2, IIL-10, IL-6, *etc.* It is a potentially fatal immune reaction typically consisting of a positive feedback loop between cytokines and immune cells, with highly elevated levels of various cytokines. Other adverse side effects described to be associated with CRS are fatigue, vomiting, tachycardia, hypertension, back pain, but also central nervous system reactions (CNS reactions), such as seizures, encephalopathy, cerebral edema, aseptic meningitis, and headache. In some embodiments, the MSFP is administered at a dose that does not induce cytokine release syndrome, such as cytokine storm. In some embodiments, the MSFP is administered at a dose that does not induce significant release of one or more cytokines selected from the group consisting of IL-2, IL-4, IL-5, IL-6, TNF, and INF- γ . In some embodiments, a significant release of a cytokine is sustained release of a cytokine over the course of at least about any of 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 18 hours, 24 hours, or more. In some embodiments, a significant release of a cytokine is a serum or blood level of a cytokine at a concentration of at least about any of 1, 5, 10, 20, 50, 100, 200, 500, 1000 or more pg/mL. Without being bound by any theory, the MSFPs described herein require binding to EpCAM on the target tumor cell in order to recruit and activate T cells. Such requirement can greatly reduce unwanted cytokine storms, and unwanted activation of T cells in the absence of the desired target tumor cell.

[174] In some embodiments, the MSFP is administered at a dose of no more than about any one of 0.01 $\mu\text{g/kg}$, 0.05 $\mu\text{g/kg}$, 0.1 $\mu\text{g/kg}$, 0.5 $\mu\text{g/kg}$, 1 $\mu\text{g/kg}$, 2 $\mu\text{g/kg}$, 5 $\mu\text{g/kg}$, 10 $\mu\text{g/kg}$, 15 $\mu\text{g/kg}$, 20 $\mu\text{g/kg}$, 25 $\mu\text{g/kg}$, 30 $\mu\text{g/kg}$, 50 $\mu\text{g/kg}$, 100 $\mu\text{g/kg}$, 150 $\mu\text{g/kg}$, 200 $\mu\text{g/kg}$, 250 $\mu\text{g/kg}$, 300 $\mu\text{g/kg}$, 400 $\mu\text{g/kg}$, 500 $\mu\text{g/kg}$, 600 $\mu\text{g/kg}$, 700 $\mu\text{g/kg}$, 800 $\mu\text{g/kg}$, 900 $\mu\text{g/kg}$, or 1 mg/kg. In some embodiments, the dose of the MSFP is within any of the following range, wherein the ranges have an upper limit of any of: 0.05 $\mu\text{g/kg}$, 0.1 $\mu\text{g/kg}$, 0.5 $\mu\text{g/kg}$, 1 $\mu\text{g/kg}$, 2 $\mu\text{g/kg}$, 5 $\mu\text{g/kg}$, 10 $\mu\text{g/kg}$, 15 $\mu\text{g/kg}$, 20 $\mu\text{g/kg}$, 25 $\mu\text{g/kg}$, 30 $\mu\text{g/kg}$, 50 $\mu\text{g/kg}$, 100 $\mu\text{g/kg}$, 150 $\mu\text{g/kg}$, 200 $\mu\text{g/kg}$, 250 $\mu\text{g/kg}$, 300 $\mu\text{g/kg}$, 400 $\mu\text{g/kg}$, 500 $\mu\text{g/kg}$, 600 $\mu\text{g/kg}$, 700 $\mu\text{g/kg}$, 800 $\mu\text{g/kg}$, 900 $\mu\text{g/kg}$, or 1 mg/kg, and an independently selected lower limit of any of 0.01 $\mu\text{g/kg}$, 0.05 $\mu\text{g/kg}$, 0.1 $\mu\text{g/kg}$, 0.5 $\mu\text{g/kg}$, 1 $\mu\text{g/kg}$, 2 $\mu\text{g/kg}$, 5 $\mu\text{g/kg}$, 10 $\mu\text{g/kg}$, 15 $\mu\text{g/kg}$, 20 $\mu\text{g/kg}$, 25 $\mu\text{g/kg}$, 30 $\mu\text{g/kg}$, 50 $\mu\text{g/kg}$, 100 $\mu\text{g/kg}$, 150 $\mu\text{g/kg}$, 200 $\mu\text{g/kg}$, 250 $\mu\text{g/kg}$, 300 $\mu\text{g/kg}$, 400 $\mu\text{g/kg}$, 500 $\mu\text{g/kg}$, 600 $\mu\text{g/kg}$, 700 $\mu\text{g/kg}$, 800 $\mu\text{g/kg}$, or 900 $\mu\text{g/kg}$, and wherein the lower limit is less than the upper limit. In some embodiments, the MSFP is administered at a dose of any one of about 0.01 $\mu\text{g/kg}$

to about 0.05 µg/kg, about 0.05 µg/kg to about 0.1 µg/kg, about 0.1 µg/kg to about 0.5 µg/kg, about 0.5 µg/kg to about 1 µg/kg, about 0.01 µg/kg to about 0.1 µg/kg, about 0.1 µg/kg to about 1 µg/kg, about 1 µg/kg to about 5 µg/kg, about 5 µg/kg to about 10 µg/kg, about 10 µg/kg to about 15 µg/kg, about 15 µg/kg to about 20 µg/kg, about 20 µg/kg to about 25 µg/kg, about 25 µg/kg to about 30 µg/kg, about 5 µg/kg to about 15 µg/kg, about 10 µg/kg to about 30 µg/kg, about 30 µg/kg to about 50 µg/kg, about 50 µg/kg to about 100 µg/kg, about 0.01 µg/kg to about 1 µg/kg, about 0.01 µg/kg to about 5 µg/kg, about 0.01 µg/kg to about 30 µg/kg, about 0.01 µg/kg to about 250 µg/kg, about 0.1 µg/kg to about 10 µg/kg, about 0.1 µg/kg to about 30 µg/kg, about 0.1 µg/kg to about 250 µg/kg, about 1 µg/kg to about 10 µg/kg, about 1 µg/kg to about 20 µg/kg, about 1 µg/kg to about 30 µg/kg, about 1 µg/kg to about 250 µg/kg, about 100 µg/kg to about 250 µg/kg, about 5 µg/kg to about 250 µg/kg, about 250 µg/kg to about 500 µg/kg, about 500 µg/kg to about 1000 µg/kg, or about 0.01 µg/kg to about 1000 µg/kg. The doses described herein may refer to a suitable dose for cynomolgus monkeys, a human equivalent dose thereof, or an equivalent dose for the specific species of the individual. In some embodiments, the MSFP is administered at a dose equivalent to about 0.1 µg/kg to about 100 µg/kg (such as such as about 0.3 µg/kg to about 5 µg/kg, or about 5 µg/kg to about 20 µg/kg) for a cynomolgus monkey. In some embodiments, the MSFP is administered at a dose equivalent to no more than about 30 µg/kg (such as no more than about 20, 15, or 10 µg/kg) for a cynomolgus monkey.

[175] In some embodiments, the MSFP is administered at a dose of about 0.1 µg/kg to about 10 µg/kg, such as about any one of 0.3, 0.5, 0.6, 1, 1.2, 2, 2.4, 3.6, or 4 µg/kg.

[176] The effective amount of the MSFP may be administered in a single dose or in multiple doses. For methods that comprises administration of the MSFP in multiple doses, exemplary dosing frequencies include, but are not limited to, daily, daily without break, weekly, weekly without break, weekly for two out of three weeks, weekly for three out of four weeks, once every three weeks, once every two weeks, monthly, every six months, yearly, etc. In some embodiments, the MSFP is administered about once every 2 weeks, once every 3 weeks, once every 4 weeks, once every 6 weeks, or once every 8 weeks. In some embodiments, the MSFP is administered at least about any of 1x, 2x, 3x, 4x, 5x, 6x, or 7x (*i.e.*, daily) a week. In some embodiments, the intervals between each administration are less than about any of 3 years, 2 years, 12 months, 11 months, 10 months, 9 months, 8 months, 7 months, 6 months, 5 months, 4 months, 3 months, 2 months, 1 month, 4 weeks, 3 weeks, 2 weeks, 1 week, 6 days, 5 days, 4 days,

3 days, 2 days, or 1 day. In some embodiments, the intervals between each administration are more than about any of 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 2 years, or 3 years. In some embodiments, there is no break in the dosing schedule.

[177] In some embodiments, the MSFP is administered at a first dose for a first period of time, and consecutively the MSFP is administered at a second dose for a second period of time, wherein the second dose exceeds the first dose. The first period of time and the second period of time may be of any suitable length, including for example about any one of 1, 2, 3, 4, 5, 6, or more weeks. In some embodiments, the second period of time exceeds the first period of time. In some embodiments, the first period of time is at least about 7 days. In some embodiments, the second period of time is at least about any one of 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, or more. The first dose and the second dose can be of any suitable dose as described above. In some embodiments, the first dose is no more than about any one of 2, 1.5, 1, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 $\mu\text{g/kg}$ or less. In some embodiments, the second dose is about 0.1 $\mu\text{g/kg}$ to about 10 $\mu\text{g/kg}$, such as about 0.3 $\mu\text{g/kg}$ to about 5 $\mu\text{g/kg}$. In some embodiments, the second dose is about any one of 0.3, 0.6, 1.2, 2.4, or 3.6 $\mu\text{g/kg}$.

[178] In some embodiments, the MSFP is administered at a low frequency, for example, any one of no more frequent than once per month, once per 2 months, once per 3 months, once per 4 months, once per 5 months, once per 6 months, once per 7 months, once per 8 months, once per 9 months, once per 10 months, once per 11 months, once per year, once per 18 months, once per two years, once per three years, or less. In some embodiments, the MSFP is administered in a single dose. In some embodiments, the MSFP is administered twice weekly.

[179] The administration of the MSFP can be extended over an extended period of time, such as from 1 day to about a week, from about a week to about a month, from about a month to about a year, from about a year to about several years. In some embodiments, the MSFP is administered over a period of at least any of about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 1 year, 2 years, 3 years, 4 years, or more.

[180] In some embodiments, the method further comprises administration of one or more glucocorticoids. Glucocorticoids (GC) are a class of steroid hormones that bind to the glucocorticoid receptor (GR), which is present in almost every vertebrate animal cell, including humans. These compounds are potent anti-inflammatory agents, regardless of the inflammation's cause. In some embodiments, the glucocorticoid suppresses release of one or more cytokines selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8 and IFN- γ . Suitable glucocorticoids include, but are not limited to, cortisone, hydrocortisone, cortisol, cloprednol, prednisone, prednisolone, methylprednisolone, deflazacort, fludrocortisone, triamcinolone, dexamethasone, and betamethasone. In some embodiments, the glucocorticoid is selected from the group consisting of cortisone, hydrocortisone, cortisol, cloprednol, prednisone, prednisolone, methylprednisolone, deflazacort, fludrocortisone, triamcinolone, dexamethasone, betamethasone and a pharmaceutically acceptable ester, salt, or complex of thereof. In some embodiments, the glucocorticoid is dexamethasone. In some embodiments, the glucocorticoid is a pharmaceutically acceptable ester, salt, or complex of dexamethasone.

[181] The glucocorticoid may be administered to the individual simultaneously with the MSFP or prior to the administration of the MSFP. The glucocorticoid may be administered at a time no longer than about any one of 3 hours, 2 hours, 1 hours, 30 minutes, or less from the administration of the MSFP. In some embodiments, the glucocorticoid is administered simultaneously or prior to each dose of the MSFP. In some embodiments, the glucocorticoid is administered simultaneously or prior to the first dose of the MSFP. In some embodiments, wherein the MSFP is administered at a first dose for a first period of time, and consecutively the MSFP is administered at a second dose for a second period of time, the glucocorticoid is administered prior (such as about 1 hour prior) to the first administration of the MSFP for the first period of time, and the glucocorticoid is administered prior (such as about 1 hour prior) to the first administration of the MSFP for the second period of time. In some embodiments, the glucocorticoid is administered prior to the administration of the MSFP when the individual has an elevated liver enzyme level (such as ALT, TBil, and/or ALP) and/or an elevated cytokine level (such as IL-6). The glucocorticoid may be administered at any suitable dosage, including, for example, at least about any one of 0.1, 0.5, 1, 2, 3, 4, 5 mg/kg or more. In some embodiments, the glucocorticoid is administered at a dose of at least about any one of 1, 2, 5, 10, 15, 20, 25 mg or more.

[182] In some embodiments, the glucocorticoid is dexamethasone. In some embodiments, the method comprises administering dexamethasone to the individual prior to the administration of the first dose of the MSFP. In some embodiments, the dexamethasone is administered at a dose of about 0.1 mg/kg to about 5 mg/kg.

Multispecific Fab Fusion Proteins

[183] The multispecific Fab fusion proteins (MSFP) used in the methods described herein comprise an anti-CD3 Fab fragment fused to a first EpCAM binding domain at the N-terminal end of the VH of the Fab fragment and/or a second EpCAM binding domain at the N-terminal end of the VL of the Fab fragment. In some embodiments, the EpCAM binding domain is an anti-EpCAM scFv. In some embodiments, the EpCAM binding domain is connected to the VH or VL of the anti-CD3 Fab fragment via a linker. An exemplary bispecific Fab fusion protein useful for methods of the present invention is shown in FIG. 1.

Fab fragment

[184] The Fab fragment of the MSFPs described herein specifically binds to CD3, such as human CD3. "CD3" is known in the art as a multi-protein complex of six chains (see, Abbas and Lichtman, 2003; Janeway *et al.*, p172 and 178, 1999). In mammals, the complex comprises a CD3 gamma chain, a CD3 delta chain, two CD3 epsilon chains, and a homodimer of CD3 zeta chains. The CD3 gamma, CD3 delta, and CD3 epsilon chains are highly related cell surface proteins of the immunoglobulin superfamily containing a single immunoglobulin domain. The transmembrane regions of the CD3 gamma, CD3 delta, and CD3 epsilon chains are negatively charged, which is a characteristic that allows these chains to associate with the positively charged T cell receptor chains. The intracellular tails of the CD3 gamma, CD3 delta, and CD3 epsilon chains each contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM, whereas each CD3 zeta chain has three. Without being bound by theory, it is believed the ITAMs are important for the signaling capacity of a TCR complex. CD3 as used herein may be from various animal species, including human, primate, mouse, rat, or other mammals.

[185] In some embodiments, the Fab fragment of the MSFP specifically binds to an individual CD3 chain, such as CD3 gamma chain, CD3 delta chain, or CD3 epsilon chain. In some embodiments, the Fab fragment specifically binds to a complex formed from two or more

individual CD3 chains (*e.g.*, a complex of more than one CD3 epsilon chains, a complex of a CD3 gamma and CD3 epsilon chain, a complex of a CD3 delta and CD3 epsilon chain). In some embodiments, the Fab fragment specifically binds to a CD3 epsilon chain. In some embodiments, the Fab fragment specifically binds to the N-terminus of CD3 epsilon. In some embodiments, the Fab fragment specifically binds to amino acids 1-27 of CD3 epsilon.

[186] The Fab fragments can be generated by a variety of methods known in the art (see, *e.g.*, U.S. Pat. Nos. 6,291,161; 6,291,158). Sources of Fabs include monoclonal antibody or antigen-binding fragments thereof from various species, including human, camelid (from camels, dromedaries, or llamas; Hamers-Casterman et al. (1993) *Nature*, 363:446 and Nguyen et al. (1998) *J. Mol. Biol.*, 275:413), shark (Roux et al. (1998) *Proc. Nat'l. Acad. Sci. (USA)* 95:11804), fish (Nguyen et al. (2002) *Immunogenetics*, 54:39), rodent, avian, or ovine. In some embodiments, the Fab fragment is derived from a human or humanized antibody.

[187] In some embodiments, the Fab fragment specifically binds to both human and non-human primates (such as cynomolgus monkey) CD3. Exemplary anti-human CD3 antibody with cross reactivity to monkey CD3 include, but are not limited to, SP34 mouse monoclonal antibody, (see, for example, Pressano, S. *The EMBO J.* 4:337-344, 1985; Alarcon, B. *EMBO J.* 10:903-912, 1991; Salmeron A. et al., *J. Immunol.* 147:3047-52, 1991; Yoshino N. et al., *Exp. Anim* 49:97-110, 2000; Conrad M L. et al., *Cytometry* 71A:925-33, 2007; and Yang et al., *J. Immunol.* 137:1097-1100: 1986). MSFPS having anti-CD3 Fab fragments with cross-reactivity to monkey CD3 may facilitate toxicity studies in non-human primates, which can provide more relevant safety assessments for human clinical trial candidates, without having to perform toxicity studies in chimpanzees or using surrogate molecules.

[188] In some embodiments, the Fab fragment is derived from an anti-CD3 antibody that does not have cross-reactivity to non-human primates. Exemplary anti-CD3 antibodies include the Cris-7 monoclonal antibody (Reinherz, E. L. et al. (eds.), *Leukocyte typing II*, Springer Verlag, New York, (1986)), BC3 monoclonal antibody (Anasetti et al. (1990) *J. Exp. Med.* 172:1691), OKT3 (Ortho multicenter Transplant Study Group (1985) *N. Engl. J. Med.* 313:337) and derivatives thereof such as OKT3 ala-ala (Herold et al. (2003) *J. Clin. Invest.* 11:409), visilizumab (Carpenter et al. (2002) *Blood* 99:2712), and 145-2C11 monoclonal antibody (Hirsch et al. (1988) *J. Immunol.* 140: 3766). Further CD3 binding molecules contemplated

herein include UCHT-1 (Beverley, P C and Callard, R. E. (1981) Eur. J. Immunol. 11: 329-334) and CD3 binding molecules described in WO2004/106380; WO2010/037838; WO2008/119567; WO2007/042261; WO2010/0150918.

[189] In some embodiments, the Fab fragment comprises one constant and one variable region of an immunoglobulin heavy, and one constant and one variable region of an immunoglobulin light chain. In some embodiments, the heavy chain constant and variable regions heterodimerize with the light chain variable and constant regions, and are covalently linked by a disulfide bond between the heavy and light chain constant regions. In some embodiments, the Fab fragment has the basic structure NH₂-VL-CL-S-S-CH1-VH-NH₂. In some embodiments, the CH1 and the CL of the Fab fragment are connected by one or more disulfide bonds. In some embodiments, the number of disulfide bonds between the first constant region of heavy chain (CH1) and the light constant (CL) of the Fab is at least one, such as 2, 3, 4, or more. In some embodiments, cysteine residues are engineered in the Fab fragment (such as in the CH1 and CL regions) to introduce disulfide bonds.

[190] In some embodiments, the Fab fragment of the MSFP does not comprise a disulfide bond. For example, the heavy and light chains may be engineered in such a way so as to stably interact without the need for disulfide bonds. In some embodiments, the heavy chain or light chain can be engineered to remove a cysteine residue, and wherein the heavy and light chains still stably interact and function as a Fab. In some embodiments, mutations are made to facilitate stable interactions between the heavy and light chains. For example, a “knobs into holes” engineering strategy can be used to facilitate dimerization between the heavy and light chains of a Fab (see *e.g.*, 1996 Protein Engineering, 9:617 - 621). Also contemplated for use herein are variant Fab fragments designed for a particular purpose, for example, amino acid changes in the constant domains of CH1 and/or CL, and removal of a disulfide bond or addition of tags for purification, *etc.*

[191] In some embodiments, the configuration of the variable and constant regions within the Fab fragment may be different from what is found in a native Fab. In some embodiments, the orientation of the variable and constant regions may be VH-CL in one chain, and VL-CH1 in another chain (see, for example, Shaefer *et al.* (2011), PNAS, 108:111870-92).

[192] In some embodiments, the Fab fragments of the MSFP are derived from monoclonal antibodies. Suitable monoclonal antibodies may be of any type, including IgA, IgM, IgD, IgG, IgE and subtypes thereof, such as IgG1, IgG2, IgG3, and IgG4. The light chain domains may be derived from the kappa or lambda chain. In some embodiments, the Fab fragment is designed recombinantly.

[193] In some embodiments, the Fab fragment comprises a human immunoglobulin CH1. In some embodiments, the human immunoglobulin CH1 comprises the amino acid sequence of SEQ ID NO: 9. In some embodiment, the Fab fragment comprises a human lambda light chain constant region. In one embodiment, the human lambda light chain constant region comprises the amino acid sequence of SEQ ID NO:10.

SEQ ID NO: 9 (human CH1)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS

SEQ ID NO: 10 (human lambda CL)

GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPS
KQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTE

[194] The Fab fragment of the MSFP specifically binds to CD3 via an antigen-binding site formed between the heavy chain variable region and the light chain variable region (VH and VL). The antigen-binding site comprises at least one (such as 1, 2, or 3) HVR of an immunoglobulin heavy chain and/or at least one (such as 1, 2, or 3) HVR of an immunoglobulin light chain. In some embodiments, the MSFP comprises 1, 2, 3, 4, 5, or all 6 HVRs of a VH and VL sequence of a full-length antibody that specifically binds to CD3.

[0100] In some embodiments, the Fab fragment is derived from SP34. In some embodiments, the Fab fragment is a CD3 Fab fragment described in U.S. Patent No. 8,846,042. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising one, two or three HVRs (or CDRs) from SEQ ID NO: 7, and/or a light chain variable region (VL) comprising one, two or three HVRs (or CDRs) from SEQ ID NO: 8. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising three HVRs from SEQ ID NO: 7, and/or a light chain variable region (VL) comprising three HVRs from SEQ ID NO: 8.

In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising one, two or three HVRs selected from SED ID Nos: 1-3, and/or a light chain variable region (VL) comprising one, two or three HVRs selected from SED ID NOs: 4-6. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence 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%, or at least about 99% identical to a sequence selected from the group consisting of SEQ ID NOs:7 and 39-43. In some embodiments, the Fab fragment comprises a light chain variable region (VL) comprising an amino acid sequence 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%, or at least about 99% identical to a sequence selected from the group consisting of SEQ ID NO: 8 and 44-47. In some embodiments, a V_H or VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but the Fab fragment comprising that sequence retains the ability to bind to CD3. In some embodiments, one or two amino acids have been substituted, inserted and/or deleted in any one or more of the HVRs. In some embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising an amino acid sequence selected from SEQ ID NOs: 7 and 39-43. In some embodiments, the Fab fragment comprises a light chain variable region (VL) comprising an amino acid sequence selected from SEQ ID NOs: 8 and 44-47. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 7, and/or a light chain variable region

(VL) comprising the amino acid sequence of SEQ ID NO: 8. In some embodiments, the Fab fragment comprises a heavy chain polypeptide comprising the amino acid sequence of SEQ ID NO: 11, and/or a light chain polypeptide comprising the amino acid sequence of SEQ ID NO: 12.

[195]

SEQ ID NO: 1 (CD3 HVR-H1)
TYAMN

SEQ ID NO: 2 (CD3 HVR-H2)
RIRSKYNNYATYYADSVKD

SEQ ID NO: 3 (CD3 HVR-H3)
HGNFGNSYVSWFAY

SEQ ID NO: 4 (CD3 HVR-L1)
RSSTGAVTTSNYAN

SEQ ID NO: 5 (CD3 HVR-L2)
GTNKRAP

SEQ ID NO: 6 (CD3 HVR-L3)
ALWYSNLWV

SEQ ID NO: 7 (CD3 VH)
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN
YATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAY
WGQGTMTVTVSS

SEQ ID NO: 8 (CD3 VL)
QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAP
GTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO: 11 (CD3 Heavy chain)
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN
YATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAY
WGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS

SEQ ID NO: 12 (CD3 Light chain)
QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAP
GTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGQPKAAP
SVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKY
AASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTE

SEQ ID NO: 39 (CD3 VH)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVSRIRSKYNNY
ATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARHGNFGNSYVSWFAYW
GQGTMTVTVSS

SEQ ID NO: 40 (CD3 VH)

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVSRIRSKYNN
YATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARHGNFGNSYVSWFAY
WGQGTMTVTVSS

SEQ ID NO: 41 (CD3 VH)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVARIRSKYNN
YATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARHGNFGNSYVSWFAY
WGQGTMTVTVSS

SEQ ID NO: 42 (CD3 VH)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVARIRSKYNN
YATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAY
WGQGTMTVTVSS

SEQ ID NO: 43 (CD3 VH)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQASGKGLEWVGRIRSKYNN
YATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRHGNFGNSYVSWFAY
WGQGTMTVTVSS

SEQ ID NO: 44 (CD3 VL)

QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWFQQKPGQAPRGLIGGTNKRAPG
TPARFSGSLLGGKAAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO: 45 (CD3 VL)

QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAP
WTPARFSGSLLGGKAAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO: 46 (CD3 VL)

QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWFQQKPGQAPRGLIGGTNKRAP
WTPARFSGSLLGGKAAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO: 47 (CD3 VL)

QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWYQQKPGQAPRGLIGGTNKRAP
WTPARFSGSLLGGKAAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL

[196] In some embodiments, a specific VH and/or VL of an anti-CD3 Fab fragment may be used to screen a library of the complementary variable region to identify VH/VL with desirable properties, such as increased affinity for CD3. Such methods are described, for example, in Portolano *et al.*, J. Immunol. (1993) 150:880-887; Clarkson *et al.*, Nature (1991) 352:624-628; and Klimka *et al.*, British Journal of Cancer (2000) 83:252-260; Beiboer *et al.*, J. Mol. Biol. (2000) 296:833-849; and Rader *et al.*, PNAS (1998) 95:8910-8915.

EpCAM binding domain

[197] The MSFPs described herein comprises one or two binding domains that specifically bind to EpCAM. Epithelial cell adhesion molecule (EpCAM, CD326), also known as 17-1A, ESA, AUA1, EGP40, *etc.*, is a 40 kD transmembrane glycoprotein composed of 314 amino acid.

EpCAM is involved in cell signaling, migration, proliferation, and differentiation. EpCAM is specifically expressed in various types of epithelial cells and main types of human malignancies. For example, EpCAM is highly expressed in colon cancer, lung cancer, prostate cancer, liver cancer, pancreatic cancer, breast cancer and ovarian cancer, thus can be used as diagnostic marker for various cancers. EpCAM is also a potential target for immunotherapeutic strategies, including vaccines, murinized or humanized monoclonal antibodies, and antibodies conjugated with bacterial toxins or chemotherapy drugs, such as EpCAM specific antibodies ING-1, adecatumumab, edrecolomab, *etc.*

[198] The binding domains, including the EpCAM binding domains, in the MSFP not only provide additional binding specificities and enhanced properties (*e.g.*, increased serum half-life, or activation of immune activation cascades), but also create steric hindrance to significantly reduce the binding affinity of the Fab fragment to CD3 due to fusion to the N terminus of the VH and/or VL chains. This is in direct contrast to other Fab fusion proteins, such as TRIBODIES™, which fuses additional binding domains at the C terminus of the Fab fragment (see, *e.g.*, Journal of Immunology, 2000, 165: 7050-7057). The binding domains are not intended to dimerize, unlike other known fusion proteins, such as those described in WO2008/024188 and WO2009/149185. A further distinguishing characteristic of the MSFP is that the binding domains reduce the binding affinity of the Fab to CD3 when the MSFP is not bound to cell surface targets, such as EpCAM, on tumor cells.

[199] In some embodiments, the MSFP has a prolonged *in vivo* half-life compared to the anti-CD3 Fab alone. In some embodiments, the half-life of the MSFP is at least about any of 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more times the half-life of the anti-CD3 Fab fragment alone.

[200] The binding domains, including EpCAM, may be selected from antigen-binding domains, such as scFv or scTCR, extracellular domains of receptors, ligands for cell surface molecules/receptors, or receptor binding domains thereof, and tumor binding proteins. In some embodiments, the antigen binding domain is selected from an scFv, a VH, a VL, a domain antibody variant (dAb), a camelid antibody (VHH), a fibronectin 3 domain variant, an ankyrin repeat variant, and other antigen-specific binding domains derived from other protein scaffolds.

[201] In some embodiments, the EpCAM binding domain is an scFv that specifically binds to EpCAM (also referred herein as anti-EpCAM scFv). In some embodiments, the VH and VL of

the anti-EpCAM scFv are connected to each other via a peptide linker, such as a flexible linker comprising glycines and/or serines. In some embodiments, the VH and VL of the anti-EpCAM scFv are connected to each other directly. In some embodiments, the anti-EpCAM scFv comprises an N-VH-VL-C fusion polypeptide. In some embodiments, the anti-EpCAM scFv comprises an N-VL-VH-C fusion polypeptide.

[202] The EpCAM binding domain (such as scFv) may be derived from any suitable anti-EpCAM antibody. In some embodiments, the anti-EpCAM antibody is a human, humanized, or chimeric antibody. In some embodiments, the EpCAM binding domain specifically binds to both human and non-human primates (such as cynomolgus monkey). In some embodiments, the EpCAM binding domain specifically recognizes human EpCAM, but does not have cross-reactivity to non-human primates. Exemplary anti-EpCAM antibodies are known in the art, for example, see, U.S. Patent NO. 8,884,602. The anti-EpCAM binding domain may comprise at least one (such as 1, 2, or 3) HVR of an immunoglobulin heavy chain and/or at least one (such as 1, 2, or 3) HVR of an immunoglobulin light chain. In some embodiments, the anti-EpCAM binding domain comprises 1, 2, 3, 4, 5, or all 6 HVRs of a VH and VL sequence of a full-length antibody that specifically binds to EpCAM.

[203] In some embodiments, the anti-EpCAM scFv comprises a heavy chain variable region (VH) comprising one, two or three HVRs (or CDRs) from SEQ ID NO: 19, and/or a light chain variable region (VL) comprising one, two or three HVRs (or CDRs) from SEQ ID NO: 20. In some embodiments, the anti-EpCAM scFv comprises a heavy chain variable region (VH) comprising three HVRs from SEQ ID NO: 19, and/or a light chain variable region (VL) comprising three HVRs from SEQ ID NO: 20. In some embodiments, the anti-EpCAM scFv comprises a heavy chain variable region (VH) comprising one, two or three HVRs selected from SEQ ID Nos: 13-15, and/or a light chain variable region (VL) comprising one, two or three HVRs selected from SEQ ID Nos: 16-18. In some embodiments, the anti-EpCAM scFv comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the anti-EpCAM

scFv comprises a heavy chain variable region (VH) comprising an amino acid sequence 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%, or at least about 99% identical to the sequence of SEQ ID NO:19, and/or a light chain variable region (VL) comprising an amino acid sequence 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%, or at least about 99% identical to the sequence of SEQ ID NO:20. In some embodiments, the anti-EpCAM scFv comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 19, and/or a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the anti-EpCAM scFv comprises the amino acid sequence of SEQ ID NO:21.

SEQ ID NO: 13 (EpCAM HVR-H1)
NYWMS

SEQ ID NO: 14 (EpCAM HVR-H2)
NIKQDGSEKIFYADSVKG

SEQ ID NO: 15 (EpCAM HVR-H3)
VGPSWEQDY

SEQ ID NO: 16 (EpCAM HVR-L1)
TGSSSNIGSYYGVBH

SEQ ID NO: 17 (EpCAM HVR-L2)
SDTNRPS

SEQ ID NO: 18 (EpCAM HVR-L3)
QSYDKGFGHRV

SEQ ID NO: 19 (EpCAM VH)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVANIKQDGSE
KIFYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVGPSWEQDYWGQGT
LVSA

SEQ ID NO: 20 (EpCAM VL)
GAQSVLTQPPSVSGAPGQRVTISCTGSSSNIGSYYGVBHWYQQLPGTAPKLLIYSDTNRPS
GVPDRFSGSKSGTSASLAITGLQAEDADYYCQSYDKGFGHRVFGGGTKLTVL

SEQ ID NO: 21 (EpCAM scFv)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVANIKQDGSE
KIFYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVGPSWEQDYWGQGT
TVSAGGGGSGGGGSGGGGSGAQSGLTQPPSVSGAPGQRVTISCTGSSSNIGSYYGVBHWY

QQLPGTAPKLLIYSDTNRP SGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDKGFG
 HRVFGGGTKLTVL

Linkers

[204] The MSFPs described herein may comprise a linker (such as a peptide linker) situated between the VH or the VL of the Fab fragment and the binding domains. In some embodiments, the linker between the VH of the Fab fragment and the first binding domain is the same as the linker between the VL of the Fab fragment and the first binding domain. In some embodiments, the linker between the VH of the Fab fragment and the first binding domain is different from the linker between the VL of the Fab fragment and the first binding domain. In some embodiments, the anti-EpCAM scFv comprises a linker (such as peptide linker) situated between the VH and VL of the scFv, which may be the same or different from any of the linkers between the VH and VL of the Fab fragment and the binding domains.

[205] The linkers can be peptide linkers of any length. In some embodiments, the peptide linker is from 1 amino acids to 10 amino acids long, from 2 amino acids to 15 amino acids long, from 3 amino acids to 12 amino acids long, from 4 amino acids to 10 amino acids long, from 5 amino acids to 9 amino acids long, from 6 amino acids to 8 amino acids long, or from 1 amino acids to 20 amino acids long. In some embodiments, the peptide linker is any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids long. In some embodiments, the peptide linker is any of 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 amino acids long. In some embodiments, the N-terminus of the peptide linker is covalently linked to the C-terminal of the binding domain, and the C terminus of the peptide linker is covalently linked to the N-terminus of the VH or VL of the Fab fragment.

[206] In some embodiments, the linker is a flexible linker. Exemplary flexible linkers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n, (GSGGS)_n and (GGGS)_n, where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, Rev. Computational Chem. 11 173-142 (1992)). Exemplary flexible linkers include, but are not limited to Gly-Gly, Gly-Gly-Ser-Gly (SEQ ID NO: 24), Gly- Gly-Ser-Gly-Gly (SEQ ID NO: 25), Gly-Ser-Gly-Ser-Gly (SEQ

ID NO: 26), Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 27), Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 28), Gly-Ser-Ser-Ser-Gly (SEQ ID NO: 29), and the like. In some embodiments, the linker between the VH of the anti-CD3 Fab fragment and the EpCAM binding domain (such as scFv) is Gly-Gly. In some embodiments, the linker between the VL of the anti-CD3 Fab fragment and the EpCAM binding domain (such as scFv) is Gly-Gly. The ordinarily skilled artisan will recognize that design of an MSFP can include linkers that are all or partially flexible, such that the linker can include a flexible linker portion as well as one or more portions that confer less flexible structure to provide a desired MSFP structure.

[207] In some embodiments, the linker between the Fab and the binding domains is a stable linker (not cleavable by protease, especially MMPs).

[208] In some embodiments, the linker is a cleavable linker. In some embodiments, the linker between the Fab VH or VL and the binding domain comprises a protease substrate cleavage sequence, for example, an MMP substrate cleavage sequence. A well-known peptide sequence of PLGLAG (SEQ ID NO: 30) in a substrate can be cleaved by most MMPs. Substrate sequences that can be cleaved by MMPs have been extensively studied. For example, the sequence of PLGLAG (SEQ ID NO: 30) can be cleaved by most MMPs. In some embodiments, the protease cleavage site is recognized by MMP-2, MMP-9 or a combination thereof.

[209] In some embodiments, the MSFP comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the MSFP comprises a second polypeptide comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the MSFP comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 22, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 23. Further provided are MSFPs and compositions (such as pharmaceutical compositions) thereof comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 22, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 23.

SEQ ID NO: 22

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVANIKQDGSE
KFYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARVGPSWEQDYWGQGT
TVSAGGGGSGGGGSGGGGSGAQSVLTPPPSVSGAPGQRVTISCTGSSSNIGSYYG
QQLPGTAPKLLIYSDTNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDKGFG
HRVFGGGTKLTVLGGEVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPG
KGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCV

RHGNFGNSYVSWFAYWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNT
KVDDKKVEPKSCPPCS

SEQ ID NO: 23

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVANIKQDGSE
KFYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARVGPSWEQDYWGQGTLV
TVSAGGGGSGGGGSGGGGSGAQSFLTQPPSVSGAPGQRVTISCTGSSSNIGSYYGVHWY
QQLPGTAPKLLIYSDTNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDKGFG
HRVFGGGTKLTVLGGAQAVVTQEPSTLVSPGGTVTLTCRSSTGAVTTSNYANWVQKPG
QAPRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFG
GGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPKA
GVETTTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECPPCS

III. EpCAM Antibodies

[210] Further provided by the present application are novel anti-EpCAM antibodies or antigen-binding fragments thereof, including multispecific (such as bispecific) Fab fusion proteins comprising an antigen-binding fragment derived from the anti-EpCAM antibody. Compared to anti-EpCAM antibodies and fragments known in the art, the anti-EpCAM antibodies and antigen-binding fragments thereof described herein have enhanced stability and ability to be expressed. Additionally, the anti-EpCAM antibodies and antigen-binding fragments thereof of the present invention have cross-reactivity towards EpCAM from both human and non-human primates (such as cynomolgus monkeys), which is beneficial for extrapolating results from toxicity and efficacy studies in monkeys to human clinical studies for evaluating the EpCAM antibodies or derivatives thereof (such as MSFPs).

[211] The antibodies of the present invention bind to an EpCAM epitope with an equilibrium binding constant (K_d) of $\leq 1 \mu\text{M}$, *e.g.*, $\leq 100 \text{ nM}$, preferably $\leq 10 \text{ nM}$, and more preferably $\leq 1 \text{ nM}$. For example, the anti-EpCAM antibodies provided herein exhibit a K_d in the range approximately between $\leq 1 \text{ nM}$ to about 1 pM .

[212] The anti-EpCAM antibodies of the invention serve to completely or partially modulate, block, inhibit, reduce, antagonize, neutralize or otherwise interfere with the functional activity of the widely distributed EpCAM. The EpCAM antibodies are considered to completely modulate, block, inhibit, reduce, antagonize, neutralize or otherwise interfere with EpCAM functional activity when the level of EpCAM functional activity in the presence of EpCAM antibody is decreased by at least 95%, *e.g.*, by 96%, 97%, 98%, 99% or 100% as compared to the level of EpCAM functional activity in the absence of binding with an anti-EpCAM antibody described

herein. The anti-EpCAM antibodies are considered to significantly block, inhibit, reduce, antagonize, neutralize or otherwise interfere with EpCAM functional activity when the level of EpCAM activity in the presence of the anti-EpCAM antibody is decreased by at least 50%, *e.g.*, 55%, 60%, 75%, 80%, 85% or 90% as compared to the level of EpCAM activity in the absence of binding with an anti- EpCAM antibody described herein. The anti-EpCAM antibodies are considered to partially modulate, block, inhibit, reduce, antagonize, neutralize or otherwise interfere with EpCAM functional activity when the level of EpCAM activity in the presence of the anti- EpCAM antibody is decreased by less than 95%, *e.g.*, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 75%, 80%, 85% or 90% as compared to the level of EpCAM activity in the absence of binding with an anti-EpCAM antibody described herein.

[213] In some embodiments, the anti-EpCAM antibody moiety specifically binds to an EpCAM present on the surface of a cell. In some embodiments, the cell presents on its surface abnormally high levels of EpCAM. In some embodiments, the cell is a cancer cell. In some embodiments, the cancer cell is in a solid tumor. In some embodiments, the cancer cell is a metastatic cancer cell.

[214] The anti-EpCAM antibody moieties in some embodiments comprise specific sequences or certain variants of such sequences. In some embodiments, the amino acid substitutions in the variant sequences do not substantially reduce the ability of the anti-EpCAM antibody moiety to bind the EpCAM. For example, alterations that do not substantially reduce EpCAM binding affinity may be made. Alterations that substantially improve EpCAM binding affinity or affect some other property, such as specificity, immunogenicity, ADCC or CDC, and/or cross-reactivity with related variants of the EpCAM, are also contemplated.

[215] In some embodiments, the anti-EpCAM antibody or antigen-binding fragment thereof (such as an scFv) comprising a heavy chain variable region (VH) comprising one, two or three HVRs (or CDRs) from SEQ ID NO: 19, and/or a light chain variable region (VL) comprising one, two or three HVRs (or CDRs) from SEQ ID NO: 20. In some embodiments, the anti-EpCAM antibody or antigen-binding fragment thereof (such as an scFv) comprises a heavy chain variable region (VH) comprising three HVRs from SEQ ID NO: 19, and/or a light chain variable region (VL) comprising three HVRs from SEQ ID NO: 20.

[216] In some embodiments, the anti-EpCAM antibody or antigen-binding fragment thereof (such as an scFv) comprises a heavy chain variable region (VH) comprising a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18.

[217] In some embodiments, the anti-EpCAM antibody or antigen-binding fragment comprises a VH comprising: (1) a HVR-H1 having one or two amino acid substitutions of SEQ ID NO: 13; (2) a HVR-H2 having one or two amino acid substitutions of SEQ ID NO: 14; and/or (3) a HVR-H3 having one or two amino acid substitutions of SEQ ID NO: 15. In some embodiments, the anti-EpCAM antibody or antigen-binding fragment comprises a VL comprising: (1) a HVR-L1 having one or two amino acid substitutions of SEQ ID NO: 16; (2) a HVR-L2 having one or two amino acid substitutions of SEQ ID NO: 17; and/or (3) a HVR-L3 having one or two amino acid substitutions of SEQ ID NO: 18.

[218] In some embodiments, the anti-EpCAM antibody or antigen-binding fragment thereof (such as an scFv) comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18.

[219] In some embodiments, the anti-EpCAM antibody or antigen-binding fragment thereof (such as an scFv) comprises a heavy chain variable region (VH) comprising an amino acid sequence 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%, or at least about 99% identical to the sequence of SEQ ID NO:19, and/or a light chain variable region (VL) comprising an amino acid sequence 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%, or at least about 99% identical to the sequence of SEQ ID NO:20. In some embodiments, a V_H or VL sequence having at least 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but the Fab fragment comprising that sequence retains the ability to bind to EpCAM. In some embodiments, one or two amino acids have been substituted, inserted and/or deleted in any one or more of the HVRs. In some embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). In some embodiments, the anti-EpCAM antibody or antigen-binding fragment thereof (such as an scFv) comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 19, and/or a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 20.

[220] In some embodiments, the anti-EpCAM antibody is a full-length antibody. In some embodiments, the full-length anti-EpCAM antibody comprises an Fc sequence from an immunoglobulin, such as IgA, IgD, IgE, IgG, and IgM. In some embodiments, the full-length anti-EpCAM antibody comprises an Fc sequence of IgG, such as any of IgG1, IgG2, IgG3, or IgG4. In some embodiments, the full-length anti-EpCAM antibody comprises an Fc sequence of a human immunoglobulin. In some embodiments, the full-length anti-EpCAM antibody comprises an Fc sequence that has been altered or otherwise changed so that it has enhanced antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) effector function.

[221] Also provided is an isolated antibody or an antigen-binding fragment thereof which competes with any of the anti-EpCAM antibodies described herein for binding with EpCAM. In some embodiments, there is provided an isolated antibody or an antigen-binding fragment thereof which binds to the same epitope as any of the anti-EpCAM antibodies described herein. Methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme linked immunosorbent assay (ELISA) and other immunologically mediated techniques known within the art.

[222] Those skilled in the art will recognize that it is possible to determine, without undue experimentation, if a monoclonal antibody has the same specificity as a monoclonal antibody of the invention (*e.g.*, the anti-EpCAM antibody having a variable heavy chain of SEQ ID NO:19, and a variable light chain of SEQ ID NO:20) by ascertaining whether the former prevents the latter from binding to EpCAM. If the monoclonal antibody being tested competes with the

monoclonal antibody of the invention, as shown by a decrease in binding by the monoclonal antibody of the invention, then the two monoclonal antibodies bind to the same, or a closely related, epitope.

[223] An alternative method for determining whether a monoclonal antibody has the specificity of monoclonal antibody of the invention is to pre-incubate the monoclonal antibody of the invention with soluble EpCAM protein (with which it is normally reactive), and then add the monoclonal antibody being tested to determine if the monoclonal antibody being tested is inhibited in its ability to bind EpCAM. If the monoclonal antibody being tested is inhibited then, in all likelihood, it has the same, or functionally equivalent, epitopic specificity as the monoclonal antibody of the invention.

[224] In some embodiments, the anti-EpCAM antibody is a monoclonal antibody, such as a monovalent antibody. In some embodiments, the anti-EpCAM antigen-binding fragment is in the form of a Fab, Fab', a F(ab)'2, single-chain Fv (scFv), an Fv fragment, a diabody, or a linear antibody.

[225] In some embodiments, there is provided an anti-EpCAM scFv comprising a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the anti-EpCAM scFv comprises a VH comprising the amino acid sequence of SEQ ID NO: 19. In some embodiments, the anti-EpCAM scFv comprises a VL comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the anti-EpCAM scFv comprises the amino acid sequence of SEQ ID NO: 21.

[226] In some embodiments, the anti-EpCAM antibody is a multispecific antibody that binds to EpCAM, but also binds one or more other targets and optionally inhibits their function. Multispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for two or more different antigens (*e.g.*, bispecific antibodies have binding specificities for at least two antigens). For example, one of the binding specificities can be for the EpCAM protein, the other one can be for any other antigen. In some embodiments, the other

antigen is a cell-surface protein or receptor or receptor subunit. For example, the cell-surface protein can be CD3, such as CD3 epsilon. Thus, according to one embodiment, a bispecific antibody of this invention can bind both EpCAM and, *e.g.*, a second cell surface receptor.

[227] In some embodiments, the multi-specific anti-EpCAM molecule is, for example, a diabody (Db), a single-chain diabody (scDb), a tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), a circular dimeric scDb (CD-scDb), a di-diabody, a tandem scFv, a tandem di-scFv (*e.g.*, a bispecific T cell engager), a tandem tri-scFv, a tri(a)body, a bispecific Fab₂, a di-miniantibody, a tetrabody, an scFv-Fc-scFv fusion, a dual-affinity retargeting (DART) antibody, a dual variable domain (DVD) antibody, an IgG-scFab, an scFab-ds-scFv, an Fv2-Fc, an IgG-scFv fusion, a dock and lock (DNL) antibody, a knob-into-hole (KiH) antibody (bispecific IgG prepared by the KiH technology), a DuoBody (bispecific IgG prepared by the Duobody technology), a heteromultimeric antibody, or a heteroconjugate antibody. In some embodiments, the multi-specific anti-EpCAM molecule is a tandem scFv (*e.g.*, a tandem di-scFv, such as a bispecific T cell engager).

[228] Further provided are fusion proteins, conjugates, or isolated cells comprising any of the anti-EpCAM antibodies or antigen-binding fragments thereof described above.

[229] In some embodiments, there is provided a multispecific (such as bispecific) Fab fusion protein comprising an anti-EpCAM antigen-binding fragment comprising a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18.

[230] In some embodiments, the multispecific Fab fusion protein comprises a single anti-EpCAM antigen-binding fragment, wherein the anti-EpCAM antigen-binding fragment is fused to the N-terminus of the heavy chain polypeptide of the Fab fragment or the light chain polypeptide of the Fab fragment. In some embodiments, the multispecific Fab fusion protein comprises two anti-EpCAM antigen-binding fragment or two copies of anti-EpCAM antigen-binding fragments.

[231] In some embodiments, there is provided a multispecific (such as bispecific) Fab fusion protein comprising a Fab fragment and an anti-EpCAM antigen-binding fragment, wherein the N-terminus of the heavy chain polypeptide or the N-terminus of the light chain polypeptide of the Fab fragment is fused to the anti-EpCAM antigen-binding fragment, and wherein the anti-EpCAM antigen-binding fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18. In some embodiments, the multispecific Fab fusion protein comprises a Fab fragment, a first anti-EpCAM antigen-binding fragment, and a second anti-EpCAM antigen-binding fragment, wherein the N-terminus of the heavy chain polypeptide of the Fab fragment is fused to the first anti-EpCAM antigen-binding fragment, and wherein the N-terminus of the light chain polypeptide of the Fab fragment is fused to the second anti-EpCAM antigen-binding fragment. In some embodiments, the first anti-EpCAM antigen-binding fragment has the same sequence as the second anti-EpCAM antigen-binding fragment. In some embodiments, the anti-EpCAM antigen-binding fragment comprises a VH comprising the amino acid sequence of SEQ ID NO: 19. In some embodiments, the anti-EpCAM antigen-binding fragment comprises a VL comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the anti-EpCAM antigen-binding fragment is an scFv, such as an scFv comprising the amino acid sequence of SEQ ID NO: 21.

[232] The multispecific Fab fusion proteins comprising one or more anti-EpCAM antigen-binding fragments described herein may further comprise one or more features of the multispecific Fab fusion proteins described in subsection “Multispecific Fab fusion protein” of section II “Methods of treating cancer” described above.

[233] In some embodiments, the multispecific Fab fusion protein comprises a Fab fragment that specifically binds to an immune effector molecule. In some embodiments, the Fab fragment binds to a T cell receptor. In some embodiments, the Fab fragment binds to CD3 ϵ chain. In some embodiments, the Fab fragment binds to a cell surface target selected from Fc γ RI, Fc γ RIIa,

FcγRIIb, FcγRIIIa, FcγRIIIa, NKG2D, CD25, CD28, CD137, CTLA-4, FAS, FGFR1, FGFR2, FGFR3, FGFR4, GITR, LTβR, TLR, TRAIL receptor 1, TRAIL receptor 2, EGFR, Her2/neu, and ErbB3.

[234] In some embodiments, the Fab fragment specifically binds to CD3, such as the N-terminus of CD3 epsilon, for example, the N-terminal 1-27 amino acids of CD3 epsilon. In some embodiments, the Fab fragment comprises a heavy chain variable region (VH) comprising: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and/or a light chain variable region (VL) comprising: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the Fab fragment comprises a VH comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:7 and 39-43, and/or a VL comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 44-47. In some of the embodiments, the multispecific Fab fusion protein comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO:22, and a second polypeptide comprising the amino acid sequence of SEQ ID NO:23.

[235] In some embodiments, the anti-EpCAM antibody or antigen-binding fragment thereof is conjugated to a therapeutic agent (*e.g.*, cytotoxic agent, a radioisotope and a chemotherapeutic agent) or a label for detecting EpCAM in patient samples or *in vivo* by imaging (*e.g.*, radioisotope, fluorescent dye and enzyme). In some embodiments, the anti-EpCAM antibody or antigen-binding fragment thereof is conjugated to a toxin.

[236] In some embodiments, there is provided an anti-EpCAM chimeric antigen receptor (CAR) comprising: a) an extracellular domain comprising any of the anti-EpCAM antibodies or antigen-binding fragments described herein; and b) an intracellular signaling domain. A transmembrane domain may be present between the extracellular domain and the intracellular domain. Between the extracellular domain and the transmembrane domain of the anti-EpCAM CAR, or between the intracellular domain and the transmembrane domain of the anti-EpCAM CAR, there may be a spacer domain, such as peptide linker (*e.g.*, a flexible peptide linker). Examples of intracellular signaling domains for use in the anti-EpCAM CAR of the invention

include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability. Any method for producing a CAR may be used herein. *See*, for example, US6,410,319, US7,446, 191, US7,514,537, WO 2002/077029, US2010/065818, US 2010/025177, US 2007/059298, and Berger C. *et al.*, J. Clinical Investigation 118: 1 294-308 (2008).

[237] In some embodiments, there is provided an anti-EpCAM recombinant T cell receptor (TCR) comprising an extracellular domain comprising any of the anti-EpCAM antibodies or antigen-binding fragments described herein. Methods of engineering TCRs have been described, for example, in see Stone J.D. *et al.* T Cell receptor engineering, Methods Enzymol. (2012) 503:189-222.

[238] Also provided are isolated cells expressing the anti-EpCAM CAR or TCR, such as CAR-T or TCR-T cells, and methods of treating a disease (such as cancer) using the anti-EpCAM CAR or TCR, or cells expressing the anti-EpCAM or TCR thereof.

[239] The anti-EpCAM antibodies or antigen-binding fragments described herein can be used in a variety of therapeutic and diagnostic methods. Further provided are methods of treating cancer in an individual, comprising administering an effective amount of the anti-EpCAM antibody or antigen-binding fragment thereof described above or pharmaceutical compositions thereof to the individual. For example, the anti-EpCAM antibodies (or antigen-binding fragments thereof) can be used alone or in combination with other agents in treating a disease characterized by abnormal EpCAM expression, including, but not limited to, head and neck cancer, pancreatic cancer, colorectal cancer, and lung cancer. The antibodies provided herein can also be used for detecting EpCAM protein in patients or patient samples.

Monoclonal antibodies

[240] Screening of monoclonal antibodies of the invention, can be also carried out, *e.g.*, by measuring EpCAM-mediated signaling, and determining whether the test monoclonal antibody is able to modulate, block, inhibit, reduce, antagonize, neutralize or otherwise interfere with EpCAM-mediated signaling. These assays can include competitive binding assays. Additionally, these assays can measure a biologic readout.

[241] Various procedures known within the art may be used for the production of monoclonal antibodies directed against EpCAM, or against derivatives, fragments, analogs homologs or orthologs thereof. (*See, for example*, Antibodies: A Laboratory Manual, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Fully human antibodies are antibody molecules in which the entire sequence of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed “human antibodies” or “fully human antibodies” herein. Human monoclonal antibodies are prepared, for example, using the procedures described in the Examples provided below. Human monoclonal antibodies can be also prepared by using the trioma technique; the human B-cell hybridoma technique (*see* Kozbor, et al., 1983 Immunol Today 4: 72); and the EBV hybridoma technique to produce human monoclonal antibodies (*see* Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized and may be produced by using human hybridomas (*see* Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*see* Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

[242] Antibodies are purified by well-known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

[243] The EpCAM antibodies of the invention are monoclonal antibodies. Monoclonal antibodies that modulate, block, inhibit, reduce, antagonize, neutralize or otherwise interfere with EpCAM-mediated cell signaling are generated, *e.g.*, by immunizing an animal with membrane bound and/or soluble EpCAM, such as, for example, human EpCAM or an immunogenic fragment, derivative or variant thereof. Alternatively, the animal is immunized with cells transfected with a vector containing a nucleic acid molecule encoding EpCAM such that EpCAM is expressed and associated with the surface of the transfected cells. Alternatively, the antibodies are obtained by screening a library that contains antibody or antigen binding domain

sequences for binding to EpCAM. This library is prepared, *e.g.*, in bacteriophage as protein or peptide fusions to a bacteriophage coat protein that is expressed on the surface of assembled phage particles and the encoding DNA sequences contained within the phage particles (*i.e.*, “phage displayed library”). Hybridomas resulting from myeloma/B cell fusions are then screened for reactivity to EpCAM.

[244] Monoclonal antibodies are prepared, for example, using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized *in vitro*.

[245] The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (“HAT medium”), which substances prevent the growth of HGPRT-deficient cells.

[246] Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the

production of monoclonal antibodies. (See Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63)).

[247] The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980). Moreover, in therapeutic applications of monoclonal antibodies, it is important to identify antibodies having a high degree of specificity and a high binding affinity for the target antigen.

[248] After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods. (See Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown *in vivo* as ascites in a mammal.

[249] The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[250] Monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as Chinese hamster ovary (CHO) cells, Human Embryonic Kidney (HEK) 293 cells, simian COS cells, PER.C6, NS0 cells, SP2/0, YB2/0, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of

monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (*see* U.S. Patent No. 4,816,567; Morrison, Nature 368, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

Human Antibodies and Humanization of Antibodies

[251] Monoclonal antibodies of the invention include fully human antibodies or humanized antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin.

[252] An anti-EpCAM antibody can be generated using any procedures known in the art. For example, anti-EpCAM antibodies can be identified using a modified RIMMS (Repetitive Immunization Multiple Sites) immunization strategy in mice and subsequent hybridoma generation. In other, alternative methods, an anti-EpCAM antibody is developed, for example, using phage-display methods using antibodies containing only human sequences. Such approaches are well-known in the art, *e.g.*, in WO92/01047 and U.S. Pat. No. 6,521,404, which are hereby incorporated by reference. In this approach, a combinatorial library of phage carrying random pairs of light and heavy chains are screened using natural or recombinant source of EpCAM or fragments thereof. In another approach, an anti-EpCAM antibody can be produced by a process wherein at least one step of the process includes immunizing a transgenic, non-human animal with human EpCAM protein. In this approach, some of the endogenous heavy and/or kappa light chain loci of this xenogenic non-human animal have been disabled and are incapable of the rearrangement required to generate genes encoding immunoglobulins in response to an antigen. In addition, at least one human heavy chain locus and at least one human light chain locus have been stably transfected into the animal. Thus, in response to an administered antigen, the human loci rearrange to provide genes encoding human variable regions immunospecific for the antigen. Upon immunization, therefore, the xenomouse produces B-cells that secrete fully human immunoglobulins.

[253] A variety of techniques are well-known in the art for producing xenogenic non-human animals. For example, see U.S. Pat. No. 6,075,181 and No. 6,150,584, which is hereby incorporated by reference in its entirety. This general strategy was demonstrated in connection with generation of the first XenoMouse™ strains as published in 1994. *See* Green *et al.* Nature Genetics 7:13-21 (1994), which is hereby incorporated by reference in its entirety. *See also*, U.S. Patent Nos. 6,162,963; 6,150,584; 6,114,598; 6,075,181; and 5,939,598 and Japanese Patent Nos. 3 068 180 B2, 3 068 506 B2, and 3 068 507 B2 and European Patent No., EP 0 463 151 B1 and International Patent Applications No. WO 94/02602, WO 96/34096, WO 98/24893, WO 00/76310 and related family members.

[254] In an alternative approach, others have utilized a “minilocus” approach in which an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. *See e.g.*, U.S. Patent Nos. 5,545,806; 5,545,807; 5,591,669; 5,612,205; 5,625,825; 5,625,126; 5,633,425; 5,643,763; 5,661,016; 5,721,367; 5,770,429; 5,789,215; 5,789,650; 5,814,318; 5,877,397; 5,874,299; 6,023,010; and 6,255,458; and European Patent No. 0 546 073 B1; and International Patent Application Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884 and related family members.

[255] Generation of human antibodies from mice in which, through microcell fusion, large pieces of chromosomes, or entire chromosomes, have been introduced, has also been demonstrated. *See* European Patent Application Nos. 773 288 and 843 961.

[256] Human anti-mouse antibody (HAMA) responses have led the industry to prepare chimeric or otherwise humanized antibodies. While chimeric antibodies have a human constant region and an immune variable region, it is expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody. Thus, it would be desirable to provide fully human antibodies against EpCAM in order to vitiate or otherwise mitigate concerns and/or effects of HAMA or HACA response.

[257] The production of antibodies with reduced immunogenicity is also accomplished via humanization, chimerization and display techniques using appropriate libraries. It will be

appreciated that murine antibodies or antibodies from other species can be humanized or primatized using techniques well known in the art. *See e.g.*, Winter and Harris *Immunol Today* 14:43-46 (1993) and Wright *et al.* *Crit. Reviews in Immunol.* 12:125-168 (1992). The antibody of interest may be engineered by recombinant DNA techniques to substitute the CH1, CH2, CH3, hinge domains, and/or the framework domain with the corresponding human sequence (*See* WO 92/102190 and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,761; 5,693,792; 5,714,350; and 5,777,085). Also, the use of Ig cDNA for construction of chimeric immunoglobulin genes is known in the art (Liu *et al.* *P.N.A.S.* 84:3439 (1987) and *J. Immunol.* 139:3521 (1987)). mRNA is isolated from a hybridoma or other cell producing the antibody and used to produce cDNA. The cDNA of interest may be amplified by the polymerase chain reaction using specific primers (U.S. Pat. Nos. 4,683,195 and 4,683,202). Alternatively, a library is made and screened to isolate the sequence of interest. The DNA sequence encoding the variable region of the antibody is then fused to human constant region sequences. The sequences of human constant region genes may be found in Kabat *et al.* (1991) *Sequences of Proteins of Immunological Interest*, N.I.H. publication no. 91-3242. Human C region genes are readily available from known clones. The choice of isotype will be guided by the desired effector functions, such as complement fixation, or activity in antibody-dependent cellular cytotoxicity. Preferred isotypes are IgG1, IgG2, IgG3, and IgG4. Either of the human light chain constant regions, kappa or lambda, may be used. The chimeric, humanized antibody is then expressed by conventional methods.

[258] Antibody fragments, such as Fv, F(ab')₂ and Fab may be prepared by cleavage of the intact protein, *e.g.*, by protease or chemical cleavage. Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the F(ab')₂ fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

[259] Consensus sequences of H and L J regions may be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

[260] Expression vectors include plasmids, retroviruses, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL

immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter, including retroviral LTRs, *e.g.*, SV-40 early promoter, (Okayama *et al.* Mol. Cell. Bio. 3:280 (1983)), Rous sarcoma virus LTR (Gorman *et al.* P.N.A.S. 79:6777 (1982)), and moloney murine leukemia virus LTR (Grosschedl *et al.* Cell 41:885 (1985)). Also, as will be appreciated, native Ig promoters and the like may be used.

[261] Further, human antibodies or antibodies from other species can be generated through display type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules can be subjected to additional maturation, such as affinity maturation, as such techniques are well known in the art. Wright *et al.* Crit. Reviews in Immunol. 12:125-168 (1992), Hanes and Plückthun PNAS USA 94:4937-4942 (1997) (ribosomal display), Parmley and Smith Gene 73:305-318 (1988) (phage display), Scott, TIBS, vol. 17:241-245 (1992), Cwirla *et al.* PNAS USA 87:6378-6382 (1990), Russel *et al.* Nucl. Acids Research 21:1081-1085 (1993), Hoganboom *et al.* Immunol. Reviews 130:43-68 (1992), Chiswell and McCafferty TIBTECH; 10:80-8A (1992), and U.S. Patent No. 5,733,743. If display technologies are utilized to produce antibodies that are not human, such antibodies can be humanized as described above.

[262] Using these techniques, antibodies can be generated to EpCAM expressing cells, soluble forms of EpCAM, epitopes or peptides thereof, and expression libraries thereto (*See e.g.*, U.S. Patent No. 5,703,057) which can thereafter be screened as described above for the activities described herein.

[263] The EpCAM antibodies of the invention can be expressed by a vector containing a DNA segment encoding the single chain antibody described above.

[264] These can include vectors, liposomes, naked DNA, adjuvant-assisted DNA, gene gun, catheters, *etc.* Vectors include chemical conjugates such as described in WO 93/64701, which has targeting moiety (*e.g.* a ligand to a cellular surface receptor), and a nucleic acid binding

moiety (*e.g.* polylysine), viral vector (*e.g.* a DNA or RNA viral vector), fusion proteins such as described in PCT/US95/02140 (WO 95/22618) which is a fusion protein containing a target moiety (*e.g.* an antibody specific for a target cell) and a nucleic acid binding moiety (*e.g.* a protamine), plasmids, phage, etc. The vectors can be chromosomal, non-chromosomal or synthetic.

[265] Preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include moloney murine leukemia viruses. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as a herpes simplex I virus (HSV) vector (*see* Geller, A. I. et al., J. Neurochem, 64:487 (1995); Lim, F., et al., in DNA Cloning: Mammalian Systems, D. Glover, Ed. (Oxford Univ. Press, Oxford England) (1995); Geller, A. I. et al., Proc Natl. Acad. Sci.: U.S.A. 90:7603 (1993); Geller, A. I., et al., Proc Natl. Acad. Sci USA 87:1149 (1990), Adenovirus Vectors (*see* LeGal LaSalle et al., Science, 259:988 (1993); Davidson, et al., Nat. Genet 3:219 (1993); Yang, et al., J. Virol. 69:2004 (1995) and Adeno-associated Virus Vectors (*see* Kaplitt, M. G., et al., Nat. Genet. 8:148 (1994)).

[266] Pox viral vectors introduce the gene into the cells cytoplasm. Avipox virus vectors result in only a short term expression of the nucleic acid. Adenovirus vectors, adeno-associated virus vectors and herpes simplex virus (HSV) vectors are preferred for introducing the nucleic acid into neural cells. The adenovirus vector results in a shorter term expression (about 2 months) than adeno-associated virus (about 4 months), which in turn is shorter than HSV vectors. The particular vector chosen will depend upon the target cell and the condition being treated. The introduction can be by standard techniques, *e.g.* infection, transfection, transduction or transformation. Examples of modes of gene transfer include *e.g.*, naked DNA, CaPO₄ precipitation, DEAE dextran, electroporation, protoplast fusion, lipofection, cell microinjection, and viral vectors.

[267] The vector can be employed to target essentially any desired target cell. For example, stereotaxic injection can be used to direct the vectors (*e.g.* adenovirus, HSV) to a desired location. Additionally, the particles can be delivered by intracerebroventricular (icv) infusion using a minipump infusion system, such as a SynchroMed Infusion System. A method based on bulk flow, termed convection, has also proven effective at delivering large molecules to extended

areas of the brain and may be useful in delivering the vector to the target cell. (*See* Bobo et al., Proc. Natl. Acad. Sci. USA 91:2076-2080 (1994); Morrison et al., Am. J. Physiol. 266:292-305 (1994)). Other methods that can be used include catheters, intravenous, parenteral, intraperitoneal and subcutaneous injection, and oral or other known routes of administration.

[268] These vectors can be used to express large quantities of antibodies that can be used in a variety of ways. For example, to detect the presence of EpCAM in a sample. The antibody can also be used to try to bind to and disrupt EpCAM-mediated signaling.

[269] Techniques can be adapted for the production of single-chain antibodies specific to an antigenic protein of the invention (*see e.g.*, U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of Fab expression libraries (*see e.g.*, Huse, et al., 1989 Science 246: 1275-1281) to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an F(ab')₂ fragment produced by pepsin digestion of an antibody molecule; (ii) an Fab fragment generated by reducing the disulfide bridges of an F(ab')₂ fragment; (iii) an Fab fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

[270] The invention also includes F_v, Fab, Fab' and F(ab')₂ anti-EpCAM fragments, single chain EpCAM antibodies, single domain antibodies (*e.g.*, nanobodies or VHHs), multispecific (such as bispecific) anti-EpCAM antibodies, and heteroconjugate anti-EpCAM antibodies.

[271] Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

[272] Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 121:210 (1986).

[273] According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory “cavities” of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[274] Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* F(ab')₂ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., *Science* 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[275] Additionally, Fab' fragments can be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody.

[276] Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., *J. Immunol.* 152:5368 (1994).

[277] Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., *J. Immunol.* 147:60 (1991).

[278] Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a

radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

[279] Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (*see* U.S. Patent No. 4,676,980), and for treatment of HIV infection (*see* WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

[280] It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating diseases and disorders associated with aberrant EpCAM signaling. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). (*See* Caron et al., J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992)). Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. (*See* Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989)).

[281] The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

[282] Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolacca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are

available for the production of radioconjugated antibodies. Examples include ^{212}Bi , ^{131}I , ^{131}In , ^{90}Y , and ^{186}Re .

[283] Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. (See WO94/11026).

[284] Those of ordinary skill in the art will recognize that a large variety of possible moieties can be coupled to the resultant antibodies of the invention. (See, for example, "Conjugate Vaccines", Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr (eds), Carger Press, New York, (1989), the entire contents of which are incorporated herein by reference).

[285] Coupling may be accomplished by any chemical reaction that will bind the two molecules so long as the antibody and the other moiety retain their respective activities. This linkage can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. The preferred binding is, however, covalent binding. Covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as the antibodies of the present invention, to other molecules. For example, representative coupling agents can include organic compounds such as thioesters, carbodiimides, succinimide esters, diisocyanates, glutaraldehyde, diazobenzenes and hexamethylene diamines. This listing is not intended to be exhaustive of the various classes of coupling agents known in the art but, rather, is exemplary of the more common

coupling agents. (See Killen and Lindstrom, Jour. Immun. 133:1335-2549 (1984); Jansen et al., Immunological Reviews 62:185-216 (1982); and Vitetta et al., Science 238:1098 (1987).

[286] Preferred linkers are described in the literature. (See, for example, Ramakrishnan, S. et al., Cancer Res. 44:201-208 (1984) describing use of MBS (M-maleimidobenzoyl-N-hydroxysuccinimide ester). See also, U.S. Patent No. 5,030,719, describing use of halogenated acetyl hydrazide derivative coupled to an antibody by way of an oligopeptide linker. Particularly preferred linkers include: (i) EDC (1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride; (ii) SMPT (4-succinimidylloxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)-toluene (Pierce Chem. Co., Cat. (21558G); (iii) SPDP (succinimidyl-6 [3-(2-pyridyldithio) propionamido]hexanoate (Pierce Chem. Co., Cat #21651G); (iv) Sulfo-LC-SPDP (sulfosuccinimidyl 6 [3-(2-pyridyldithio)-propionamide] hexanoate (Pierce Chem. Co. Cat. #2165-G); and (v) sulfo-NHS (N-hydroxysulfo-succinimide: Pierce Chem. Co., Cat. #24510) conjugated to EDC.

[287] The linkers described above contain components that have different attributes, thus leading to conjugates with differing physio-chemical properties. For example, sulfo-NHS esters of alkyl carboxylates are more stable than sulfo-NHS esters of aromatic carboxylates. NHS-ester containing linkers are less soluble than sulfo-NHS esters. Further, the linker SMPT contains a sterically hindered disulfide bond, and can form conjugates with increased stability. Disulfide linkages, are in general, less stable than other linkages because the disulfide linkage is cleaved *in vitro*, resulting in less conjugate available. Sulfo-NHS, in particular, can enhance the stability of carbodimide couplings. Carbodimide couplings (such as EDC) when used in conjunction with sulfo-NHS, forms esters that are more resistant to hydrolysis than the carbodimide coupling reaction alone.

[288] The antibodies disclosed herein can also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

[289] Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-

derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin et al., J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction.

Use of antibodies against EpCAM

[290] It will be appreciated that administration of therapeutic entities in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. *See also* Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." Int. J. Pharm. 203(1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J Pharm Sci. 89(8):967-78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" PDA J Pharm Sci Technol. 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[291] In one embodiment, antibodies of the invention, which include a monoclonal antibody of the invention, may be used as therapeutic agents. Such agents will generally be employed to diagnose, prognose, monitor, treat, alleviate, and/or prevent a disease or pathology associated with aberrant EpCAM expression, activity and/or signaling in a subject. A therapeutic regimen

is carried out by identifying a subject, *e.g.*, a human patient suffering from (or at risk of developing) a disease or disorder associated with aberrant EpCAM expression, activity and/or signaling, *e.g.*, a cancer or other neoplastic disorder, using standard methods. An antibody preparation, preferably one having high specificity and high affinity for its target antigen, is administered to the subject and will generally have an effect due to its binding with the target. Administration of the antibody may abrogate or inhibit or interfere with the expression, activity and/or signaling function of the target (*e.g.*, EpCAM). Administration of the antibody may abrogate or inhibit or interfere with the binding of the target (*e.g.*, EpCAM) with an endogenous ligand to which it naturally binds. For example, the antibody binds to the target and modulates, blocks, inhibits, reduces, antagonizes, neutralizes, or otherwise interferes with EpCAM expression, activity and/or signaling.

[292] Diseases or disorders related to aberrant EpCAM expression, activity and/or signaling include, by way of non-limiting example, hematological cancer and/or solid tumors.

Hematological cancers include, *e.g.*, leukemia, lymphoma and myeloma. Certain forms of leukemia include, by way of non-limiting example, acute lymphocytic leukemia (ALL); acute myeloid leukemia (AML); chronic lymphocytic leukemia (CLL); chronic myelogenous leukemia (CML); Myeloproliferative disorder/neoplasm (MPDS); and myelodysplasia syndrome. Certain forms of lymphoma include, by way of non-limiting example, Hodgkin's lymphoma, both indolent and aggressive non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell). Certain forms of myeloma include, by way of non-limiting example, multiple myeloma (MM), giant cell myeloma, heavy-chain myeloma, and light chain or Bence-Jones myeloma. Solid tumors include, *e.g.*, breast tumors, ovarian tumors, lung tumors, pancreatic tumors, prostate tumors, melanoma tumors, colorectal tumors, lung tumors, head and neck tumors, bladder tumors, esophageal tumors, liver tumors, and kidney tumors.

[293] Symptoms associated with cancers and other neoplastic disorders include, for example, inflammation, fever, general malaise, fever, pain, often localized to the inflamed area, loss of appetite, weight loss, edema, headache, fatigue, rash, anemia, muscle weakness, muscle fatigue and abdominal symptoms such as, for example, abdominal pain, diarrhea or constipation.

[294] A therapeutically effective amount of an antibody of the invention relates generally to the amount needed to achieve a therapeutic objective. As noted above, this may be a binding

interaction between the antibody and its target antigen that, in certain cases, interferes with the functioning of the target. The amount required to be administered will furthermore depend on the binding affinity of the antibody for its specific antigen, and will also depend on the rate at which an administered antibody is depleted from the free volume other subject to which it is administered. Common ranges for therapeutically effective dosing of an antibody or antibody fragment of the invention may be, by way of nonlimiting example, from about 0.1 mg/kg body weight to about 100 mg/kg body weight. Common dosing frequencies may range, for example, from twice daily to once a week.

[295] Efficaciousness of treatment is determined in association with any known method for diagnosing or treating the particular inflammatory-related disorder. Alleviation of one or more symptoms of the inflammatory-related disorder indicates that the antibody confers a clinical benefit.

[296] In another embodiment, antibodies directed against EpCAM may be used in methods known within the art relating to the localization and/or quantitation of EpCAM (*e.g.*, for use in measuring levels of EpCAM within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies specific to EpCAM, or derivative, fragment, analog or homolog thereof, that contain the antibody derived antigen binding domain, are utilized as pharmacologically active compounds (referred to hereinafter as “Therapeutics”).

[297] In another embodiment, an antibody specific for EpCAM can be used to isolate a EpCAM polypeptide, by standard techniques, such as immunoaffinity, chromatography or immunoprecipitation. Antibodies directed against the EpCAM protein (or a fragment thereof) can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein,

fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

[298] In yet another embodiment, an antibody according to the invention can be used as an agent for detecting the presence of EpCAM (or a protein fragment thereof) in a sample. In some embodiments, the antibody contains a detectable label. Antibodies are polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab, scFv, or F(ab')₂) is used. The term “labeled”, with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term “biological sample” is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. Included within the usage of the term “biological sample”, therefore, is blood and a fraction or component of blood including blood serum, blood plasma, or lymph. That is, the detection method of the invention can be used to detect an analyte mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of an analyte mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of an analyte protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of an analyte genomic DNA include Southern hybridizations. Procedures for conducting immunoassays are described, for example in “ELISA: Theory and Practice: Methods in Molecular Biology”, Vol. 42, J. R. Crowther (Ed.) Human Press, Totowa, NJ, 1995; “Immunoassay”, E. Diamandis and T. Christopoulos, Academic Press, Inc., San Diego, CA, 1996; and “Practice and Theory of Enzyme Immunoassays”, P. Tijssen, Elsevier Science Publishers, Amsterdam, 1985. Furthermore, *in vivo* techniques for detection of an analyte protein include introducing into a subject a labeled anti-analyte protein antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

IV. Methods of preparation

[299] The MSFPs or the anti-EpCAM antibodies (or antigen-binding fragments thereof) described herein may be prepared by any of the known protein expression and purification methods in the art.

[300] In some embodiments, the present application provides isolated nucleic acids encoding one or more of the polypeptide chains of any one of the MSFPs or the anti-EpCAM antibodies (or antigen-binding fragments thereof) described herein. In some embodiments, the isolated nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 31 or SEQ ID NO: 32. The isolated nucleic acids may be DNA or RNA.

SEQ ID NO: 31 (Nucleic acid encoding SEQ ID NO: 22)

gaggtgcagctggtggagtcagggggaggttgggtccagcctgggggatccctgagactctcctgtgcagcctctggattcacctttagta
attattggatgagctgggtccgccaggctccagggaaggggctggagtggtggccaacataaagcaagatggaagtgagaaattctatg
cggactctgtgaagggccgattcaccatctccagagacaacgccaagaactcactgtatctgcaaatgaacagcctgagagccgaagaca
cggctgtctattactgtgcgagagtggtggccgtcctgggagcaggactactggggccagggaaccctggcactgtctcgccggtggcg
gtggcagcggcggtggtgggtccggtggcgccggtatctggcgcgagctgtactgactcaaccgccctcagtgtctggggccccagg
cagagggtcaccatctcctgcaactgggagcagctccaacatcggtgttattatggtgtgcaactgggtaccagcagcttcagggaacagccc
ccaaactcctcatctattctgacactaatcgacctcaggggtccctgaccgattctctggctccaagtctggcacctcgccctcctggccat
cactgggtccaggctgaggatgaggtgattattactgccagtcgtatgacaagggcttcgggcaccgggtgttcggcgaggaggaccaa
gtgaccgtcctagggggcgaggtgcagctggtggagctctgggggaggttggtagcctgggggtccctgagactctcctgtgcag
cctctggattcaccttaacacctacgccatgaactgggtccgccagggtccagggaaggggctggagtggtgcacgcataagaagta
aatataataattatgcaacatattatgccgattcagtgaagaccgggtccaccatctccagagacgattccaagaacacgctgtatctgcaaat
gaacagcctgagagccgaggacacggcgtatattactgtgtgagacatgggaacttcggtaatagctacgtttcctggttgcctactgggg
ccaagggacaatggtcaccgtctctcagctagcaccaagggcccacgtcttccccctggcacctcctccaagagcacctctgggggc
acagcggcctgggtgctgtcaaggactactccccgaaccgggtgacggtgtctggaactcaggcgccctgaccagcggcggtgca
caccttcccgggtgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcaccagacctaca
tctgcaacgtgaatcaccaagcccagcaacaccaaggtggacaagaaagttgagcccaaatctgtccaccgtgctcatga

SEQ ID NO: 32 (Nucleic acid encoding SEQ ID NO: 23)

gaggtgcagctggtggagtcagggggaggttgggtccagcctgggggatccctgagactctcctgtgcagcctctggattcacctttagta
attattggatgagctgggtccgccaggctccagggaaggggctggagtggtggccaacataaagcaagatggaagtgagaaattctatg
cggactctgtgaagggccgattcaccatctccagagacaacgccaagaactcactgtatctgcaaatgaacagcctgagagccgaagaca
cggcctgtctattactgtgcgagagtggtggccgtcctgggagcaggactactggggccagggaaccctggcactgtctcgccggtggc
ggtggcagcggcggtggtgggtccggtggcgccggtatctggcgcgagctgtactgactcaaccgccctcagtgtctggggccccagg
gcagagggtcaccatctcctgcaactgggagcagctccaacatcggtgttattatggtgtgcaactgggtaccagcagcttcagggaacagcc
cccaaactcctcatctattctgacactaatcgacctcaggggtccctgaccgattctctggctccaagtctggcacctcgccctcctggcc
atcactgggtccaggctgaggatgaggtgattattactgccagtcgtatgacaagggcttcgggcaccgggtgttcggcgaggaggacc
aagctgaccgtcctagggggccaggctgtggtgactcaggagccctcactgactgtgtccccaggaggagacagtcactctcacctgtcgt
caagtactggggctgttacaactagtaactatgccaactgggtccagcagaacctggacaagcaccagggggtctgattggtgtaccaaa
caagcgagctccaggtaacctgcccgggtctcaggctccctccttggggggcaaggtgccctgacactgtcaggtgtgcagcctgagga
cgaggctgagtattactgcgctctatggtacagcaacctctgggtgttcggcgaggaggaccaagctgaccgtcctaggccaaccgaaagc
ggcgccctgggtcactctgttcccgcctcctctgaggagcttcaagccaacaaggccacactgggtgtgtctcataagtgaacttctaccgg

gagccgtgacagtggcctggaaggcagatagcagcccgtaaggcgggagtgagaccaccacaccctccaaacaagcaacaaca
 agtacgcccagcagctatctgagcctgacgcctgagcagtggagtcacacagaagctacagctgccaggtcacgcatgaagggag
 caccgtggagaagacagtggcccctacagaatgtccaccgtgtcatga

[301] In some embodiments, the isolated nucleic acid is inserted into a vector, such as an expression vector, a viral vector, or a cloning vector. For expression of the nucleic acids, the vector may be introduced into a host cell to allow expression of the nucleic acids within the host cell. The expression vectors may contain a variety of elements for controlling expression, including without limitation, promoter sequences, transcription initiation sequences, enhancer sequences, selectable markers, and signal sequences. These elements may be selected as appropriate by a person of ordinary skill in the art. For example, the promoter sequences may be selected to promote the transcription of the polynucleotide in the vector. Suitable promoter sequences include, without limitation, T7 promoter, T3 promoter, SP6 promoter, beta-actin promoter, EF1a promoter, CMV promoter, and SV40 promoter. Enhancer sequences may be selected to enhance the transcription of the nucleic acids. Selectable markers may be selected to allow selection of the host cells inserted with the vector from those not, for example, the selectable markers may be genes that confer antibiotic resistance. Signal sequences may be selected to allow the expressed polypeptide to be transported outside of the host cell. In some embodiments, the isolated nucleic acids further comprise a nucleic acid sequence encoding a signal peptide. In some embodiments, the signal peptide comprises the amino acid sequence of SEQ ID NO: 33. In some embodiments, the nucleic acid sequence encoding the signal peptide comprises the nucleic acid sequence of SEQ ID NO: 34.

SEQ ID NO: 33 (Signal peptide)
 MEWSWVFLFFLSVTTGVHS

SEQ ID NO: 34 (Nucleic acid encoding signal peptide)
 atggaatggagctgggtcttctcttctctctgtcagtaacgactgggtgtccactcc

[302] In some embodiments, there is provided an isolated host cell containing the vector described above. The host cells containing the vector may be useful in expression or cloning of the isolated nucleic acids. Suitable host cells can include, without limitation, prokaryotic cells, fungal cells, yeast cells, or higher eukaryotic cells such as mammalian cells. The expression of antibodies and antigen-binding fragments in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Pluckthun, A. *BioTechnology* 9: 545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option

for production of antibodies or antigen-binding fragments thereof, see recent reviews, for example Ref, M. E. (1993) Curr. Opinion Biotech. 4: 573-576; Trill J. J. et al. (1995) Curr. Opinion Biotech 6: 553-560. Higher eukaryotic cells, in particular, those derived from multicellular organisms can be used for expression of glycosylated polypeptides. Suitable higher eukaryotic cells include, without limitation, invertebrate cells and insect cells, and vertebrate cells.

[303] The vector can be introduced to the host cell using any suitable methods known in the art, including, but not limited to, DEAE-dextran mediated delivery, calcium phosphate precipitate method, cationic lipids mediated delivery, liposome mediated transfection, electroporation, microprojectile bombardment, receptor-mediated gene delivery, delivery mediated by polylysine, histone, chitosan, and peptides. Standard methods for transfection and transformation of cells for expression of a vector of interest are well known in the art. In some embodiments, the host cells comprise a first vector encoding a first polypeptide and a second vector encoding a second polypeptide. In some embodiments, the host cells comprise a single vector comprising isolated nucleic acids encoding a first polypeptide and a second polypeptide.

[304] In some embodiments, the present application provides methods of expressing any of the MSFPs or the anti-EpCAM antibodies (or antigen-binding fragments thereof) described herein, comprising culturing the isolated host cell containing the vector and recovering the MSFP or anti-EpCAM antibody (or antigen-binding fragment thereof) from the cell culture. The isolated host cells are cultured under conditions that allow expression of the isolated nucleic acids inserted in the vectors. Suitable conditions for expression of polynucleotides may include, without limitation, suitable medium, suitable density of host cells in the culture medium, presence of necessary nutrients, presence of supplemental factors, suitable temperatures and humidity, and absence of microorganism contaminants. A person with ordinary skill in the art can select the suitable conditions as appropriate for the purpose of the expression.

[305] In some embodiments, the polypeptides expressed in the host cell can form a dimer and thus produce an MSFP or the anti-EpCAM antibody (or antigen-binding fragment thereof) described herein. In some embodiments, the polypeptide expressed in the host cell can form a polypeptide complex which is a homodimer. In some embodiments, wherein the host cells

express a first polynucleotide and a second polynucleotide, the first polynucleotide and the second polynucleotide can form a polypeptide complex which is a heterodimer.

[306] In some embodiments, the polypeptide complex (such as the MSFP or the anti-EpCAM antibody or antigen-binding fragment thereof) may be formed inside the host cell. For example, the dimer may be formed inside the host cell with the aid of relevant enzymes and/or cofactors. In some embodiments, the polypeptide complex may be secreted out of the cell. In some embodiments, a first polypeptide and a second polypeptide may be secreted out of the host cell and form a dimer (such as the MSFP or the anti-EpCAM antibody or antigen-binding fragment thereof) outside of the host cell.

[307] In some embodiments, a first polypeptide and a second polypeptide may be separately expressed and allowed to dimerize to form the MSFP or the anti-EpCAM antibody (or antigen-binding fragment thereof) under suitable conditions. For example, the first polypeptide and the second polypeptide may be combined in a suitable buffer and allow the first protein monomer and the second protein monomer to dimerize through appropriate interactions such as hydrophobic interactions. In some embodiments, the first polypeptide and the second polypeptide may be combined in a suitable buffer containing an enzyme and/or a cofactor which can promote the dimerization of the first polypeptide and the second polypeptide. In some embodiments, the first polypeptide and the second polypeptide may be combined in a suitable vehicle and allow them to react with each other in the presence of a suitable reagent and/or catalyst.

[308] The expressed polypeptide(s) and/or the polypeptide complex can be collected using any suitable methods. The polypeptide(s) and/or the polypeptide complex can be expressed intracellularly, in the periplasmic space or be secreted outside of the cell into the medium. If the polypeptide and/or the polypeptide complex are expressed intracellularly, the host cells containing the polypeptide and/or the polypeptide complex may be lysed and polypeptide and/or the polypeptide complex may be isolated from the lysate by removing the unwanted debris by centrifugation or ultrafiltration. If the polypeptide and/or the polypeptide complex is secreted into periplasmic space of *E. coli*, the cell paste may be thawed in the presence of agents such as sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) for about 30 min, and cell debris can be removed by centrifugation (Carter et al., *BioTechnology* 10:163-167

(1992)). If the polypeptide and/or the polypeptide complex is secreted into the medium, the supernatant of the cell culture may be collected and concentrated using a commercially available protein concentration filter, for example, an Amincon or Millipore Pellicon ultrafiltration unit. A protease inhibitor and/or a antibiotics may be included in the collection and concentration steps to inhibit protein degradation and/or growth of contaminated microorganisms.

[309] The expressed polypeptide(s) and/or the polypeptide complex can be further purified by a suitable method, such as without limitation, affinity chromatography, hydroxylapatite chromatography, size exclusion chromatography, gel electrophoresis, dialysis, ion exchange fractionation on an ion-exchange column, ethanol precipitation, reverse phase HPLC, chromatography on silica, chromatography on heparin sepharose, chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation (see, for review, Bonner, P. L., Protein purification, published by Taylor & Francis. 2007; Janson, J. C., et al, Protein purification: principles, high resolution methods and applications, published by Wiley-VCH, 1998).

[310] In some embodiments, the polypeptides and/or polypeptide dimer complexes can be purified by affinity chromatography. In some embodiments, protein A chromatography or protein A/G (fusion protein of protein A and protein G) chromatography can be useful for purification of polypeptides and/or polypeptide complexes comprising a component derived from antibody CH2 domain and/or CH3 domain (Lindmark et al., J. Immunol. Meth. 62:1-13 (1983)); Zettlitz, K. A., Antibody Engineering, Part V, 531-535, 2010). In some embodiments, protein G chromatography can be useful for purification of polypeptides and/or polypeptide complexes comprising IgG $\gamma 3$ heavy chain (Guss et al., EMBO J. 5:1567 1575 (1986)). In some embodiments, protein L chromatography can be useful for purification of polypeptides and/or polypeptide complexes comprising κ light chain (Sudhir, P., Antigen engineering protocols, Chapter 26, published by Humana Press, 1995; Nilson, B. H. K. et al, J. Biol. Chem., 267, 2234-2239 (1992)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABX resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification.

V. Pharmaceutical compositions, Unit dosages, articles of manufacture, and kits

[311] Further provided by the present application are pharmaceutical compositions comprising any one of the MSFPs or the anti-EpCAM antibodies (or antigen-binding fragments thereof) as described herein, and a pharmaceutically acceptable carrier.

[312] The pharmaceutical compositions may be suitable for a variety of modes of administration described herein, including for example systemic or localized administration. In some embodiments, the pharmaceutical composition is formulated for intravenous administration. In some embodiments, the pharmaceutical composition is formulated for subcutaneous administration. In some embodiments, the pharmaceutical composition is formulated for local administration to a tumor site. In some embodiments, the pharmaceutical composition is formulated for intratumoral injection.

[313] “Carriers” as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[314] In some embodiments, the pharmaceutical composition is formulated to have a pH in the range of about 4.5 to about 9.0, including for example pH ranges of about any one of 5.0 to about 8.0, about 6.5 to about 7.5, or about 6.5 to about 7.0. In some embodiments, the

pharmaceutical composition can also be made to be isotonic with blood by the addition of a suitable tonicity modifier, such as glycerol.

[315] The pharmaceutical compositions to be used for *in vivo* administration are generally formulated as sterile, substantially isotonic, and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration. Sterility is readily accomplished by filtration through sterile filtration membranes. In some embodiments, the composition is free of pathogen. For injection, the pharmaceutical composition can be in the form of liquid solutions, for example in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the pharmaceutical composition can be in a solid form and re-dissolved or suspended immediately prior to use. Lyophilized compositions are also included.

[316] In some embodiment, the pharmaceutical composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for injection intravenously, introperitoneally, or intravitreally. Typically, compositions for injection are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[317] In some embodiments, the pharmaceutical composition is suitable for administration to a human. In some embodiments, the pharmaceutical composition is contained in a single-use vial, such as a single-use sealed vial. In some embodiments, the pharmaceutical composition is contained in a multi-use vial. In some embodiments, the pharmaceutical composition is contained in bulk in a container. In some embodiments, the pharmaceutical composition is cryopreserved.

[318] Also provided are unit dosage forms of the MSFPs, anti-EpCAM antibodies (or antigen-binding fragments thereof), or compositions thereof. Each dosage may contain from about 0.01 μ g to about 10 mg, including for example any of about 0.01 μ g to about 10 mg, about 0.01 μ g to about 5 mg, about 0.01 μ g to about 1 mg, about 0.1 μ g to about 300 μ g, about 0.1 μ g to about 200 μ g, about 0.1 μ g to about 100 μ g, about 0.1 μ g to about 90 μ g, about 0.1 μ g to about 80 μ g, about 0.1 μ g to about 70 μ g, about 0.1 μ g to about 60 μ g, about 0.1 μ g to about 50 μ g, about 0.1 μ g to about 40 μ g, about 0.1 μ g to about 30 μ g, about 0.1 μ g to about 20 μ g, about 0.1 μ g to about 10 μ g, about 0.1 μ g to about 5 μ g, or about 0.1 μ g to about 1 μ g. In some embodiments, the unit dosage forms of the MSFP, anti-EpCAM antibody (or antigen-binding fragment thereof), or composition thereof is within any of the following range, wherein the ranges have an upper limit of any of: 0.1 μ g, 0.2 μ g, 0.3 μ g, 0.4 μ g, 0.5 μ g, 0.6 μ g, 0.7 μ g, 0.8 μ g, 0.9 μ g, 1 μ g, 5 μ g, 10 μ g, 15 μ g, 20 μ g, 25 μ g, or 30 μ g, 35 μ g, 40 μ g, 45 μ g, 50 μ g, 55 μ g, 60 μ g, 65 μ g, 70 μ g, 75 μ g, 80 μ g, 85 μ g, 90 μ g, 95 μ g, 100 μ g, 150 μ g, 200 μ g, 250 μ g, 300 μ g, 350 μ g, 400 μ g, 450 μ g, 500 μ g, 550 μ g, 600 μ g, 650 μ g, 700 μ g, 750 μ g, 800 μ g, 850 μ g, 900 μ g, 1000 μ g, 1500 μ g, 2000 μ g, 2500 μ g, 3000 μ g, 6000 μ g, or 10000 μ g, and an independently selected lower limit of any of 0.1 μ g, 0.2 μ g, 0.3 μ g, 0.4 μ g, 0.5 μ g, 0.6 μ g, 0.7 μ g, 0.8 μ g, 0.9 μ g, 1 μ g, 5 μ g, 10 μ g, 15 μ g, 20 μ g, 25 μ g, or 30 μ g, 35 μ g, 40 μ g, 45 μ g, 50 μ g, 55 μ g, 60 μ g, 65 μ g, 70 μ g, 75 μ g, 80 μ g, 85 μ g, 90 μ g, 95 μ g, 100 μ g, 150 μ g, 200 μ g, 250 μ g, 300 μ g, 350 μ g, 400 μ g, 450 μ g, 500 μ g, 550 μ g, 600 μ g, 650 μ g, 700 μ g, 750 μ g, 800 μ g, 850 μ g, 900 μ g, 1000 μ g, 1500 μ g, 2000 μ g, 2500 μ g, 3000 μ g, 6000 μ g, or 10000 μ g, and wherein the lower limit is less than the upper limit. The term “unit dosage form” refers to a physically discrete unit suitable as unitary dosages for an individual, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier, diluent, or excipient. These unit dosage forms can be stored in a suitable packaging in single or multiple unit dosages and may also be further sterilized and sealed.

[319] The present application further provides articles of manufacture comprising the compositions (such as pharmaceutical compositions) described herein in suitable packaging. Suitable packaging for compositions (such as MSFP or anti-EpCAM antibody compositions) described herein are known in the art, and include, for example, vials (such as sealed vials), vessels, ampules, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

The present application also provides kits comprising compositions (such as pharmaceutical compositions) described herein and may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein.

EXEMPLARY EMBODIMENTS

[320] The invention provides the following embodiments:

[321] 1. A method of treating a cancer in an individual, comprising administering to the individual an effective amount of a multispecific Fab fusion protein comprising: a Fab fragment that specifically binds to CD3, and a binding domain that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment; and wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$.

[322] 2. The method of embodiment 1, wherein the binding domain is an scFv.

[323] 3. The method of embodiment 2, wherein the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment.

[324] 4. The method of embodiment 3, wherein the first scFv and the second scFv have the same sequence.

[325] 5. The method of any one of embodiments 1-4, wherein the multispecific Fab fusion protein is administered intravenously.

[326] 6. The method of any one of embodiments 1-5, wherein the multispecific Fab fusion protein is administered at a low frequency.

[327] 7. The method of embodiment 6, wherein the multispecific Fab fusion protein is administered twice weekly.

- [328] 8. The method of any one of embodiments 1-7, wherein the multispecific Fab fusion protein is administered at a dose equivalent to about 0.1 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$ for a cynomolgus monkey.
- [329] 9. The method of any one of embodiments 1-8, wherein the multispecific Fab fusion protein is administered at a dose that does not induce cytokine storm.
- [330] 10. The method of embodiment 9, wherein multispecific Fab fusion protein is administered at a dose equivalent to no higher than about 30 $\mu\text{g/kg}$ for a cynomolgus monkey.
- [331] 11. The method of any one of embodiments 1-10, wherein the multispecific Fab fusion protein is administered at a first dose for a first period of time to the individual, and consecutively, the multispecific Fab fusion protein is administered at a second dose for a second period of time to the individual, and wherein the second dose exceeds the first dose.
- [332] 12. The method of embodiment 11, wherein the second period of time exceeds the first period of time.
- [333] 13. The method of embodiment 11 or embodiment 12, wherein the first period of time is at least about 7 days.
- [334] 14. The method of any one of embodiments 11-13, wherein the second period of time is at least about 2 weeks.
- [335] 15. The method of any one of embodiments 11-14, wherein the first dose is no more than about 1 $\mu\text{g/kg}$.
- [336] 16. The method of any one of embodiments 11-14, wherein the second dose is about 0.1 $\mu\text{g/kg}$ to about 10 $\mu\text{g/kg}$.
- [337] 17. The method of any one of embodiments 1-16, further comprising administering a glucocorticoid to the individual.
- [338] 18. The method of embodiment 17, wherein the glucocorticoid is dexamethasone.
- [339] 19. The method of embodiment 17 or embodiment 18, wherein the glucocorticoid is administered prior to the first dose of the multispecific Fab fusion protein.
- [340] 20. The method of any one of embodiments 17-19, wherein the glucocorticoid is administered at a dose of about 0.1 mg/kg to about 5 mg/kg.

- [341] 21. The method of any one of embodiments 1-20, wherein the individual is a human individual.
- [342] 22. The method of any one of embodiments 1-21, wherein the Fab fragment specifically binds to the N-terminus of CD3 epsilon.
- [343] 23. The method of embodiment 22, wherein the Fab fragment specifically binds to an epitope within amino acids 1-27 of CD3 epsilon.
- [344] 24. The method of embodiment 23, wherein the VH of the Fab fragment comprises a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3.
- [345] 25. The method of embodiment 23 or embodiment 24, wherein the VL of the Fab fragment comprises: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.
- [346] 26. The method of any one of embodiments 23-25, wherein the VH of the Fab fragment comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs:7 and 39-43.
- [347] 27. The method of any one of embodiments 23-26, wherein the VL of the Fab fragment comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 44-47.
- [348] 28. The method of any one of embodiments 23-27, wherein the Fab fragment comprises a human immunoglobulin heavy chain constant region 1 (CH1) comprising the amino acid sequence of SEQ ID NO:9.
- [349] 29. The method of any one of embodiments 23-28, wherein the Fab fragment comprises a human lambda light chain constant region comprising the amino acid sequence of SEQ ID NO:10.

[350] 30. The method of any one of embodiments 23-29, wherein the CH1 and the CL of the Fab fragment are connected by one or more disulfide bonds.

[351] 31. The method of embodiment 30, wherein the Fab fragment comprises a first polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:11.

[352] 32. The method of embodiment 30 or embodiment 31, wherein the Fab fragment comprises a second polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:12.

[353] 33. The method of any one of embodiments 1-32, wherein the cancer is an EpCAM-positive solid cancer.

[354] 34. The method of embodiment 33, wherein the EpCAM-positive solid cancer is a carcinoma or adenocarcinoma.

[355] 35. The method of any one of embodiments 1-34, wherein the cancer is selected from the group consisting of small intestine cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, endometrial cancer, breast cancer, bile duct cancer, and head and neck cancer.

[356] 36. The method of embodiment 35, wherein the cancer is colorectal adenocarcinoma.

[357] 37. The method of embodiment 35, wherein the cancer is lung adenocarcinoma.

[358] 38. The method of any one of embodiments 2-37, wherein the scFv comprises an N-VH-VL-C fusion polypeptide.

[359] 39. The method of any one of embodiments 2-38, wherein the VH of the scFv comprises a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15.

[360] 40. The method of any one of embodiments 2-39, wherein the VL of the scFv comprises a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2

comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18.

[361] 41. The method of any one of embodiments 2-40, wherein the VH of the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO: 19.

[362] 42. The method of any one of embodiments 2-41, wherein the VL of the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO: 20.

[363] 43. The method of embodiment 42, wherein the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:21.

[364] 44. The method of any one of embodiments 1-43, wherein the multispecific Fab fusion protein comprises a first polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:22.

[365] 45. The method of any one of embodiments 1-44, wherein the multispecific Fab fusion protein comprises a second polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:23.

[366] 46. An anti-EpCAM antibody or antigen-binding fragment thereof, comprising a heavy chain variable region comprising: (1) a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; (2) a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and (3) a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15; and a light chain variable region comprising: (1) a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; (2) a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and (3) a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18.

[367] 47. The anti-EpCAM antibody or antigen-binding fragment thereof according to embodiment 46, wherein the heavy chain variable domain sequence comprises a VH comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:19.

[368] 48. The anti-EpCAM antibody or antigen-binding fragment thereof according to embodiment 46 or embodiment 47, wherein the light chain variable domain sequence comprises a VL comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:20.

[369] 49. The anti- EpCAM antibody or antigen-binding fragment thereof according to any one of embodiments 46-48, wherein the anti-EpCAM antibody comprises an Fc sequence of a human IgG.

[370] 50. The anti-EpCAM antibody of any one of embodiments 46-49, wherein the anti-EpCAM antibody is a multispecific antibody.

[371] 51. The antigen-binding fragment of the anti-EpCAM antibody according to any one of embodiments 46-48, wherein the antigen-binding fragment is a single-chain Fv (scFv).

[372] 52. The antigen-binding fragment of the anti-EpCAM antibody of embodiment 51, wherein the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:21.

[373] 53. A multispecific Fab fusion protein comprising the anti-EpCAM antigen-binding fragment of any one of embodiments 46-48 and 51-52.

[374] 54. The multispecific Fab fusion protein of embodiment 53, comprising a Fab fragment that specifically binds to CD3, a first copy of the anti-EpCAM antigen-binding fragment, and a second copy of the anti-EpCAM antigen binding fragment; wherein the first copy of the anti-EpCAM antigen-binding fragment is fused to the N-terminus of the VH of the Fab fragment; and wherein the second copy of the anti-EpCAM antigen binding fragment is fused to the N-terminus of the VL of the Fab fragment.

[375] 55. The multispecific Fab fusion protein of embodiment 54, wherein the Fab fragment specifically binds to the N-terminus of CD3 epsilon.

[376] 56. The multispecific Fab fusion protein of embodiment 55, wherein the Fab fragment specifically binds to an epitope within amino acids 1-27 of CD3 epsilon.

[377] 57. The multispecific Fab fusion protein of embodiment 56, wherein the VH of the Fab fragment comprises: a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-

H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3.

[378] 58. The multispecific Fab fusion protein of embodiment 56 or embodiment 57, wherein the VL of the Fab fragment comprises: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

[379] 59. The multispecific Fab fusion protein of any one of embodiments 56-58, wherein the VH of the Fab fragment comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs:7 and 39-43.

[380] 60. The multispecific Fab fusion protein of any one of embodiments 56-59, wherein the VL of the Fab fragment comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 44-47.

[381] 61. The multispecific Fab fusion protein of any one of embodiments 53-60, wherein the Fab fragment comprises a human immunoglobulin heavy chain constant region 1 (CH1) comprising the amino acid sequence of SEQ ID NO:9.

[382] 62. The multispecific Fab fusion protein of any one of embodiments 53-61, wherein the Fab fragment comprises a human lambda light chain constant region comprising the amino acid sequence of SEQ ID NO:10.

[383] 63. The multispecific Fab fusion protein of any one of embodiments 53-62, wherein the CH1 and the CL of the Fab fragment are connected by one or more disulfide bonds.

[384] 64. The multispecific Fab fusion protein of any one of embodiments 56-63, wherein the Fab fragment comprises a first polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:11.

[385] 65. The multispecific Fab fusion protein of any one of embodiments 56-64, wherein the Fab fragment comprises a second polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:12.

- [386] 66. An isolated nucleic acid molecule that encodes the anti-EpCAM antibody or antigen-binding fragment thereof or the multispecific Fab fusion protein according to any one of embodiments 46-65.
- [387] 67. An expression vector encoding the isolated nucleic acid molecule of embodiment 66.
- [388] 68. An isolated host cell comprising the expression vector of embodiment 67.
- [389] 69. A method of producing an anti-EpCAM antibody or antigen-binding fragment thereof or the multispecific Fab fusion protein comprising culturing the isolated host cell of embodiment 68 and recovering the anti-EpCAM antibody or antigen-binding fragment thereof or the multispecific Fab fusion protein from the cell culture.
- [390] 70. A composition comprising the anti-EpCAM antibody or antigen-binding fragment thereof or the multispecific Fab fusion protein according to any one of embodiments 46-65 and a pharmaceutically acceptable carrier.
- [391] 71. A method of treating cancer in an individual, comprising administering an effective amount of the composition of embodiment 70 to the individual.
- [392] 72. Use of the anti-EpCAM antibody or antigen-binding fragment thereof or the multispecific Fab fusion protein of any one of embodiments 46-65 in the preparation of a medicament for treating a cancer in an individual.
- [393] 73. Use of a multispecific Fab fusion protein in the preparation of a medicament for of treating a cancer in an individual, wherein the multispecific Fab fusion protein comprises a Fab fragment that specifically binds to CD3, and a binding domain that specifically binds to EpCAM; wherein the binding domain is fused to an N-terminus of the Fab fragment.
- [394] 74. The use of embodiment 73, wherein the binding domain is an scFv.
- [395] 75. The use of embodiment 74, wherein the multispecific Fab fusion protein comprises a first scFv that specifically binds to EpCAM, and a second scFv that specifically binds to EpCAM; wherein the first scFv is fused to the N-terminus of the VH of the Fab fragment; and wherein the second scFv is fused to the N-terminus of the VL of the Fab fragment.

[396] 76. The use of embodiment 75, wherein the first scFv and the second scFv have the same sequence.

[397] 77. The use of any one of embodiments 73-76, wherein the multispecific Fab fusion protein is administered intravenously.

[398] 78. The use of any one of embodiments 73-77, wherein the multispecific Fab fusion protein is administered at a low frequency.

[399] 79. The use of embodiment 78, wherein the multispecific Fab fusion protein is administered twice weekly.

[400] 80. The use of any one of embodiments 73-79, wherein the multispecific Fab fusion protein is administered at a dose of about 0.1 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$.

[401] 81. The use of embodiment 80, wherein the multispecific Fab fusion protein is administered at a dose equivalent to about 0.1 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$ for a cynomolgus monkey.

[402] 82. The use of any one of embodiments 63-81, wherein the multispecific Fab fusion protein is administered at a dose that does not induce cytokine storm.

[403] 83. The use of any one of embodiments 63-82, wherein the multispecific Fab fusion protein is administered at a first dose for a first period of time to the individual, and consecutively, the multispecific Fab fusion protein is administered at a second dose for a second period of time to the individual, and wherein the second dose exceeds the first dose.

[404] 84. The use of embodiment 83, wherein the second period of time exceeds the first period of time.

[405] 85. The use of embodiment 83 or embodiment 84, wherein the first period of time is at least about 7 days.

[406] 86. The use of any one of embodiments 83-85, wherein the second period of time is at least about 2 weeks.

[407] 87. The use of any one of embodiments 83-86, wherein the first dose is no more than about 1 $\mu\text{g/kg}$.

[408] 88. The use of any one of embodiments 83-87, wherein the second dose is about 0.1 µg/kg to about 10 µg/kg.

[409] 89. The use of any one of embodiments 83-88, further comprising administering a glucocorticoid to the individual.

[410] 90. The use of embodiment 89, wherein the glucocorticoid is dexamethasone.

[411] 91. The use of embodiment 89 or embodiment 90, wherein the glucocorticoid is administered prior to the first dose of the multispecific Fab fusion protein.

[412] 92. The use of any one of embodiments 89-91, wherein the glucocorticoid is administered at a dose of about 0.1 mg/kg to about 5 mg/kg.

[413] 93. The use of any one of embodiments 73-92, wherein the individual is a human individual.

[414] 94. The use of any one of embodiments 73-93, wherein the Fab fragment specifically binds to the N-terminus of CD3 epsilon.

[415] 95. The use of embodiment 94, wherein the Fab fragment specifically binds to an epitope within amino acids 1-27 of CD3 epsilon.

[416] 96. The use of embodiment 95, wherein the VH of the Fab fragment comprises a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3.

[417] 97. The use of embodiment 95 or embodiment 96, wherein the VL of the Fab fragment comprises: a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6.

[418] 98. The use of any one of embodiments 95-97, wherein the VH of the Fab fragment comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs:7 and 39-43.

- [419] 99. The use of any one of embodiments 95-98, wherein the VL of the Fab fragment comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 44-47.
- [420] 100. The use of any one of embodiments 95-99, wherein the Fab fragment comprises a human immunoglobulin heavy chain constant region 1 (CH1) comprising the amino acid sequence of SEQ ID NO:9.
- [421] 101. The use of any one of embodiments 95-100, wherein the Fab fragment comprises a human lambda light chain constant region comprising the amino acid sequence of SEQ ID NO:10.
- [422] 102. The use of any one of embodiments 95-101, wherein the CH1 and the CL of the Fab fragment are connected by one or more disulfide bonds.
- [423] 103. The use of embodiment 102, wherein the Fab fragment comprises a first polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:11.
- [424] 104. The use of embodiment 102 or embodiment 103, wherein the Fab fragment comprises a second polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:12.
- [425] 105. The use of any one of embodiments 73-104, wherein the cancer is an EpCAM-positive solid cancer.
- [426] 106. The use of embodiment 105, wherein the EpCAM-positive solid cancer is a carcinoma or adenocarcinoma.
- [427] 107. The use of any one of embodiments 73-106, wherein the cancer is selected from the group consisting of small intestine cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, endometrial cancer, breast cancer, bile duct cancer, and head and neck cancer.
- [428] 108. The use of embodiment 107, wherein the cancer is colorectal adenocarcinoma.

[429] 109. The use of embodiment 107, wherein the cancer is lung adenocarcinoma.

[430] 110. The use of any one of embodiments 74-109, wherein the scFv comprises an N-VH-VL-C fusion polypeptide.

[431] 111. The use of any one of embodiments 74-110, wherein the VH of the scFv comprises a HVR-H1 comprising the amino acid sequence of SEQ ID NO:13; a HVR-H2 comprising the amino acid sequence of SEQ ID NO:14; and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:15.

[432] 112. The use of any one of embodiments 74-111, wherein the VL of the scFv comprises a HVR-L1 comprising the amino acid sequence of SEQ ID NO:16; a HVR-L2 comprising the amino acid sequence of SEQ ID NO:17; and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:18.

[433] 113. The use of any one of embodiments 74-112, wherein the VH of the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO: 19.

[434] 114. The use of any one of embodiments 74-113, wherein the VL of the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO: 20.

[435] 115. The use of embodiment 114, wherein the scFv comprises an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:21.

[436] 116. The use of any one of embodiments 73-115, wherein the multispecific Fab fusion protein comprises a first polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:22.

[437] 117. The use of any one of embodiments 73-116, wherein the multispecific Fab fusion protein comprises a second polypeptide comprising an amino acid sequence having at least about 85% (such as about 100%) sequence identity to the amino acid sequence of SEQ ID NO:23.

EXAMPLES

[438] The examples below are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way. The following examples and detailed description are offered by way of illustration and not by way of limitation. For the embodiments in which details of the experimental methods are not described, such methods are carried out according to conventional conditions such as those described in Sambrook *et al.* Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989), or as suggested by the manufacturers.

Example 1: Expression and purification of an exemplary bispecific Fab fusion protein1. Transient expression

[439] Fab fragments or Fab fusion proteins were expressed using standard protocols. DNA fragments encoding the light chain and the heavy chain of the Fab fusion protein were cloned into expression vector pcDNA to generate constructs expressing the light chain and the heavy chain. The constructs also contained sequences encoding signal peptides in order to facilitate secretion of the light chain and heavy chain proteins. Sequencing results indicated correct gene insertion. The constructs were transformed into *E. coli* to obtain transfection-grade plasmid DNA. HEK293F cells were grown in EXPI293TM expression medium (Invitrogen). For transfection, 10 mL of medium containing plasmid DNA and 25 kD Polyethylenimine (PEI; DNA/linear 25 kD PEI weight ratio of 1:3) was added to 90 mL of cell culture. Transfected cells were cultured in a CO₂ incubator (37°C, 5% CO₂, 125 rpm) for about 6 days, then the supernatant was collected.

2. Stable expression

[440] DNA fragments (SEQ ID NO:32) encoding the light chain of an exemplary EpCAM×CD3 Fab fusion protein (referred hereinafter as ITAB1002) was cloned into the expression vector carrying a hygromycin resistance gene. DNA fragments (SEQ ID NO:31) encoding the heavy chain of the EpCAM×CD3 Fab fusion protein was cloned into the expression vector carrying a puromycin resistance gene. The DNA fragments encoding the light and heavy chains of the Fab fusion protein also each comprised a Kozak sequence and a signal peptide sequence (amino acid sequence is shown as SEQ ID NO: 33, nucleic acid sequence is shown as

SEQ ID NO: 34), and were cloned in between HindIII and NotI restriction enzyme sites to generate constructs expressing the light chain and heavy chain, respectively. 40 µg of DNA plasmids carrying the heavy chain gene or the light chain gene, respectively, were transfected via electroporation (MaxCyte) into CHO-S suspension cells in logarithmic growth phase, when cell numbers were $\sim 8 \times 10^8$. Cells were counted after 24 h. Transfected cells were then seeded with a density of about 1.5×10^6 cells/mL in CD OptiCHO Medium (Invitrogen) containing 5 µg/mL puromycin (Invitrogen) and 200 µg/mL hygromycin B (HyClone), and cultured in a CO₂ incubator (37°C, 5% CO₂, 125 rpm). Fresh media were replaced every 2-3 days until the transfected cells resumed normal growth rate, then cells in logarithmic growth phase were collected, stably transfected cell populations were stored, and single-cell clones were obtained by limiting dilution. Stably transfected cell populations were cultured in CD OptiCHO Medium (Invitrogen) supplemented with 1× glutamic acid and 1 g/L PF68 (37°C, 5% CO₂, 125 rpm). When cell density reached at least 3×10^6 cells/mL, cells were transferred to 32°C incubation (HF511 2.5%, HF502 0.2%, MednaBio), and media were changed every 2 days. After 2-4 times of media change, the supernatant was collected.

[441] The cell culture supernatant was purified with IgG-CH1 affinity chromatography (Thermo Fisher Scientific) to obtain target protein. The cell culture supernatant was filtered through a 0.22 µm sterile membrane, loaded onto the IgG-CH1 affinity matrix balanced with 150 mM NaCl and 10 mM phosphate buffered saline (PBS, pH 7.5), and eluted with 150 mM NaCl and 50 mM NaAc Buffer (pH 3.5). The eluate was adjusted with 2M Tris elution to a pH of 7.2, and concentrated with Vivaspin centrifugal concentrators having a 10 kD molecular weight cutoff (Sartorius). Purified protein was stored at 4°C. Protein was analyzed using 4-20% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing and/or reducing (5% β-Mercaptoethanol) conditions. Analysis results are shown in FIG. 2A and 2B. Non-reducing and/or reducing capillary electrophoresis (CE-SDS, Beckman Coulter, PA 800 plus) were performed to analyze purified Fab fusion protein, the results are shown in FIG. 3A and 3B. HPLC analysis indicated that the purity of purified Fab fusion protein is >90%.

[442] FIGs. 2A and 2B show the SDS-PAGE analysis results of the purified EpCAM×CD3 Fab fusion protein. FIG. 2A shows the results of non-reducing SDS-PAGE. Lane 1 contains protein molecular weight standard PAGERULER™ Unstained Protein Ladder (Thermo Scientific), with molecular weights of 200, 150, 120, 100, 85, 70, 60, 50, 40, 30, 25, 20, 15, 10

kD from top to bottom. Lane 2 contains the purified protein with a molecular weight of ~100 kD, similar to the theoretical molecular weight of EpCAM×CD3 Fab fusion protein. FIG. 2B shows the results of reducing SDS-PAGE. Lane 1 contains protein molecular weight standard PIERCETM Unstained Protein MW Marker (Thermo Scientific), with molecular weights of 116, 66.2, 45, 35, 25, 18.4, 14.4 from top to bottom. Lane 2 contains the purified protein, the molecular weight of which is between 45-66 kD.

[443] FIGs. 3A and 3B show results of the capillary electrophoresis (CE-SDS) of the purified EpCAM×CD3 Fab fusion protein. FIG. 3A depicts the non-reducing CE-SDS results, which show a single protein peak at migration time ~21.59 min. FIG. 3B depicts the reducing CE-SDS results, which show two single protein peaks at migration times ~18.37 min and ~18.84 min, corresponding to the light chain and heavy chain of the Fab fusion protein, respectively.

Example 2: Determination of binding affinities of an EpCAM×CD3 Fab fusion protein

Antigen binding affinity

[444] The binding affinity of the anti-EpCAM and anti-CD3 domains in an exemplary EpCAM×CD3 Fab fusion protein (*i.e.*, ITAB1002) with the corresponding human and cynomolgus monkey antigens were measured using Octet QK^e with an anti-human IgG Fc Capture (AHC) biosensor. The human EpCAM antigen construct (huEpCAM.Fc) and the cynomolgus EpCAM antigen construct (cynoEpCAM.Fc) had a full length EpCAM protein fused to a human IgG Fc. The human CD3 antigen construct (CD3εAA 1-27.Fc) and the cynomolgus CD3 antigen construct (cynoCD3εAA 1-27.Fc) had a peptide consisting of amino acids 1-27 of the CD3 epsilon fused to a human IgG Fc. The expressions of the antigen constructs is described in US 8,846,042. The antigen constructs were diluted to 0.02 mg/mL with dilution(PBS, 0.1%BSA, 0.02% Tween-20, 0.05% NaN₃), and then immobilized on an anti-hIgG Fc capture (AHC) biosensor. ITAB1002 were diluted to various concentrations, and added to a black microplate at 200 μL/well. The control wells containing only PBS were also set up. The detection results were analyzed using ForteBio Data Acquisition and ForteBio Data Analysis software.

[445] As shown in Table 1, the anti-EpCAM and anti-CD3 domains had high *in vitro* binding affinities to both human and cynomolgus monkey EpCAM and CD3 constructs respectively, indicating cross-reactivity of the EpCAM×CD3 Fab fusion protein in human and cynomolgus monkeys.

Table 1. *In vitro* binding affinities(KD)

Domain	Antigen	Kd(M)
Anti-EpCAM	huEpCAM.Fc	3.49×10^{-9}
Anti-EpCAM	cynoEpCAM.Fc	4.71×10^{-9}
Anti-CD3	CD3εAA 1-27.Fc	1.26×10^{-8}
Anti-CD3	cyno CD3εAA 1-27.Fc	1.56×10^{-8}

Cell binding affinity

[446] The following binding assay was carried out to determine the binding affinities of an exemplary EpCAM×CD3 Fab fusion protein (*i.e.*, ITAB1002) with cells expressing target antigens.

[447] The binding affinity of ITAB1002 to human and cynomolgus monkey peripheral blood mononuclear cells (hPBMC or cynoPBMC) were determined using fluorescence-activated cell sorting (FACS).

[448] hPBMC Preparation: White blood cell concentrate samples from healthy human adults were diluted with PBS buffer (Gibco), centrifuged by density gradient centrifugation (Ficoll-Paque, GE Healthcare) to obtain PBMC, washed twice with PBS, then centrifuged at room temperature, 1000 g for 10 min. Cells were collected, and resuspended in RPMI 1640 Medium (Gibco) containing 10% FBS.

[449] cynoPBMC Preparation: Whole blood samples from cynomolgus monkeys were diluted with PBS buffer (Gibco), centrifuged by density gradient centrifugation (Ficoll-Paque, GE Healthcare) to obtain PBMC, washed twice with PBS, then centrifuged at room temperature,

1000 g for 10 min. Cells were collected, and resuspended in RPMI 1640 Medium (Gibco) containing 10% FBS.

[450] ITAB1002 was diluted to various concentrations using a FACS buffer (PBS with 1% FBS), mixed with about 3.6×10^5 huPBMC, then incubated at room temperature for 45 min. A control group with no ITAB1002(1%FBS/PBS+hPBMC) was also set up. Cells were washed with the FACS buffer once, resuspended in 50 μ L FACS buffer, supplemented with CD4 Antibody (RFT-4g) APC-Cy7® conjugate(Invitrogen) and PE Mouse Anti-Human Light Chain λ (BD Pharmingen™), and incubated at room temperature for 45 min. 150 μ L FACS buffer was finally added, and the samples were analyzed using ACCURI® C6 Cytometer (BD Biosciences). The detection results are shown in FIG. 4A.

[451] ITAB1002 was diluted to various concentrations in 50 μ L FACS buffer (PBS with 1% FBS), mixed with about 4×10^5 cynoPBMC, and then incubated at room temperature for 60 min. A negative control group with no ITAB1002 was also set up. Cells were stained with CD4 Antibody (RFT-4g) APC-Cy7® conjugate(Invitrogen) and PE Mouse Anti-Human Light Chain λ (BD Pharmingen™), and incubated at room temperature for 45 min. 150 μ L FACS buffer was finally added, and the samples were analyzed using ACCURI® C6 Cytometer (BD Biosciences). The detection results are shown in FIG. 4B.

[452] As shown in FIGs. 4A and 4B, the EpCAM \times CD3 Fab fusion protein exhibited potent binding affinity to human and cynomolgus monkey PBMC, while the negative control group had substantially no binding affinity to human or cynomolgus monkey PBMC.

[453] Additionally, the following binding assay was performed to determine the binding affinity of the EpCAM \times CD3 Fab fusion protein(i.e., ITAB1002) to EpCAM+ cells.

[454] Human colon cancer cell line SW480 (Cell Bank, Committee on Type Culture Collection of Chinese Academy of Sciences) and Chinese hamster ovary (CHO) cells transfected with cynomolgus monkey EpCAM (hereinafter referred to as CyEpCAM-CHO) were used to determine the binding affinity of ITAB1002 with EpCAM. SW480 cells and CyEpCAM-CHO cells were trypsinized with 0.25% Trypsin-EDTA (Gibco), resuspended with a FACS buffer. ITAB1002 was diluted to various concentrations using the FACS buffer, and mixed with about 1×10^5 SW480 cells or CyEpCAM-CHO cells respectively at equal volume, followed by incubation at 4°C for 30 min. Cells were then washed with the FACS buffer and resuspended in

50 μ L FACS buffer. Mouse anti-Human IgG Fab Secondary Antibody PE conjugate(Invitrogen) were added, and allowed for incubation at 4°C for 30 min. 150 μ L FACS buffer was finally added, and the samples were analyzed using ACCURI[®] C6 Cytometer (BD Biosciences). The detection results are shown in FIG. 5.

[455] As shown in FIG. 5, the EpCAM \times CD3 Fab fusion protein exhibited potent binding affinities to both SW480 cells expressing human EpCAM (EC₅₀=411.2 ng/mL) and CHO cells expressing cynomolgus monkey EpCAM on the cell surface (CyEpCAM-CHO, EC₅₀=107.6 ng/mL).

[456] Therefore, the exemplary EpCAM \times CD3 Fab fusion protein ITAB1002 exhibited cross-reactivity towards human and cynomolgus monkey antigens in vitro. The cross-reactivity of the bispecific Fab fusion protein can facilitate extrapolation of toxicity and efficacy study results in cynomolgus monkeys to human clinical studies.

Example 3: Tumor-dependent activation of human PBMC by an EpCAM \times CD3 Fab fusion protein

[457] CD4 and CD8 are typical T cell surface antigens, by which T cells can be divided into CD4⁺ and CD8⁺ subtypes. CD69 is a cell surface receptor, which is upregulated upon T cell activation. The percentage of the CD4⁺CD69⁺ and CD8⁺CD69⁺ subtypes can serve as an effective indicator of the activation status of T cells. FACS-based T cell activation assays were performed to determine the ability of an exemplary EpCAM \times CD3 Fab fusion protein(*i.e.*, ITAB1002) in T cell activation.

[458] Human PBMC were prepared according to the method described in Example 2, and resuspended in RPMI 1640 Medium (Gibco) containing 10% FBS (Gibco). SW480 cells were trypsinized with 0.25% Trypsin-EDTA (Gibco), and resuspended in RPMI 1640 Medium containing 10% FBS. 50 μ L/well of cell mixture was added to each well of a 96-well plate, with a final density of 10,000 SW480 cells/well and 100,000 PBMC per well. ITAB1002 or OKT3 (Sigma) were diluted to various concentrations with RPMI 1640 Medium, and 50 μ L of the diluted ITAB1002 or OKT3 was added to each well. Control wells containing no SW480 cells(PBMC+ITAB1002) were also set up. All samples were incubated for 24 hr at 37°C.

Afterwards, the supernatant was discarded and cells were resuspended in 50 μ L FACS buffer. Antibodies CD4 Antibody (RFT-4g) APC-Cy7® conjugate (Invitrogen), CD8 Antibody (3B5) RPE conjugate(Invitrogen) and FITC Mouse Anti-Human CD69 (BD Pharmingen™) were added to the resuspended cells, and allowed to incubate at room temperature for 30 min. 150 μ L FACS buffer was added, and the samples were analyzed using ACCURI® C6 Cytometer (BD Biosciences). The detection results are shown in FIGs. 6A and 6B.

[459] As shown in FIGs. 6A and 6B, in the presence of SW480 cells, the EpCAM \times CD3 Fab fusion protein up-regulated CD69 expression in both CD4⁺ and CD8⁺ T cell populations in a dose-dependent manner (EC50=149.6 ng/mL in CD4⁺ T cells; and EC50=68.78 ng/mL in CD8⁺ T cells). In the absence of SW480 cells, the EpCAM \times CD3 Fab fusion protein did not significantly up-regulate CD69 expression on T cells. OKT3, on the other hand, up-regulated CD69 expression in both CD4⁺ and CD8⁺ T cell populations.

[460] Additionally, Ki-67 is a cell surface marker for proliferation. The percentage of the CD4⁺Ki-67⁺ subtype in the CD4⁺ cells, and the percentage of the CD8⁺ Ki-67⁺ subtype in the CD8⁺ cells can serve as an effective indicator of the proliferation status of T cells. FACS-based T cell proliferation assays were performed to determine the ability of an exemplary EpCAM \times CD3 Fab fusion protein(*i.e.*, ITAB1002) in T cell proliferation.

[461] Human PBMC were prepared according to the method described in Example 2, and resuspended in RPMI 1640 Medium (Gibco) containing 10% FBS (Gibco). SW480 cells were trypsinized with 0.25% Trypsin-EDTA (Gibco), and resuspended in RPMI 1640 Medium containing 10% FBS. cell mixture was added to each well of a 96-well plate, with a final density of 20,000 SW480 cells/well and 300,000 PBMC per well. ITAB1002 was diluted to various concentrations with RPMI 1640 Medium containing 10% FBS, and added to cells. Wells containing PBMC and ITAB1002 only (PBMC+ITAB1002), wells containing PBMC and SW480 cells only (PBMC+SW480), and wells containing only PBMC were set up as controls. 72 hr incubation was carried out at 37°C. Afterwards, the supernatant was discarded and cells were fixed and permeabilized in Fixation/Permeabilization Solution (BD Pharmingen) for 20 min at 4°C, and then resuspended in 50 μ L BD Perm/Wash™ buffer. The cells were stained with CD4 Antibody (RFT-4g) APC-Cy7® conjugate (Invitrogen), CD8 Antibody (3B5) RPE conjugate(Invitrogen) and FITC Mouse Anti- Ki-67 Set(BD Pharmingen™) for 30 min in the

dark. The samples were analyzed using ACCURI[®] C6 Cytometer (BD Biosciences). The detection results are shown in FIGs. 6C and 6D.

[462] As shown in FIGs. 6C and 6D, in the presence of SW480 cells, the EpCAM×CD3 Fab fusion protein up-regulated Ki-67 expression in both CD4⁺ and CD8⁺ T cell populations in a dose-dependent manner. In the absence of SW480 cells, the EpCAM×CD3 Fab fusion protein did not significantly up-regulate Ki-67 expression on T cells.

[463] The above data demonstrates that T cells activation by the EpCAM×CD3 Fab fusion protein is specific and dependent on its tumor antigen target.

Example 4: EpCAM×CD3 mediated PBMC cytotoxicity against tumor cells (cytotoxicity assays)

[464] Human and cynomolgus monkey PBMC were prepared according to the method described in Example 2, and resuspended in RPMI 1640 Medium (Gibco) containing 10% FBS (Gibco).

[465] SW480 cells (target cells) were trypsinized with 0.25% Trypsin-EDTA (Gibco), and resuspended in RPMI 1640 Medium containing 10% FBS. 50 μ L/well of cell mixture were added to each well of a 96-well plate, with a final density of 10,000 SW480 cells/well and 100,000 PBMC per well. An exemplary EpCAM×CD3 Fab fusion protein (*i.e.*, ITAB1002) was added to cells at concentrations according to the experimental design. Wells with no drugs (PBMC+target cells), wells containing only target cells, wells containing only PBMC, and wells containing only the medium were set up as controls. About 18 hr incubation was carried out at 37°C with 5% CO₂. CYTOTOX 96[®] Non-Radioactive Cytotoxicity Assay (Promega) was carried out to measure lactate dehydrogenase (LDH) release, and ELISA Microplate Reader (Molecular Devices, Versa Max) was used to measure OD₄₉₀. The EpCAM×CD3 Fab fusion protein mediated cytotoxicity was calculated using the following formula:

$$\text{Death rate} = (\text{OD}_{\text{sample well}} - \text{OD}_{\text{control well without drug}}) / (\text{OD}_{\text{target cell control well having maximum lysis}} - \text{OD}_{\text{target cell control well with spontaneous release}}) \times 100\%$$

[466] The GraphPad Prism 6.0 software was used for data analysis. Death rate was set as the y-axis, and drug concentration was set as the x-axis. The curves were fitted using a 4-parameter

logistic model to determine EC50. The assay results are shown in FIG. 7. The EC50 of ITB1002-mediated human PBMC cytotoxicity against SW480 cells was 41.4 ng/mL. The EC50 of ITB1002 mediated cynomolgus PBMC cytotoxicity against SW480 cells was 35.5 ng/mL.

[467] As shown in FIG. 7, the EpCAM×CD3 Fab fusion protein could mediate cynomolgus PBMC or human PBMC to kill tumor cells, such as SW480 cells. The cytotoxicity against tumor cells was comparable for human and cynomolgus monkey PBMCs.

Example 5: EpCAM×CD3 mediated human PBMC cytotoxicity against tumor cells (cytotoxicity assays)

[468] Human PBMC were prepared according to the method described in Example 2, and resuspended in RPMI 1640 Medium (Gibco) containing 10% FBS. Tumor cells (target cells) were trypsinized with 0.25% Trypsin-EDTA (Gibco), and resuspended in RPMI 1640 Medium containing 10% FBS. 50 µL/well of cell mixture was added to each well of a 96-well plate, with a final density of 10,000 tumor cells/well and 100,000 PBMC per well. An exemplary EpCAM×CD3 Fab fusion protein (*i.e.*, ITAB1002) was added to cells at concentrations according to the experimental design. Wells with no drugs (PBMC+target cells), wells containing only target cells, wells containing only PBMC, and wells containing only the medium were set up as controls. About 18 hr (48 hr for H1975 and N87 cells) incubation was carried out at 37°C with 5% CO₂. CYTOTOX 96[®] Non-Radioactive Cytotoxicity Assay (Promega) was carried out to measure LDH release, and ELISA Microplate Reader (Molecular Devices, VersaMax) was used to measure OD490. EpCAM×CD3 Fab fusion protein mediated cytotoxicity was calculated using the following formula:

$$\text{Death rate} = (\text{OD}_{\text{sample well}} - \text{OD}_{\text{control well without drug}}) / (\text{OD}_{\text{target cell control well having maximum lysis}} - \text{OD}_{\text{target cell control well with spontaneous release}}) \times 100\%$$

[469] The GraphPad Prism 6.0 software was used for data analysis. Death rate was set as the y-axis, and drug concentration was set as the x-axis. The curve was fitted using a 4-parameter logistic model to determine EC50. The assay results are shown in Table 2 and FIG. 8.

Table 2. EC50 values of EpCAM×CD3 Fab fusion protein mediated human PBMC cytotoxicity against tumor cells

cell line name	representative cancer type	EC50 (ng/mL)
SW480	human colon cancer cell	2.5
MDA-MB-453	human breast cancer cell	12.3
A549	human lung cancer cell	>5000
CFPAC-1	human pancreatic ductal cancer cell	53.2
HT29	human colon cancer cell	8.1
MiaPaCa	human pancreatic cancer	>5000
H1975	human lung cancer cell	11.9
HCC827	human lung cancer cell	15.3
N87	human gastric cancer cell	9.9
SNU-1	human gastric cancer cell	>1000
H292	human lung cancer cell	26.3
KATOIII	human gastric cancer cell	2.0
SK-OV-3	human ovarian cancer cell	34.4
MCF-7	human breast cancer cell	5.1
HepG2	human liver cancer cell	19.6
HEC-1-A	human endometrial cancer cell	1.5
OVCAR-3	human ovarian cancer cell	2.7
RBE	human liver and bile duct carcinoma	64.7
HCCC-9810	human intrahepatic cholangiocellular carcinoma	191.8

Example 6: Efficacy assay of an EpCAM×CD3 Fab fusion protein in killing subcutaneous human colon tumor xenograft in immunodeficient mice

[470] To examine the effect of an exemplary EpCAM×CD3 Fab fusion protein (i.e., ITAB1002) on inhibiting the growth of human colon tumor xenograft, *in vivo* drug efficacy assays were carried out on immunodeficient mice implanted with SW480 tumor cells.

[471] Female immunodeficient mice NOD SCID (NOD.CB17-Prkdc^{scid}/NcrCrl) were purchased from Shanghai Lingchang Biotechnology Co., Ltd., and raised in an SPF-level animal facility.

[472] Human colon cancer cells SW480 were cultured *in vitro* and collected, resuspended with serum-free L-15 Medium (Gibco) pre-cooled on ice, and placed on ice for later use. White cell concentrate donated by healthy human donors was collected, centrifuged by density gradient centrifugation (Ficoll-Paque, GE Healthcare) to obtain PBMC, resuspended in RPMI 1640 Medium (Gibco) pre-cooled on ice, and placed on ice for later use. Tumor cells and human PBMC were mixed at equal volume and inoculated subcutaneously into NOD SCID mice (about 5.0×10^6 SW480 cells and about 5.0×10^6 human PBMC per animal).

[473] One hour after the inoculation, mice were given drugs in randomly assigned groups. Each set of 30 mice were divided into 5 groups, with 6 mice per group, designated as vehicle control group, 2.5 µg/kg ITAB1002 treatment group, 25 µg/kg ITAB1002 treatment group, 250 µg/kg ITAB1002 treatment group and Cetuximab 30 mg/kg control group, respectively. The day of inoculation and grouping was defined as D0. ITAB1002 to be tested was diluted to concentrations needed using a vehicle (PBS+0.05% Tween-80), and intravenously administered to tails of the animals at a dose of 2.5 µg/kg, 25 µg/kg and 250 µg/kg, respectively. The volume of administration was 0.1 mL/10 g body weight. The animals were administered one dose per day for 5 consecutive days (D0 to D4). Animals in the vehicle control group were administered with the same volume of the vehicle. The control drug Cetuximab (Merck Serono) was diluted to concentrations needed using the vehicle, and intravenously administered to tails of the animals at a dose of 30 mg/kg, with a volume of 0.1 mL/10 g body weight, at 2 doses per week for 3 consecutive weeks.

[474] Meantime, control treatment groups were set up, in which animals were not inoculated with human PBMCs. NOD SCID mice were inoculated subcutaneously with about 5.0×10^6

SW480 tumor cells. One hour after the inoculation, the mice were randomly assigned into groups, and intravenously administered with various doses of ITAB1002 to tails of the animals, respectively.

[475] Body weight and tumor size of the animals were examined every week. Tumor volume was calculated according to the formula: tumor volume (mm^3) = length (mm) \times width (mm) \times width (mm) \times 0.5. Tumor growth inhibition rate (TGI%) was used to evaluate drug efficacy. $\text{TGI\%} = [1 - (\text{avT}_i - \text{avT}_0) / (\text{avC}_i - \text{avC}_0)] \times 100$, wherein $\text{avT}_i - \text{avT}_0$ is the average tumor size on day i minus the average tumor size on day 0 for the treatment group, and $\text{avC}_i - \text{avC}_0$ is the average tumor size on day i minus the average tumor size on day 0 for the vehicle control group. The results of tumor volume assessment are shown in FIG. 9A. Pictures of the SW480 tumors isolated from the mice at the end of the experiments are shown in FIG. 9B.

[476] As shown in FIGs. 9A and 9B, in vehicle control group, after co-inoculation of SW480 tumor cells and human PBMC, tumor cells had normal growth, with an average tumor size of 1414.06 mm^3 on day 56 after inoculation. Administration of ITAB1002 effectively inhibited growth of the SW480 tumor *in vivo* in a dose-dependent manner. The Day 56 TGI% was 46.93%, 89.90%, and 99.35% in the 2.5 $\mu\text{g/kg}$, 25 $\mu\text{g/kg}$, and 250 $\mu\text{g/kg}$ ITAB1002 treatment group, respectively. The Day 56 TGI% in the 30 mg/kg Cetuximab group was -16.97%, suggesting no inhibition of *in vivo* tumor growth of SW480. By contrast, without co-inoculation of the human PBMC, tumor cells had normal growth, and none of the control treatment groups showed signs of tumor regression (Data not shown). Thus, the cytotoxicity of the exemplary EpCAM \times CD3 Fab fusion protein against SW480 xenograft in mice is dependent on human PBMC.

[477] The results indicated the EpCAM \times CD3 Fab fusion protein could redirect immune cells to kill tumor cells, and significantly inhibit tumor growth *in vivo* in a dose-dependent manner.

Example 7: Efficacy assay of an EpCAM \times CD3 Fab fusion protein in killing subcutaneous human colon tumor xenograft in an immune-reconstructed mouse model

[478] To examine the effect of an exemplary EpCAM \times CD3 Fab fusion protein (*i.e.*, ITAB1002) on inhibiting the growth of human colon tumor xenograft, *in vivo* drug efficacy

assays were carried out on immunodeficient mice having their immune system reconstructed with human lymphocytes and implanted with SW480 tumor cells.

[479] Female immunodeficient mice NOG (NOD.Cg-Prkdc^{scid}Il2rg^{tm1Sug}/JicCr1) were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd., and raised in an SPF-level animal facility.

[480] Experiments started after the NOG mice reached a body weight of 20 g. The mice were first treated with Busulfan(Sigma) to eradicate bone marrow cells. On the second day, *in vitro* cultured human colon cancer SW480 cells were collected, thoroughly mixed and resuspended with serum-free L-15 medium (Gibco) pre-cooled on ice, and were subcutaneously inoculated into NOG mice (each animal was inoculated with about 2.5×10^6 tumor cells). The day of inoculation was defined as D0. After 2 weeks, white blood cell concentrate samples donated by healthy people were collected, centrifuged by density gradient centrifugation (Ficoll-Paque, GE Healthcare) to obtain PBMC, resuspended in RPMI 1640 Medium (Gibco) pre-cooled on ice, and inoculated subcutaneously into NOG mice (each animal was inoculated with about 3.0×10^6 tumor cells). When the tumor volume reached 150-250 mm³, the mice were randomly assigned to groups and administered with drugs. 40 animals were divided into 5 groups (8 animals per group), including model group, 2.5 µg/kg ITAB1002 treatment group, 25 µg/kg ITAB1002 treatment group, 250 µg/kg ITAB1002 treatment group, and 30 mg/kg Cetuximab control group.

[481] ITAB1002 to be tested was diluted to various concentrations using a sterile filtered vehicle (PBS+0.05% Tween-80), and intraperitoneally administered in volumes of 0.1 mL/10 g body weight (corresponding to doses of 2.5 µg/kg, 25 µg/kg and 250 µg/kg, respectively) to the mice daily for 25 consecutive days. The control drug Cetuximab (Merck Serono) was diluted to concentrations needed using a sterile filtered vehicle(PBS+0.05% Tween-80), and intraperitoneally administered at a dose of 30 mg/kg to the mice twice weekly for 25 consecutive days. Animals in the model group were administered with the same volume of the vehicle.

[482] The animals were assessed weekly for body weight and tumor size. Tumor volume was calculated according to the formula: tumor volume (mm³) = length (mm) × width (mm) × width (mm) × 0.5. Tumor growth inhibition rate (TGI%) was used to evaluate drug efficacy. $TGI\% = [1 - (avT_i - avT_0) / (avC_i - avC_0)] \times 100$, wherein $avT_i - avT_0$ is the average tumor size on day i minus the average tumor size on day 0 for the treatment group, and $avC_i - 0$ is the average tumor

size on day i minus the average tumor size on day 0 for the vehicle control group. At the end of the experiments, anticoagulated whole blood samples were collected, and stained with PE-CyTM5 Mouse Anti-Human CD3 (BD Pharmingen). The red blood cells were lysed. The percentage of human CD3⁺ T cells in the blood samples were analyzed using FACS. FIG. 10A shows tumor volume assessment results. FIG. 10B shows pictures of SW480 tumors isolated from mice at the end of the experiments.

[483] FIGs. 10A and 10B show the growth inhibitory effect of ITAB1002 against subcutaneous SW480 xenograft tumor in immune-reconstructed NOG mice inoculated with human PBMC. The results demonstrate that tumor cells could grow normally after subcutaneous inoculation of the SW480 tumor cells into NOG mice that had been immune-reconstructed with human PBMC. After 46 days of inoculation, the average tumor volume reached 738.78 mm³. Administration of ITAB1002 could effectively inhibit *in vivo* growth of the SW480 tumor, and resulted in tumor regression. In the 2.5 µg/kg ITAB1002 treatment group, the TGI% on Day 40 was 13.29%. In the 25 µg/kg ITAB1002 treatment group, the TGI% on Day 46 was 68.55%. In the 250 µg/kg ITAB1002 treatment group, the TGI% on Day 46 was 131.65%. In the 30 mg/kg Cetuximab treatment group, Cetuximab could not inhibit *in vivo* growth of the SW480 tumor, and the TGI% on Day 46 was 8.91%. As shown in FIG. 10C, the average percentage of human T cells in total white blood cells of the mice was 26.84%, indicating successful reconstruction of the human T cell immune system in the NOG mice.

[484] Therefore, after Busulfan conditioning of the bone marrow in NOG mice, and reconstruction of the immune system using human PBMC, administration of EpCAM×CD3 Fab fusion protein could effectively inhibit *in vivo* growth of the human colon cancer cells SW480 in mice, indicating the EpCAM×CD3 Fab fusion protein could mediate immune cells to kill tumor cells *in vivo*, and significantly inhibit tumor growth in a dose-dependent manner.

Example 8: Efficacy assay of an EpCAM×CD3 Fab fusion protein in killing subcutaneous human lung tumor xenograft in an immune-reconstruction mouse model

[485] To examine the effect of an exemplary EpCAM×CD3 Fab fusion protein (*i.e.*, ITAB1002) on inhibiting the growth of human lung tumor xenograft, *in vivo* drug efficacy assays

were carried out on immunodeficient mice having their immune system reconstructed with human PBMC and implanted with human lung cancer tumor cells (NCI-H1975).

[486] Female immunodeficient mice NOG (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug}/JicCrl) were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd., and raised in an SPF-level animal facility.

[487] Experiments started after the NOG mice reached a body weight of 20 g. The mice were first treated with Busulfan (Sigma) to eradicate bone marrow cells. On the second day, *in vitro* cultured human lung cancer cells NCI-H1975 were collected, thoroughly mixed and resuspended with serum-free L-15 medium (Gibco) pre-cooled on ice, and were subcutaneously inoculated into NOG mice (each animal was inoculated with about 2.5×10^6 tumor cells). The day of inoculation was defined as D0. On Day 2, white blood cell concentrate samples donated by healthy human donors were collected, centrifuged by density gradient centrifugation (Ficoll-Paque, GE Healthcare) to obtain PBMC, resuspended in RPMI 1640 Medium (Gibco) pre-cooled on ice, and inoculated subcutaneously into NOG mice (each animal was inoculated with about 3.0×10^6 PBMC, except for animals in control group). When the tumor volume reached 100-150 mm³, 18 mice were randomly assigned to three groups and administered with drugs, including control group (inoculated with tumor cells, n=6), model group (inoculated with tumor cells and PBMC, n=5), and 250 µg/kg ITAB1002 treatment group (inoculated with tumor cells and PBMC, n=7).

[488] ITAB1002 to be tested was diluted to concentrations needed using a sterile filtered vehicle (PBS+0.05% Tween-80), and intraperitoneally administered in volumes of 0.1 mL/10 g body weight (corresponding to a dose of 250 µg/kg) to the mice in the treatment group daily for 25 consecutive days. Animals in the model group and the control group were administered with the same volume of the vehicle. The animals were assessed weekly for body weight and tumor size.

[489] FIG. 11 shows the growth inhibitory effect of ITAB1002 against subcutaneous NCI-H1975 xenograft tumor in immune-reconstructed NOG mice inoculated with human PBMC. The results demonstrate that tumor cells could grow normally in both control and vehicle groups, reaching an average tumor volume of 1382.63 mm³ and 1432.15 mm³ respectively after 28 days of inoculation. Administration of ITAB1002 at 250 µg/kg could completely inhibit *in vivo*

growth of the NCI-H1975 tumor, and resulted in complete tumor regression. The average tumor volume on Day 34 in the 250 µg/kg ITAB1002 treatment group was 7.77 mm³.

[490] Therefore, administration of the exemplary EpCAM×CD3 Fab fusion protein could effectively inhibit *in vivo* growth of the human lung cancer cells NCI-H1975 in NOG mice that had been immune-reconstructed with human PBMC, indicating the EpCAM×CD3 Fab fusion protein could mediate immune cells to kill tumor cells, and significantly inhibit the tumor growth *in vivo*.

Example 9: Induced T cell redistribution, cytokine release and pharmacokinetics of an EpCAM×CD3 Fab fusion protein in cynomolgus monkeys

[491] Cynomolgus monkeys (regular grade), age: 3-5, body weight: 3.0-5.0 kg. 16 cynomolgus monkeys were divided into four groups for administration of an exemplary EpCAM×CD3 Fab fusion protein (*i.e.*, ITAB1002), designated as 0.5 µg/kg dosage group (n=3), 5 µg/kg dosage group (n=4), 15 µg/kg dosage group (n=6) and 50 µg/kg dosage group (n=3) respectively.

[492] ITAB1002 to be tested was diluted with vehicle (PBS+0.5% monkey serum) to various concentrations, and was administered at a volume of 2.0 mL/kg body weight. ITAB1002 was administered with a dosage of 0.5 µg/kg, 5 µg/kg, 15 µg/kg and 50 µg/kg, respectively, by intravenous infusion at forearms for 1 hr, single dose. Animal symptoms, behaviors, mental states, and feces, *etc.* were observed every day.

[493] Whole blood collection: EDTA-K2 coated VACUTAINER[®] anticoagulant blood collection tubes (BD Bioscience) were used to collect 0.3 mL blood samples before drug infusion and at different time points after drug infusion. The collected anticoagulant blood samples were separated into two portions: one portion (100 µL) of the blood was analyzed for lymphocyte subtypes using automated blood cell counter (Siemens, ADVIA[®] 2120); the other portion (200 µL) of the blood was stained for CD4 and CD8 antigens (APC Mouse Anti-Human CD4 and PE Mouse Anti-Human CD8, BD PHARMINGEN[™]), and subjected to analysis of the CD4⁺ and CD8⁺ lymphocyte subtypes using FACS (Beckman Coulter, Cytomics FC 500).

[494] Serum collection: Non-anticoagulant blood collection tubes were also used to collect 1.2 mL blood samples before drug infusion and at different time points after drug infusion.

Serum was collected after coagulation, and stored at -80°C. Non-Human Primate Th1/Th2 Cytokine Kit (BD PHARMINGEN™) was used to detect the secretion of IL-2, IL-4, IL-5, IL-6, TNF and IFN- γ cytokines in the serum. Drug concentration within the serum was measured using an enzyme-linked immunosorbent assay (ELISA). PK Solver 2.0 software was used to calculate pharmacokinetic parameters.

[495] No death or near-death was observed in any group of monkeys during the experimental period. All groups of monkeys had normal spontaneous behaviors, good mental status, clean skin and hair. No irritation or fester was observed at the infusion areas. EpCAM \times CD3 Fab fusion protein showed good safety and tolerance in cynomolgus monkeys.

[496] FIG. 12A shows a plot of change in the number of CD4⁺ T cells over time in the blood of cynomolgus monkeys after single intravenous administration of the EpCAM \times CD3 Fab fusion protein at various dosages (“H” is hour and “D” is day on the x-axis). After drug administration, the number of CD4⁺ T cells in the blood decreased, with the lowest cell number observed 8 hours post drug infusion, then cell number started to gradually recover.

[497] FIG. 12B shows a plot of change in the number of CD8⁺ T cells over time in the blood of cynomolgus monkeys after single intravenous administration of the EpCAM \times CD3 Fab fusion protein at various dosages (“H” is hour and “D” is day on the x-axis). After drug administration, the number of CD8⁺ T cells in the blood decreased, with the lowest cell number observed 8 hours post drug infusion, then cell number started to gradually recover.

[498] FIGs. 13A-13F shows change of the concentration of IL-2 (FIG. 13A), IL-4 (FIG. 13B), IL-5 (FIG. 13C), IL-6 (FIG. 13D), TNF (FIG. 13E) and IFN- γ (FIG. 13F) over time in the serum of cynomolgus monkeys after single intravenous administration of the EpCAM \times CD3 Fab fusion protein at various dosages. The results indicated that after the single intravenous administration of the EpCAM \times CD3 Fab fusion protein, the serum concentrations of IL-2, IL-6, TNF and IFN- γ increased to different extents, then decreased back to baseline level after reaching a peak concentration, and exhibited a dose-dependent relationship, while the serum concentrations of IL-4 and IL-5 did not change significantly.

[499] FIG. 14 depicts the change of drug concentration over time in the serum of cynomolgus monkeys after single intravenous administration of the EpCAM \times CD3 Fab fusion protein at various dosages.

[500] The results indicated that for all three dosage groups (5 µg/kg, 15 µg/kg and 50 µg/kg), the serum concentration of drug reached a peak level after 1 hour of single intravenous administration of the EpCAM×CD3 Fab fusion protein, then gradually decreased. The EpCAM×CD3 Fab fusion protein exhibited increased *in vivo* half-life in cynomolgus monkeys.

[501] Detailed pharmacokinetic parameters of the EpCAM×CD3 fusion protein are shown in Table 3

Table 3. *In vivo* pharmacokinetic parameters of the exemplary EpCAM×CD3 Fab fusion protein in cynomolgus monkeys (Mean ± SD)

Dose	t _{1/2} (hr)	C _{max} (ng/mL)	AUC 0-t (ng/mL*hr)	MRT 0-inf_obs (hr)	Cl_obs (mL/hr/kg)
ITAB1002 5 µg/kg	15.8±0.9	237.9±66.9	1781.6±338.7	13.8±1.0	2.8±0.5
ITAB1002 15 µg/kg	19.5±7.5	429.0±116.2	4378.8±937.1	20.4±4.5	3.4±0.8
ITAB1002 50 µg/kg	14.6±2.0	1182.4±271.5	20899.8±4680.9	18.2±2.0	2.4±0.5

Example 10: Comparison of ITAB1002 and ITAB1012 mediated PBMC cytotoxicity against tumor cells

[502] ITAB1012 is an EpCAMA×CD3 Fab fusion protein comprising an EpCAM scFv having different HVRs from those in the EpCAM scFv of ITAB1002. ITAB1012 was transiently expressed and purified as described in Example 1. The heavy chain of ITAB1012 has the amino acid sequence of SEQ ID NO: 35, and is encoded by the nucleic acid sequence of SEQ ID NO: 37. The light chain of ITAB1012 has the amino acid sequence of SEQ ID NO: 36, and is encoded by the nucleic acid sequence of SEQ ID No: 38. The HVRs of the EpCAM scFv fragments are underlined in the sequences below. The EpCAM scFv of ITAB1012 has previously been described in U.S. Patent No. 8,846,042.

SEQ ID No: 35 (ITAB1012 Heavy Chain)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVANIKQDGSE
KFYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDMAVYYCARVGGAWELGYWGQGT
 LVTVSAGGGGSGGGGSGGGGSGAQSFLTQPPSVSGAPGQRTISCTGSSSNIGSYYGVHW
 YQQLPGTAPKLLIYSDTNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSL
 SGRVFGGGTKLTVLGGEVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAP
 GKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYC
 VRHGNFGNSYVSWFAYWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
 YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN
 TKVDKKVEPKSCPPCS

SEQ ID No: 36 (ITAB1012 Light Chain)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMSWVRQAPGKGLEWVANIKQDGSE
KFYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDMAVYYCARVGGAWELGYWGQGT
 LVTVSAGGGGSGGGGSGGGGSGAQSFLTQPPSVSGAPGQRTISCTGSSSNIGSYYGVHW
 YQQLPGTAPKLLIYSDTNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSL
 SGRVFGGGTKLTVLGQAVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQKQP
 GQAPRGLIGGTNKRAPGTPARFSGSLLGKAALTLSGVQPEDEAEYYCALWYSNLWVF
 GGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
 AGVETTPSKQSNKYAASSYLSLTPEQWKSQRSYSQVTHEGSTVEKTVAPTECPPCS

SEQ ID NO: 37 (Nucleic acid encoding ITAB1012 Heavy Chain)

gaggtgcagctggtggagtcagggggaggcttggtccagcctgggggatcactgagactctctgtgcagcctctggattcaccttagta
 attattgtagctgggtccgccaggctccagggaagggctggagtggtggccaacataaagcaagatggaagtgagaaattctatg
 tggactctgtgaagggccgattaccatctccagagacaacgccaaagaactcactgtatctgcaaatgaacagcctgagagccgaagacat
 ggctgtctattactgtgcgagagtggtggggggcgtgggagctaggctactggggccagggaaccctggcactgtctcggccggtggcg
 gtggcagcggtggtggtgggtccggtggcgccgagctggcgcgagctgtactgactcaaccgccctcagtgctcggggcccagg
 gaggggtcaccatctctgactgggagcagctccaacatcggtcttattatggtgtgactggtaccagcagctccaggaaacagccc
 ccaaaactctatctattctgacactaatgacctcaggggtccctgaccgattctctggtccaagtctggcacctcggcctccctggccat
 cactgggtccaggctgaggatgaggctgattattactgccagctgtatgacagcagcctgagtggtggcggtgtcggcgagggaacaa
 gctgacagtactaggtggcgaggtgcagctggtggagctcggggaggcttggtacagcctgggggggtccctgagactctctgtgcagc
 ctctggattcaccttaacacctacgccatgaactgggtccgccaggctccagggaagggctggagtggtcgcacgcataagaagtaa
 atataataattatgcaacatattatgccgattcagtgaaagaccggttcacatctccagagacgattccaagaacacgctgtatctgcaaatg
 aacagcctgagagccgaggacacggcgtatattactgtgtgagacatgggaactcggtaatactacgtttctggttgccttactggggc
 caagggaacatgggtaccgtctctcagctagcaccagggcccatccgtcttcccctggcacctcctccaagagcacctctgggggca
 cagcgccctgggtgctggtcaaggactactccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggtgcac
 acctcccggctgtctacagctcaggaactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacat
 ctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaatctgtccaccgtgctcatga

SEQ ID NO: 38 (Nucleic acid encoding ITAB1012 Light Chain)

gaggtgcagctggtggagtcagggggaggcttggtccagcctgggggatcactgagactctctgtgcagcctctggattcaccttagta
 attattgtagctgggtccgccaggctccagggaagggctggagtggtggccaacataaagcaagatggaagtgagaaattctatg
 tggactctgtgaagggccgattaccatctccagagacaacgccaaagaactcactgtatctgcaaatgaacagcctgagagccgaagacat
 ggctgtctattactgtgcgagagtggtggggggcgtgggagctaggctactggggccagggaaccctggcactgtctcggccggtggcg
 gtggcagcggtggtggtgggtccggtggcgccgagctggcgcgagctgtactgactcaaccgccctcagtgctcggggcccagg
 gaggggtcaccatctctgactgggagcagctccaacatcggtcttattatggtgtgactggtaccagcagctccaggaaacagccc
 ccaaaactctatctattctgacactaatgacctcaggggtccctgaccgattctctggtccaagtctggcacctcggcctccctggccat

cactgggctccaggctgaggatgaggctgattattactgccagtcgtatgacagcagcctgagtggccgggtgttcggcggaggaccacaa
 gctgacagtactaggtggccaggctgtggtgactcaggagccctcactgactgtgtcccaggaggacagtcactctcactgtcgtccta
 tccactggggctgttacaactagtaactatgccaactgggtccagcagaaacctggacaagcaccagggggtctgattggtggtaccaaca
 agcgagctccagggtaccctgcccgggtctcaggctccctccttgggggcaaagctgcctgacactgtcaggtgtgcagcctgaggacg
 aggtgagttactgctgtctatggtacagcaacctctgggtgttcggcggaggggaccaagctgaccgtccta ggccaaccgaaagcg
 gcgcctcgggtcactctgttcccgcctcctctgaggagcttcaagccaacaagccacactgggtgtgtctcataagtacttctacccggg
 agccgtgacagtggcctggaaggcagatagcagccccgtcaaggcgggagtgaggagaccaccacacctccaaacaaagcaacaacaa
 gtacgcggccagcagctatctgagcctgacgcctgagcagtggaagtccacagaagctacagctgccaggtcacgcatgaaggggagc
 accgtggagaagacagtggtggccctacagaa tgcaccgtgctcatga

[503] ITAB1002 and ITAB1012 were tested in a cytotoxicity assay using human PBMC as described in Example 5. Results are shown in FIG. 15. The EC₅₀ of ITAB1002-mediated human PBMC cytotoxicity against SW480 cells was about 3.54 ng/mL. In comparison, the EC₅₀ of ITAB1012-mediated human PBMC cytotoxicity against SW480 cells was about 223.3 ng/mL. Thus, ITAB1002 exhibited significantly higher activity of mediating human PBMC cytotoxicity against tumor cells when compared to ITAB1012.

[504] Additionally, the binding affinity of the anti-EpCAM and anti-CD3 domains of exemplary EpCAM×CD3 Fab fusion proteins (*i.e.*, ITAB1002 and ITAB1012) with the human antigens were measured using the methods as described in example 2. As shown in table 4, ITAB1002 exhibited stronger binding affinity with EpCAM, while its binding affinity with CD3 is weaker when compared to ITAB1012.

Table 4. *In vitro* binding affinities (KD)

MSFP	Domain	Antigen	K _{on} (M ⁻¹ s ⁻¹)	K _{off} (s ⁻¹)	K _d (M)
ITAB1002	Anti-EpCAM	huEpCAM.Fc	2.00 x 10 ⁺⁴	4.98 x 10 ⁻⁵	2.49 x 10 ⁻⁹
	Anti-CD3	CD3εAA 1-27.Fc	8.64 x 10 ⁺⁴	8.50 x 10 ⁻⁴	9.84 x 10 ⁻⁹
ITAB1012	Anti-EpCAM	huEpCAM.Fc	1.35 x 10 ⁺⁵	6.47 x 10 ⁻³	4.78 x 10 ⁻⁸
	Anti-CD3	CD3εAA 1-27.Fc	8.72 x 10 ⁺⁴	3.54 x 10 ⁻⁵	4.05 x 10 ⁻¹⁰

Example 11: EpCAM×CD3 Fab fusion protein mediated human PBMC cytotoxicity against tumor cells in the presence of steroid.

[505] An *in vitro* exploratory assay was performed to assess the effect of steroid pre-treatment on the tumor cell killing activity and cytokine release by human T cells as induced by ITAB1002.

[506] The cytotoxicity assay was performed as described in Example 5. PBMCs were isolated from healthy donors and incubated with dexamethasone (DXM) at the concentration of 3 μ M for 1 hour, then added to wells of a 96-well plate, with a final density of 30,000 SW480 cells and 300,000 PBMC per well. Mixed Cells were incubated at 37°C, 5% CO₂ with final concentration of DXM at 0.15 μ M for about 18 hrs. Cell killing was measured by lactate dehydrogenase (LDH) assay and calculated. In the same assay, cytokine (*i.e.*, IL-6) release was analyzed by human IL-6 ELISA Set (BD Biosciences).

[507] FIG. 16 shows the effect of dexamethasone (DXM) on ITAB1002-mediated SW480 cells killing activity. ITAB1002 exhibited comparable killing activity against SW480 cells *in vitro* in the presence of dexamethasone compared to ITAB1002 alone.

[508] FIG. 17 shows results from the cytokine release assay. IL-6 was released by activated T cells induced by ITAB1002. However, IL-6 release was almost completely inhibited in the presence of dexamethasone, suggesting that DXM treatment might be an effective pre-medication strategy to control cytokine release syndrome (CRS) in patients receiving ITAB1002.

Example 12: Pre-clinical study of EpCAM×CD3 Fab fusion protein in cynomolgus monkeys

[509] An exploratory study was conducted to evaluate the effect of dexamethasone pre-treatment on alleviating potential toxicity in cynomolgus monkeys that received ITAB1002 treatment. 6 monkeys (ITAB1002+DXM) were treated with ITAB1002 at 0.5 μ g/kg twice (on day 1 and day 4) weekly via intravenous (IV) infusion during the first week. During the second week, the animals were treated with ITAB1002 at 1.0 μ g/kg twice (Day 8 and Day 11) weekly by IV infusion. During the 3rd week, the animals were treated with ITAB1002 at 2.0 μ g/kg twice (Day 15 and Day 18) weekly by IV infusion. During the 4th and 5th weeks, the animals were treated with ITAB1002 at 4.0 μ g/kg twice (Day 22, 25, 29 and 32) weekly by IV infusion.

Dexamethasone at a dose of 1 mg/kg was administered to each animal via intravenous injection (IV) about 1 hour prior to the first infusion at each dose level. Dexamethasone was increased to 2 mg/kg in week 5. In the group without DXM pre-treatment (n=10), ITAB1002 was administered to each monkey at 2.0 µg/kg via intravenously infusion twice weekly without any DXM pre-treatment. Blood samples were collected at different time points and serum chemistry parameters were analyzed. Serum IL-6 levels were analyzed by human IL-6 ELISA Set (BD Biosciences).

[510] Pre-treatment with DXM prior to ITAB1002 remarkably reduced the serum ALT (FIGs. 18A-18B), TBil (FIGs. 19A-19B) and ALP levels (FIGs. 20A-20B), as well as inhibited IL-6 release (FIGs. 21A-21B) when compared to treatment without DXM pre-treatment. These results demonstrated that the early induction of IL-6 release and side effects in liver in monkeys treated with ITAB1002 could be ameliorated by DXM pre-treatment.

Example 13: Clinical testing of ITAB1002 in human cancer patients

[511] The safety and efficacy of ITAB1002 is assessed in a clinical trial in human cancer patients. The aim of the trial is to evaluate the safety, tolerability, pharmacokinetics, immunogenicity and antitumor activity of ITAB1002 in adult subjects with locally advanced or metastatic solid tumor for whom standard therapy either does not exist or has proven to be ineffective or intolerable. Five dose levels at 0.3, 0.6, 1.2, 2.4 and 3.6 µg/kg are assessed by dose escalation. The conventional 3+3 design (3 patients per dose cohort, with the potential to add 3 additional patients to the same cohort to further evaluate toxicity) are applied for dose escalation and MTD determination. 5 cohorts with about 6 patients per group are enrolled.

[512] All patients receive a conditioning dose of 0.3 µg/kg of ITAB1002 twice (on day 1 and day 4) via intravenous (IV) infusion during the first week. In the next three weeks, patients in the 5 cohorts receive ITAB1002 twice weekly by IV infusion at the one of the following doses: 0.3 µg/kg, 0.6 µg/kg, 1.2 µg/kg, 2.4 µg/kg and 3.6 µg/kg, respectively. Dexamethasone at the dose of 20 mg is given 1 hour prior to the first conditioning dose of ITAB1002 and the first ramp-up dose (0.3 µg/kg to 3.6 µg/kg). Administration of dexamethasone prior to the subsequent doses of ITAB1002 is given at either 20 mg or 10 mg based on clinical assessment. Table 4 below shows the dosing schedule of the five cohorts in the study.

Table 4. Dosing schedule of human clinical trial.

Cohort No.	Week 1			Week2			Week 3		Week 4	
	1hr prior	Day 1	Day 4	1hr prior	Day 1	Day 4	Day 1	Day 4	Day 1	Day 4
1	DXM	0.3*	0.3	DXM	0.3	0.3	0.3	0.3	0.3	0.3
2	DXM	0.3	0.3	DXM	0.6	0.6	0.6	0.6	0.6	0.6
3	DXM	0.3	0.3	DXM	1.2	1.2	1.2	1.2	1.2	1.2
4	DXM	0.3	0.3	DXM	2.4	2.4	2.4	2.4	2.4	2.4
5	DXM	0.3	0.3	DXM	3.6	3.6	3.6	3.6	3.6	3.6

* Doses of ITAB1002 are in $\mu\text{g/kg}$.

[513] All references mentioned in the present invention are incorporated herein by reference as if each of those references has been incorporated by reference individually. Although the description referred to particular embodiments, it will be clear to a person skilled in the art that the present invention may be practiced with variation of these specific details. Hence this invention should not be construed as limited to the embodiments set forth herein.

CLAIMS

1. An anti-EpCAM antibody or antigen-binding fragment thereof, comprising a heavy chain variable region (VH) and a light chain variable region (VL),
wherein the VH comprises: (1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13; (2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; and (3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15;
wherein the VL comprises: (1) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 16; (2) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 17; and (3) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 18.
2. The anti-EpCAM antibody or antigen-binding fragment thereof of claim 1, wherein the VH comprises the amino acid sequence of SEQ ID NO: 19; and/or wherein the VL comprises the amino acid sequence of SEQ ID NO: 20.
3. The antigen-binding fragment of the anti-EpCAM antibody of claim 1 or claim 2, wherein the antigen-binding fragment is a single-chain Fv (scFv).
4. The antigen-binding fragment of the anti-EpCAM antibody of claim 3, wherein the scFv comprises the amino acid sequence of SEQ ID NO: 21.
5. A multispecific Fab fusion protein comprising the anti-EpCAM antigen-binding fragment of any one of claims 1-4.
6. The multispecific Fab fusion protein of claim 5, wherein the multispecific Fab fusion protein comprises:
 - (a)(i) a Fab fragment that specifically binds to CD3 ("anti-CD3 Fab fragment"), optionally wherein the anti-CD3 Fab fragment specifically binds the N-terminus of CD3 epsilon, optionally wherein the anti-CD3 Fab fragment specifically binds an epitope within amino acids 1-27 of CD3 epsilon, and (ii) an anti-EpCAM antigen-binding fragment, wherein the anti-EpCAM antigen-binding fragment is fused to the N-terminus of the VH or the VL of the anti-CD3 Fab fragment; or
 - (b)(i) a Fab fragment that specifically binds to CD3 ("anti-CD3 Fab fragment"), optionally wherein the anti-CD3 Fab fragment specifically binds the N-terminus of CD3 epsilon, optionally wherein the anti-CD3 Fab fragment specifically binds an epitope within amino acids 1-27 of

CD3 epsilon, (ii) a first copy of the anti-EpCAM antigen-binding fragment, and (iii) a second copy of the anti-EpCAM antigen-binding fragment; wherein the first copy of the anti-EpCAM antigen-binding fragment is fused to the N-terminus of the VH of the anti-CD3 Fab fragment; and wherein the second copy of the anti-EpCAM antigen-binding fragment is fused to the N-terminus of the VL of the anti-CD3 Fab fragment.

7. The multispecific Fab fusion protein of claim 6, wherein the VH of the anti-CD3 Fab fragment comprises: (1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1; (2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2; and (3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, and wherein the VL of the anti-CD3 Fab fragment comprises: (1) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4; (2) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5; and (3) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6.

8. The multispecific Fab fusion protein of claim 6 or claim 7, wherein the VH of the anti-CD3 Fab fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 7 and 39-43; and/or wherein the VL of the anti-CD3 Fab fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 44-47.

9. The multispecific Fab fusion protein of any one of claims 6-8, wherein the anti-CD3 Fab fragment comprises a human immunoglobulin heavy chain constant region 1 (CH1) comprising the amino acid sequence of SEQ ID NO: 9; and/or wherein the anti-CD3 Fab fragment comprises a human lambda light chain constant region (CL) comprising the amino acid sequence of SEQ ID NO: 10;

optionally wherein the anti-CD3 Fab fragment comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 11, and/or a second polypeptide comprising the amino acid sequence of SEQ ID NO: 12;

optionally wherein the CH1 and the CL of the anti-CD3 Fab fragment are connected by one or more disulfide bonds.

10. The multispecific Fab fusion protein of any one of claims 6-9, wherein the multispecific Fab fusion protein comprises:

(i) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 22, and/or a second polypeptide comprising the amino acid sequence of SEQ ID NO: 23;

(ii) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 11, and/or a second polypeptide comprising the amino acid sequence of SEQ ID NO: 23; or

(ii) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 22, and/or a second polypeptide comprising the amino acid sequence of SEQ ID NO: 12.

11. A composition comprising the anti-EpCAM antibody or antigen-binding fragment thereof of any one of claims 1-4 or the multispecific Fab fusion protein of any one of claims 5-10, and optionally a pharmaceutically acceptable carrier.

12. A method of treating an EpCAM-positive cancer in an individual, comprising administering to the individual an effective amount of the anti-EpCAM antibody or antigen-binding fragment thereof of any one of claims 1-4, the multispecific Fab fusion protein of any one of claims 5-10, or the composition of claim 11, optionally wherein the EpCAM-positive cancer is a carcinoma or an adenocarcinoma.

13. Use of an effective amount of the anti-EpCAM antibody or antigen-binding fragment thereof of any one of claims 1-4, the multispecific Fab fusion protein of any one of claims 5-10, or the composition of claim 11 in the preparation of a medicament for treating an EpCAM-positive cancer in an individual, optionally wherein the EpCAM-positive cancer is a carcinoma or an adenocarcinoma.

14. The method of claim 12 or the use of claim 13, wherein the effective amount is about 0.01 µg/kg to about 250 µg/kg.

15. A method of treating an EpCAM-positive cancer in an individual, comprising administering to the individual an effective amount of a multispecific Fab fusion protein comprising: (i) an anti-CD3 Fab fragment, and (ii) a binding domain that specifically binds to EpCAM ("anti-EpCAM binding domain"), wherein the anti-EpCAM binding domain comprises the anti-EpCAM antigen-binding fragment of any one of claims 1-4; wherein the anti-EpCAM binding domain is fused to an N-terminus of the anti-CD3 Fab fragment; optionally wherein the multispecific Fab fusion protein is administered at a dose of about 0.01 µg/kg to about 250 µg/kg; optionally wherein the EpCAM-positive cancer is a carcinoma or an adenocarcinoma.

16. Use of an effective amount of a multispecific Fab fusion protein in the preparation of a medicament for treating an EpCAM-positive cancer in an individual, wherein the multispecific

Fab fusion protein comprises (i) an anti-CD3 Fab fragment and (ii) an anti-EpCAM binding domain, wherein the anti-EpCAM binding domain comprises the anti-EpCAM antigen-binding fragment of any one of claims 1-4; wherein the anti-EpCAM binding domain is fused to an N-terminus of the anti-CD3 Fab fragment; optionally wherein the medicament is administered at a dose of about 0.01 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$; optionally wherein the EpCAM-positive cancer is a carcinoma or an adenocarcinoma.

17. The method of any one of claims 12, 14, and 15, or the use of any one of claims 13, 14, and 16, wherein the EpCAM-positive cancer is selected from the group consisting of small intestine cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, gastric cancer, pancreatic cancer, skin cancer, renal cancer, bladder cancer, thyroid cancer, prostate cancer, ovarian cancer, endometrial cancer, breast cancer, bile duct cancer, and head and neck cancer; optionally wherein the cancer is colorectal adenocarcinoma or lung adenocarcinoma.

18. The method of any one of claims 12, 14, 15, and 17, or the use of any one of claims 13, 14, 16, and 17, wherein the anti-EpCAM antibody or antigen-binding fragment thereof, the multispecific Fab fusion protein, the composition, or the medicament:

- (i) is administered intravenously;
- (ii) is administered at a low frequency; and/or
- (iii) is administered at a first dose for a first period of time to the individual, then administered at a second dose for a second period of time to the individual, wherein the second dose exceeds the first dose; and/or
- (iv) is administered in combination with a glucocorticoid to the individual, optionally wherein the glucocorticoid is dexamethasone.

19. An isolated nucleic acid encoding the anti-EpCAM antibody or antigen-binding fragment thereof of any one of claims 1-4 or the multispecific Fab fusion protein of any one of claims 5-10.

20. A vector comprising the isolated nucleic acid of claim 19.

21. An isolated host cell comprising the nucleic acid of claim 19, or the vector of claim 20.

22. A method of producing an anti-EpCAM antibody or antigen-binding fragment thereof or a multispecific Fab fusion protein, comprising

(i) culturing a host cell comprising the nucleic acid of claim 19, a vector of claim 20, or the isolated host cell of claim 21, under a condition suitable for the expression of the anti-EpCAM antibody or antigen-binding fragment thereof or the multispecific Fab fusion protein; and

(ii) recovering the expressed anti-EpCAM antibody or antigen-binding fragment thereof or the multispecific Fab fusion protein from the cell culture.

ITabMed [HK] Limited
Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

FIG. 1

α EpCAM X α CD3

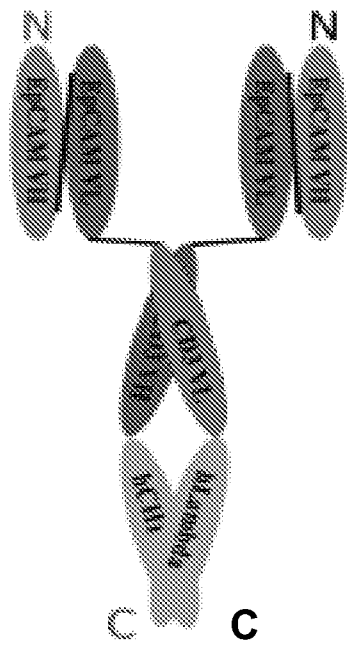


FIG. 2A

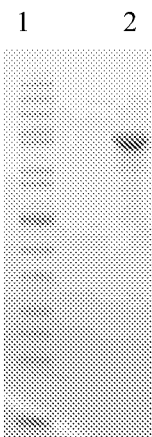


FIG. 2B

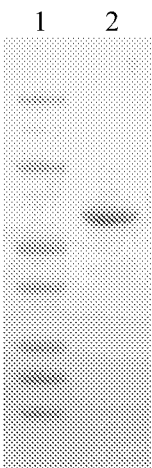


FIG. 3A

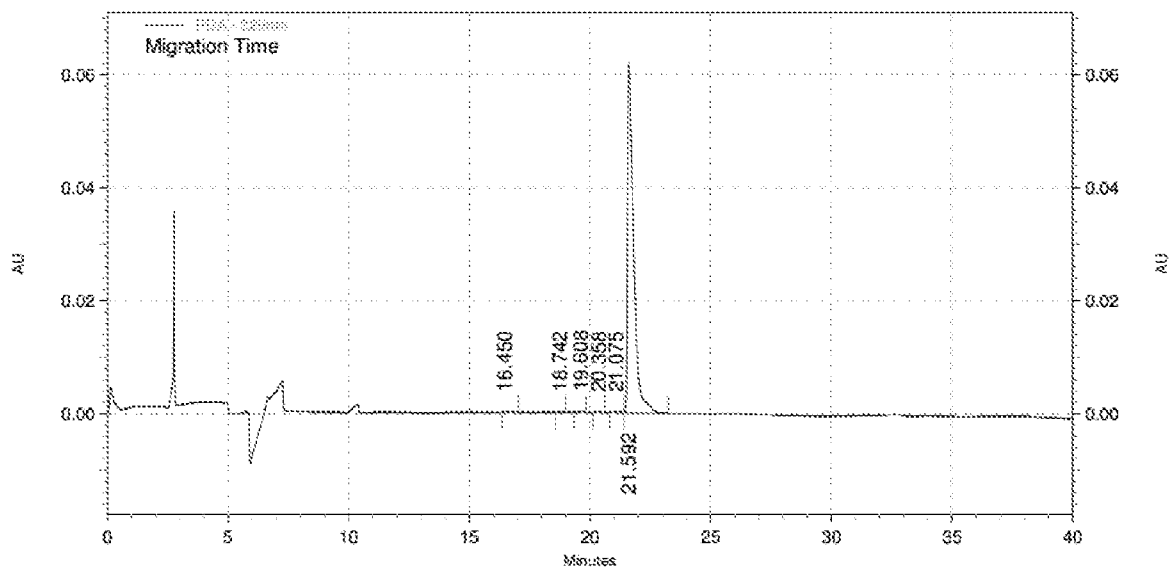


FIG. 3B

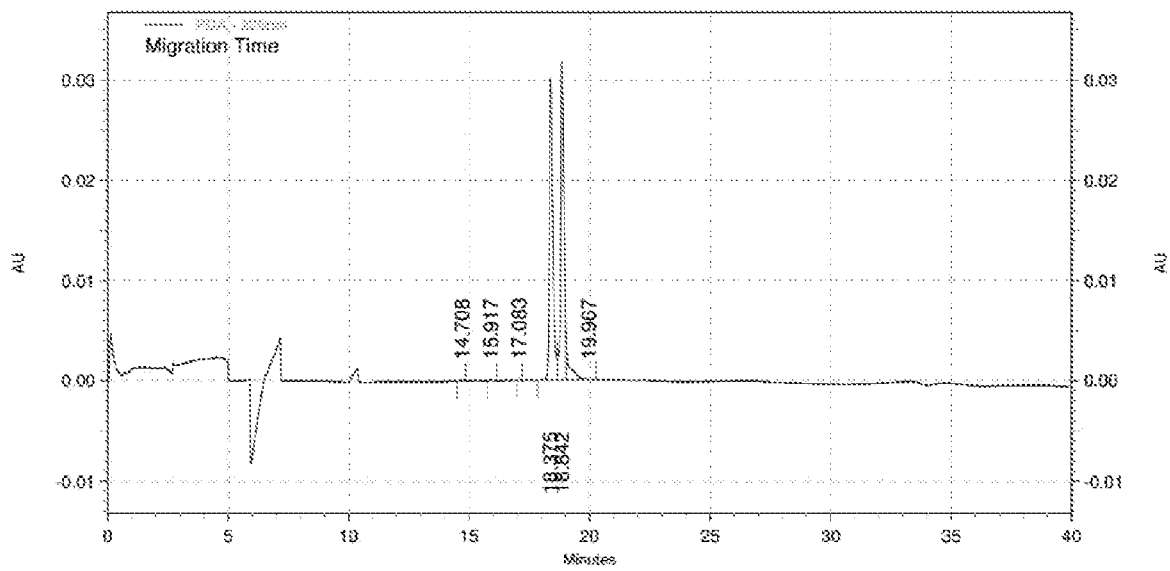


FIG. 4A

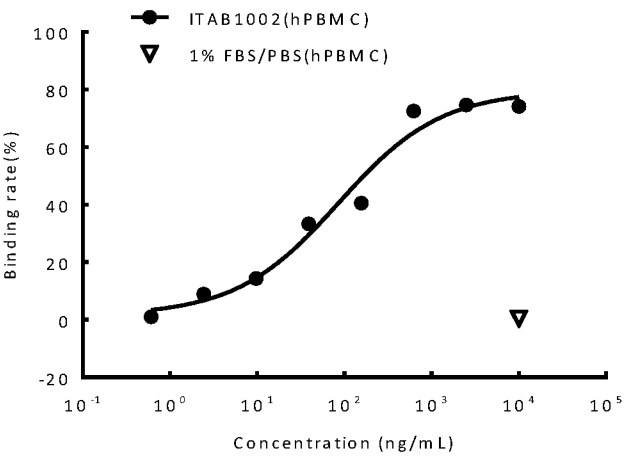


FIG. 4B

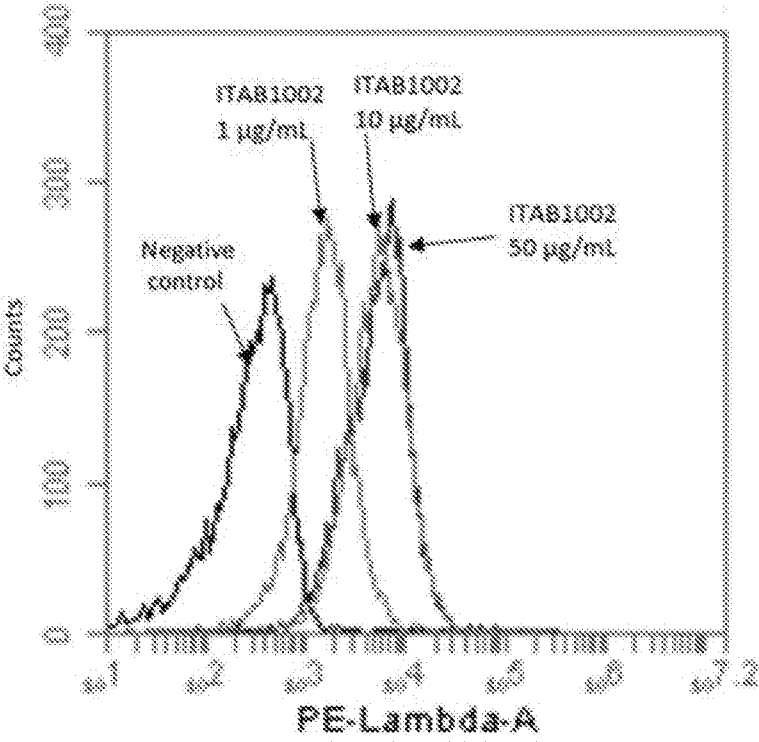


FIG. 5

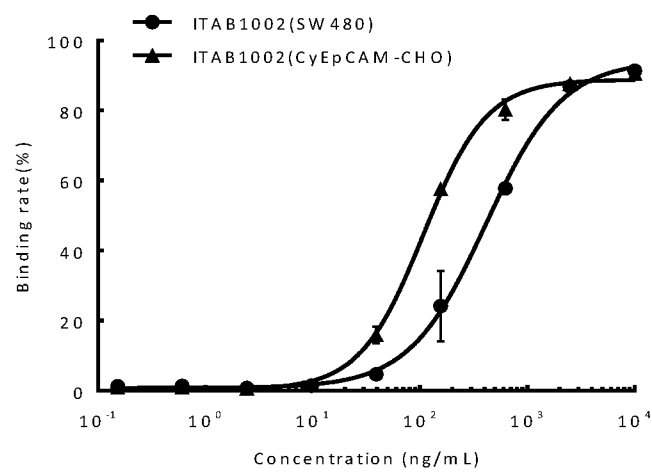


FIG. 6A

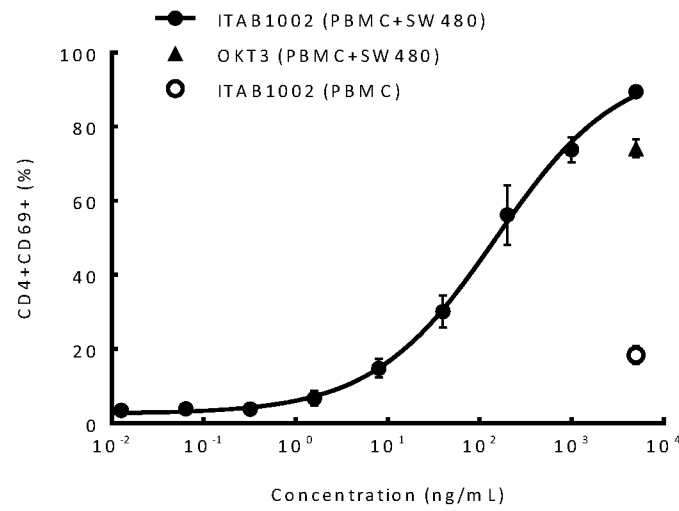


FIG. 6B

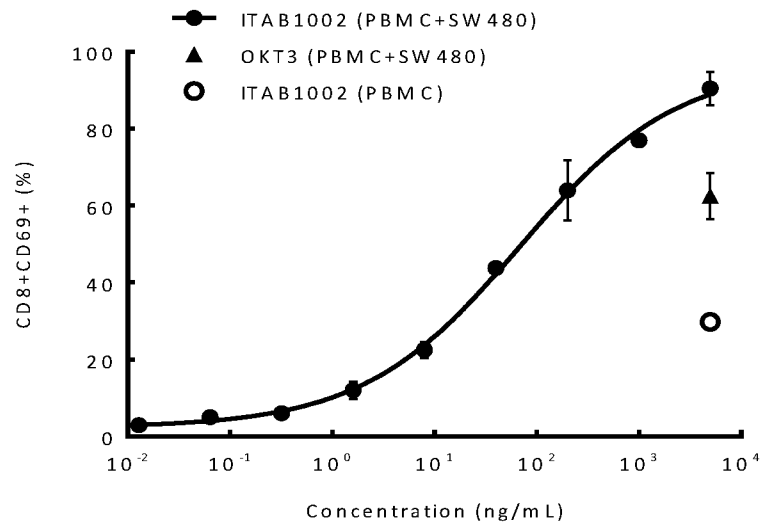


FIG. 6C

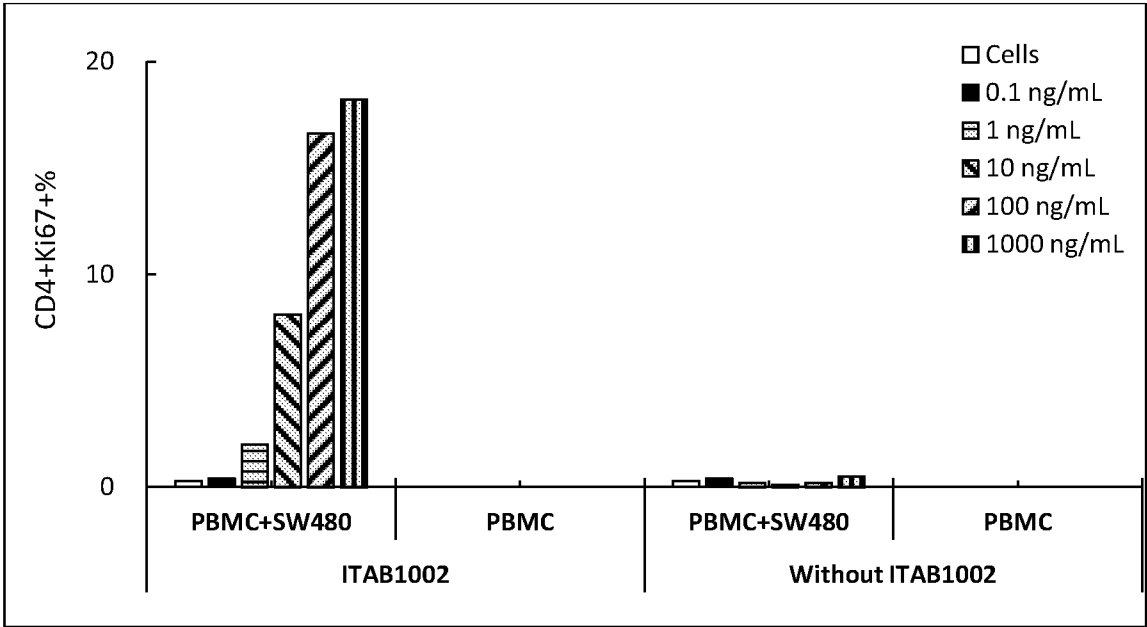


FIG. 6D

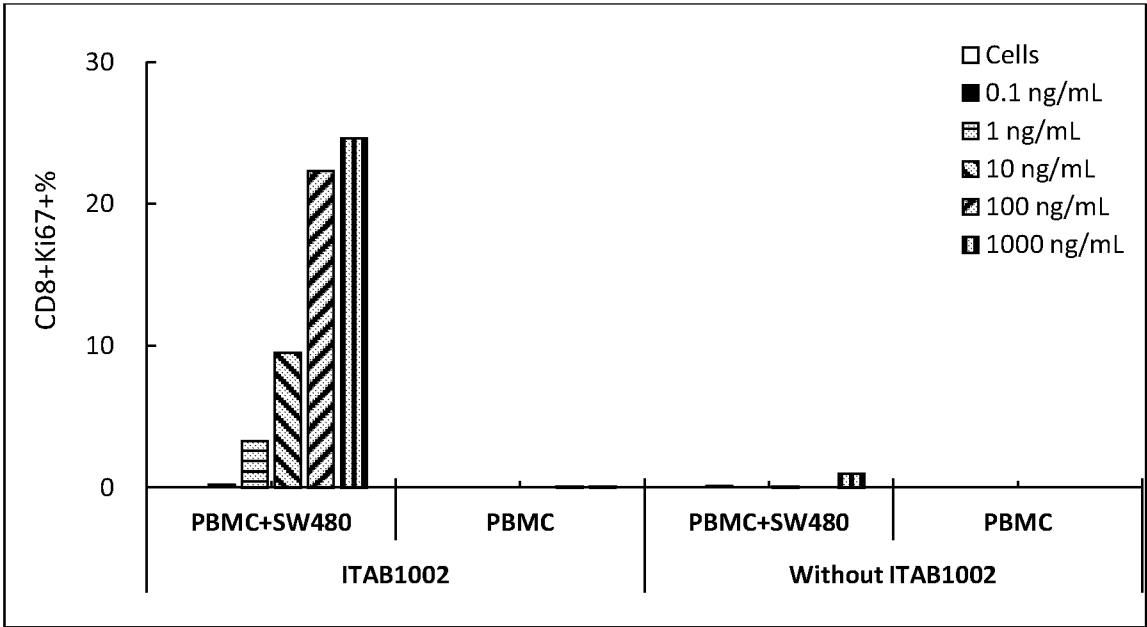


FIG. 7

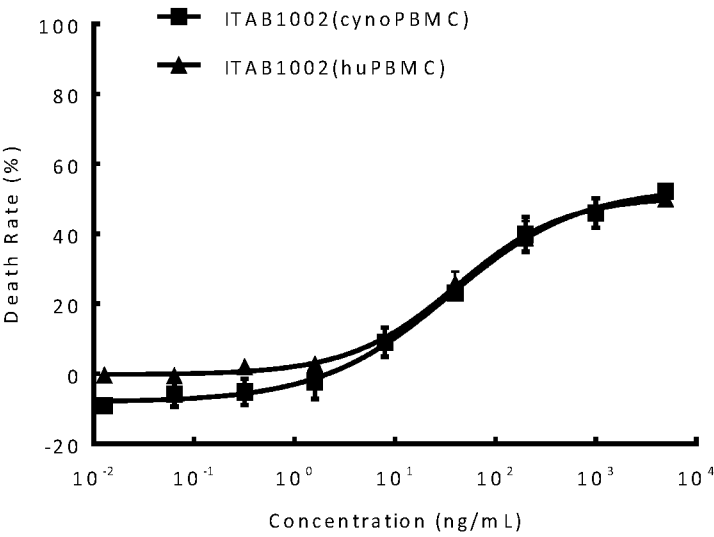


FIG. 8

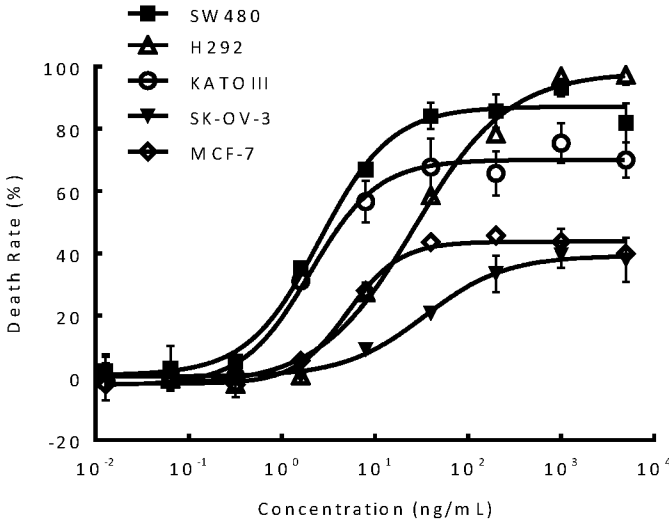


FIG. 9A

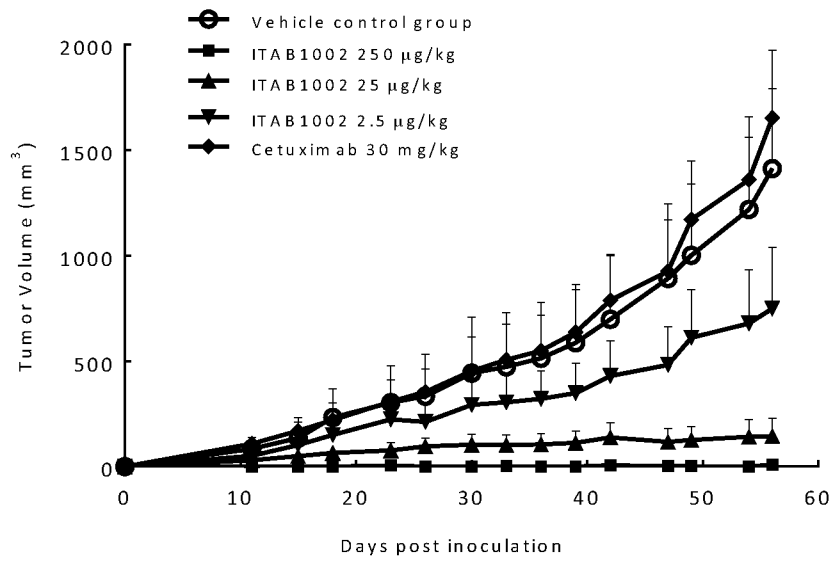


FIG. 9B

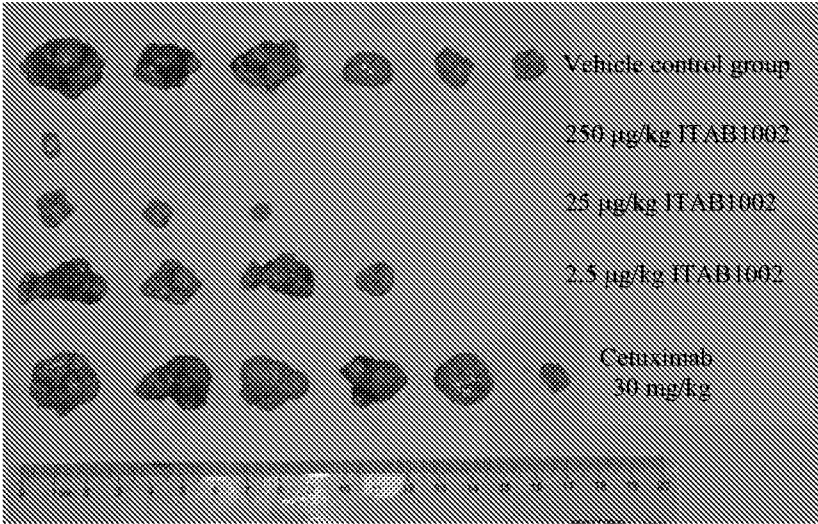


FIG. 10A

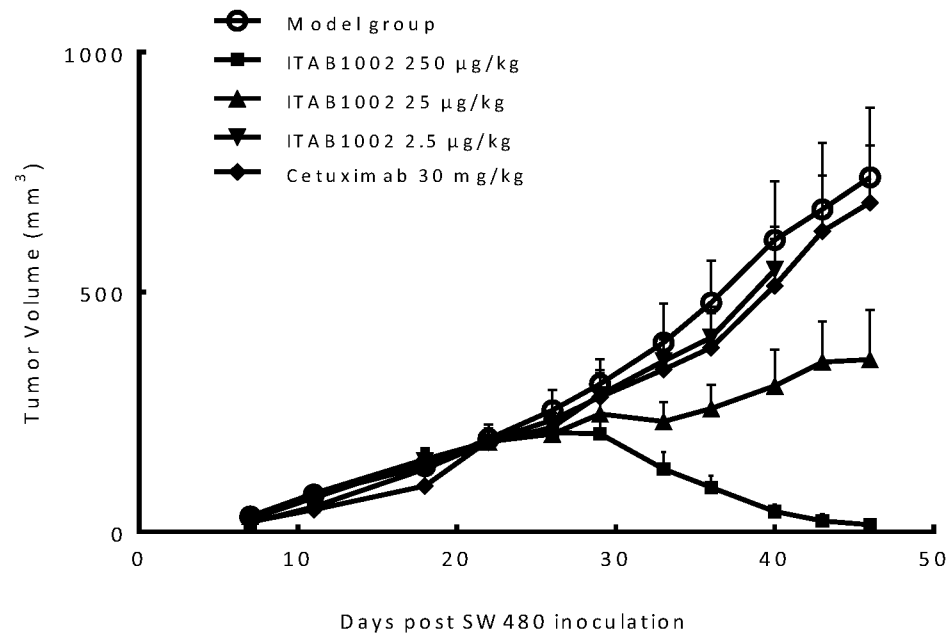


FIG. 10B

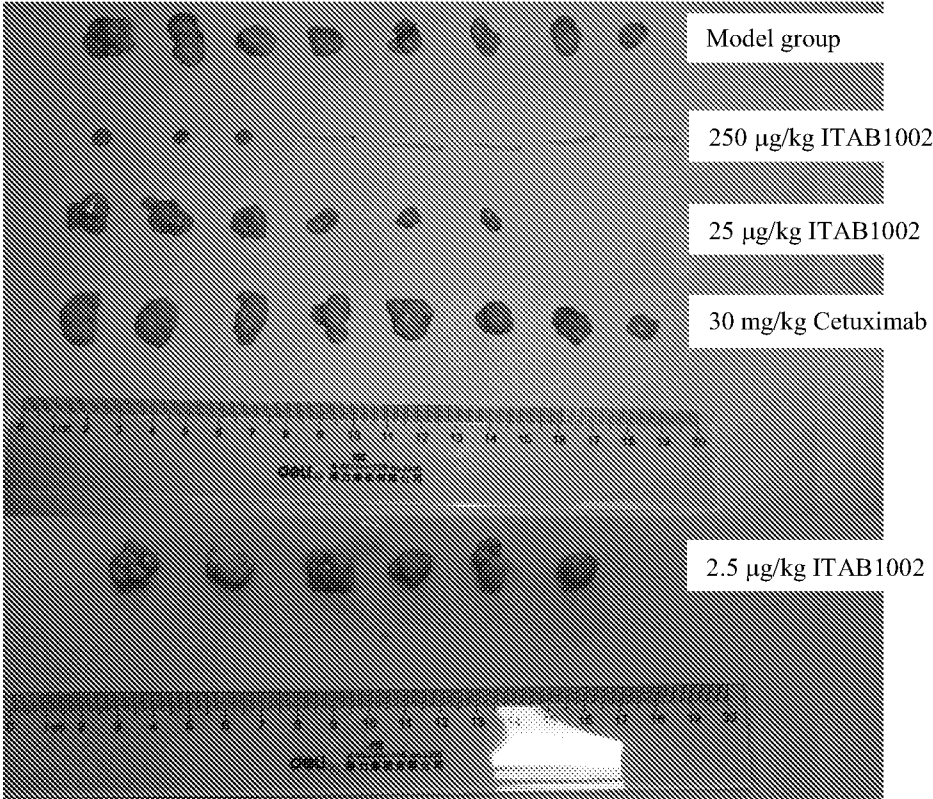


FIG. 10C

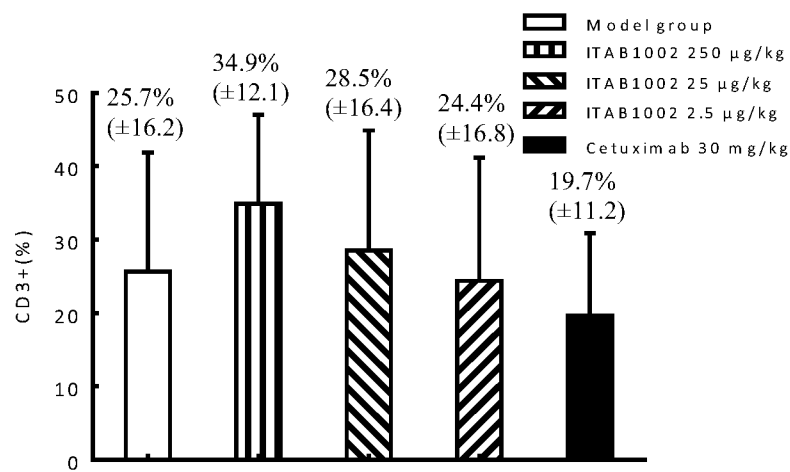


FIG. 11

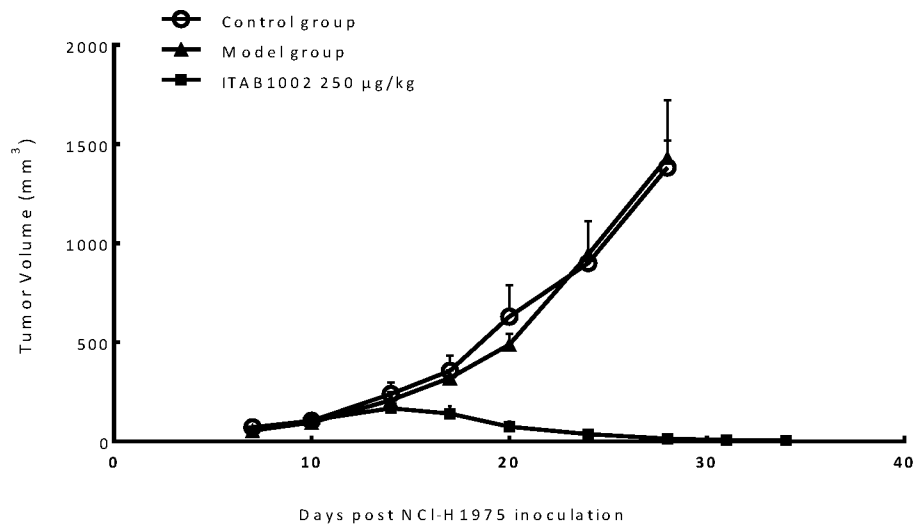


FIG. 12A

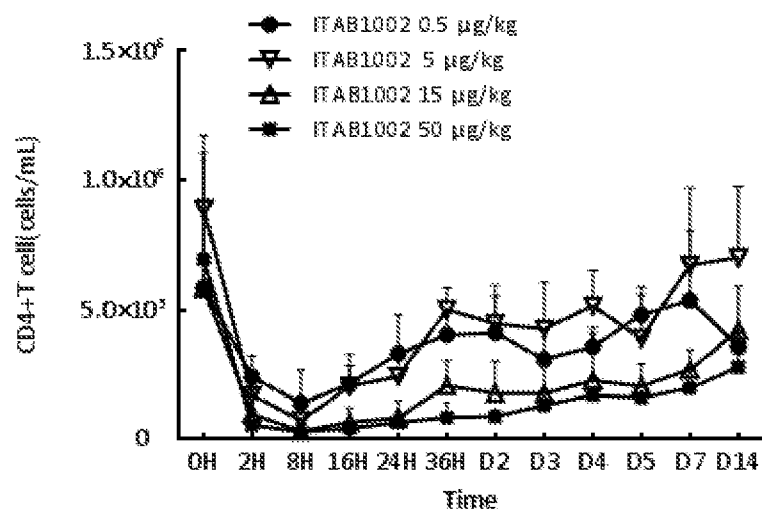


FIG. 12B

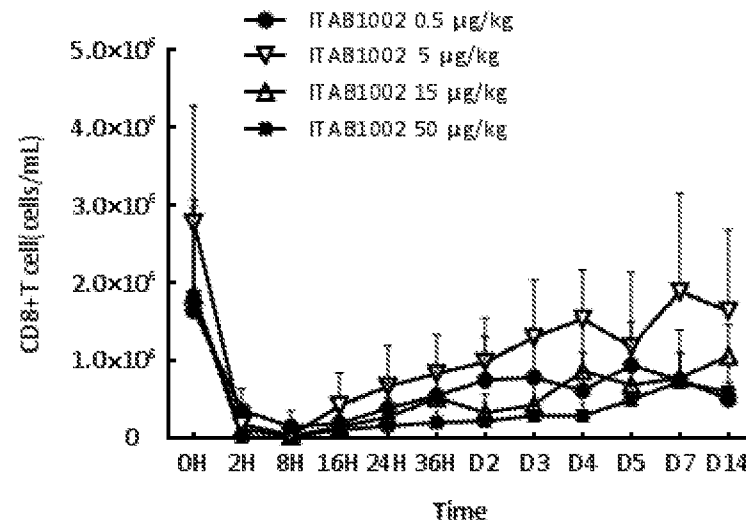


FIG. 13A

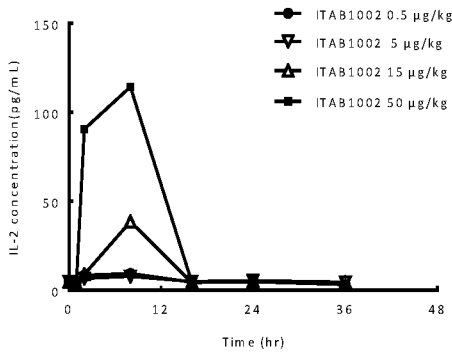


FIG. 13B

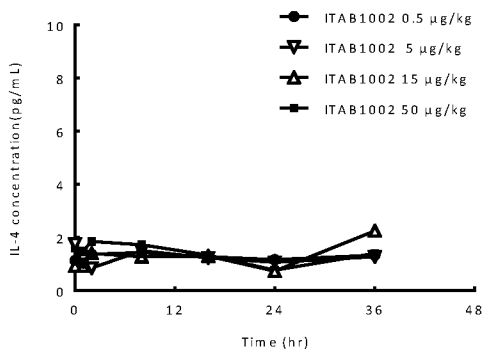


FIG. 13C

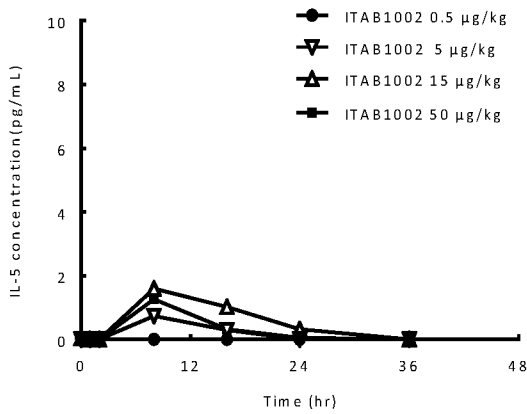


FIG. 13D

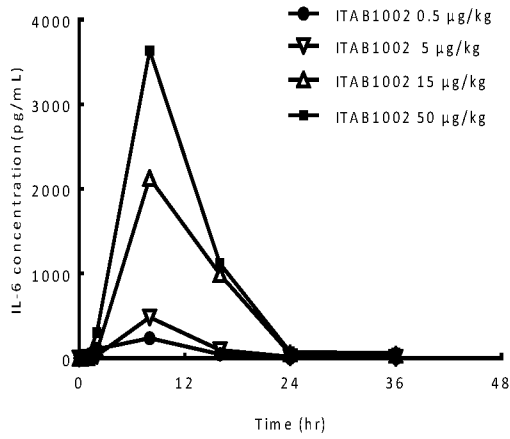


FIG. 13E

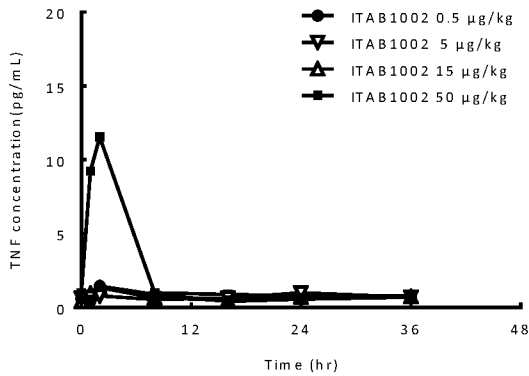


FIG. 13F

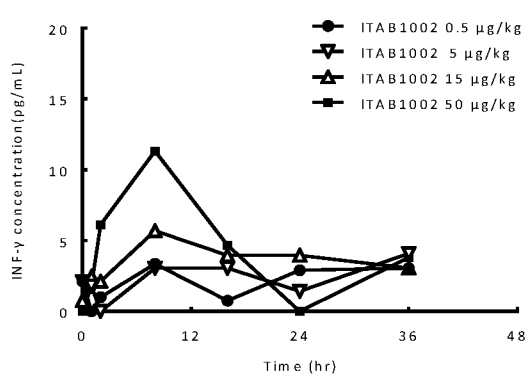


FIG. 14

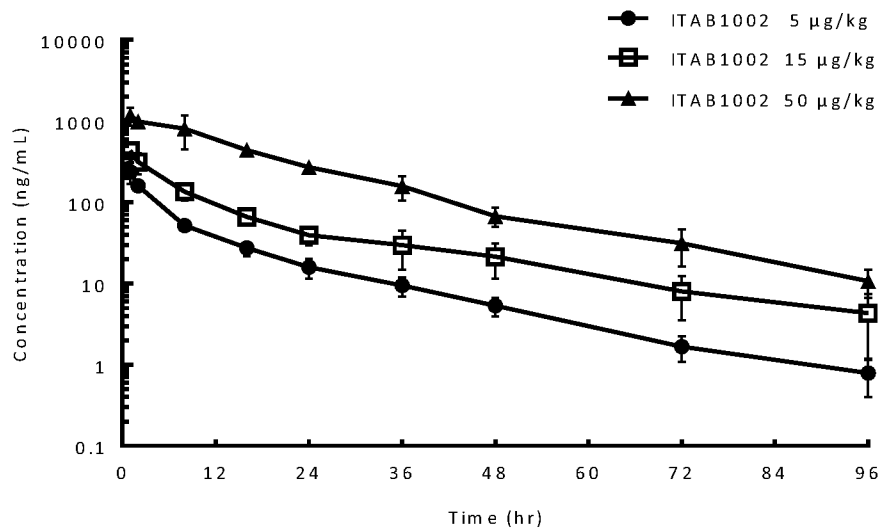


FIG. 15

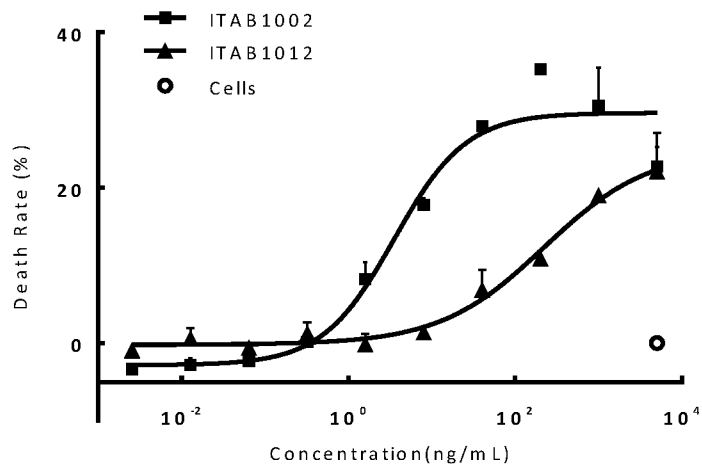


FIG. 16

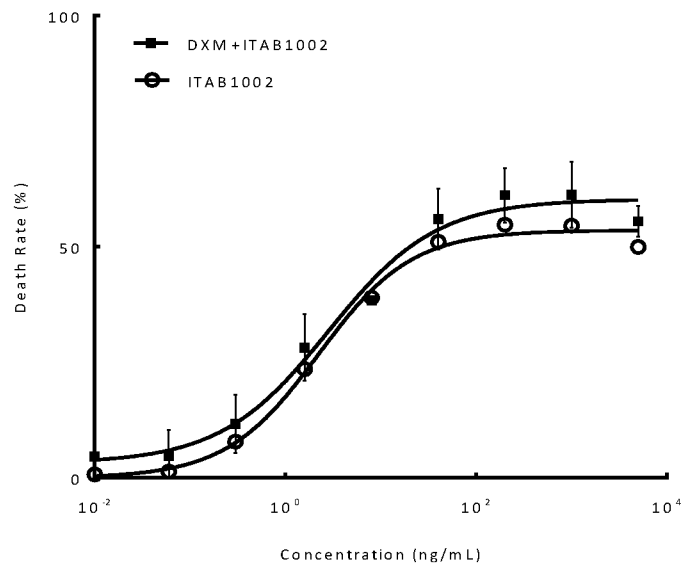


FIG. 17

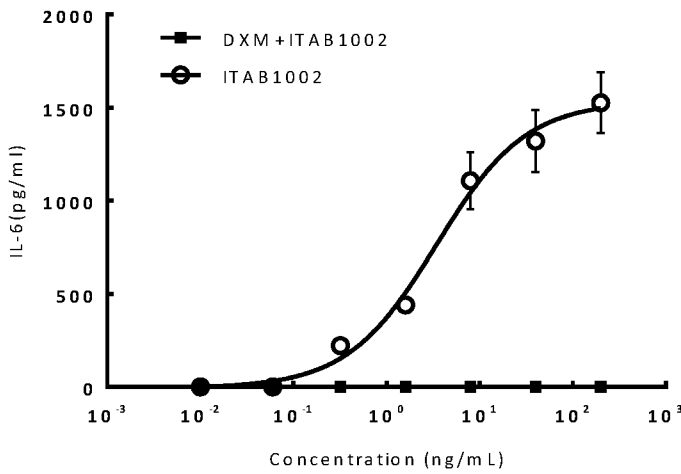


FIG. 18A

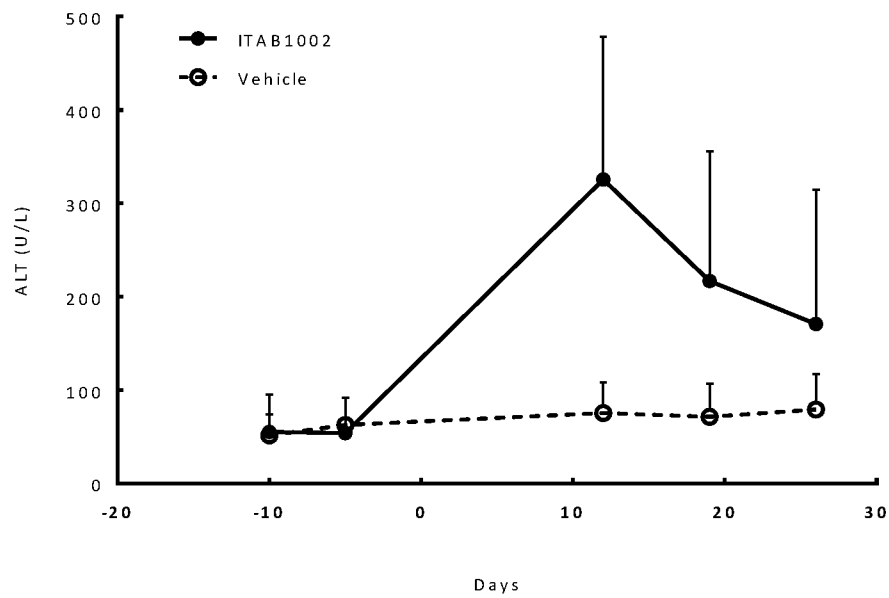


FIG. 18B

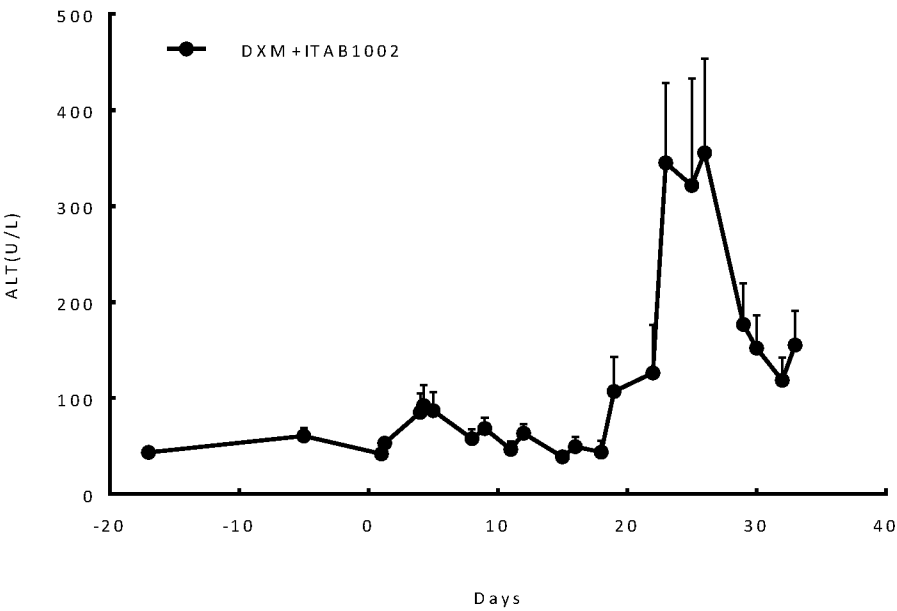


FIG. 19A

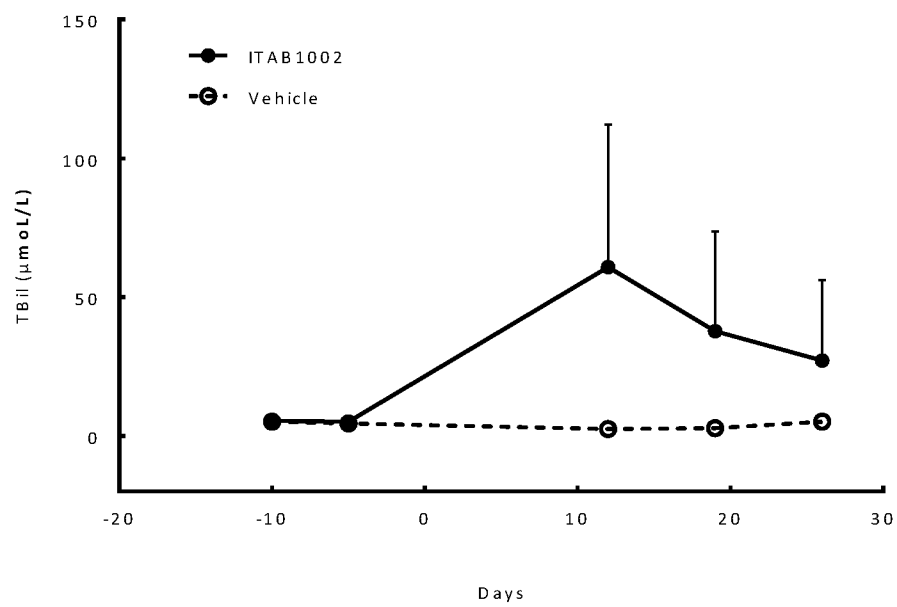


FIG. 19B

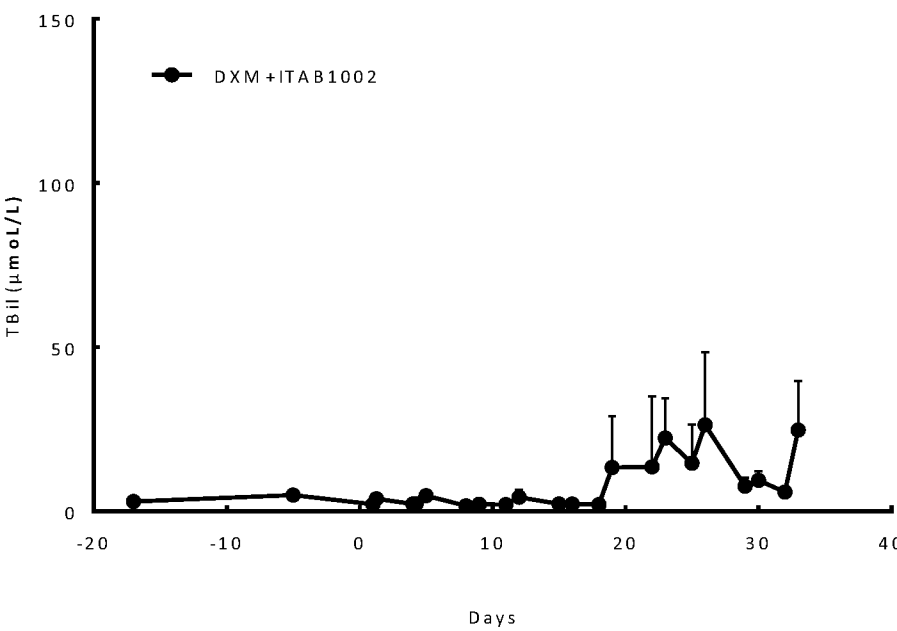


FIG. 20A

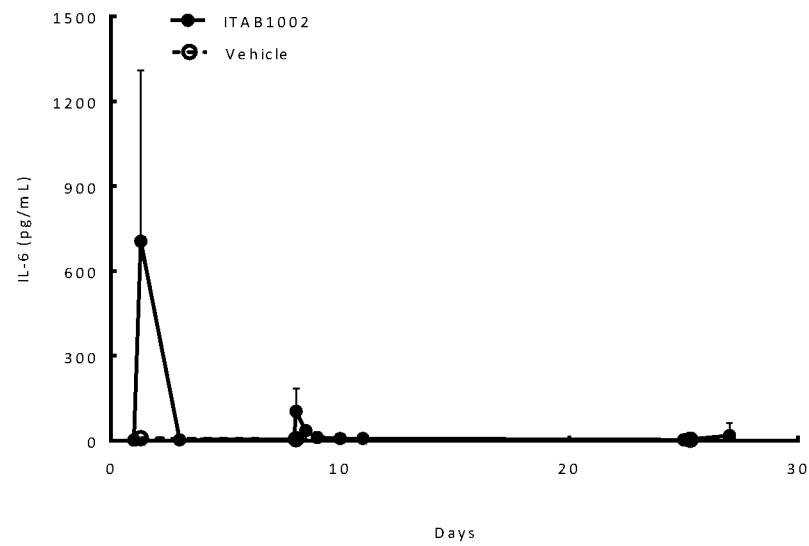


FIG. 20B

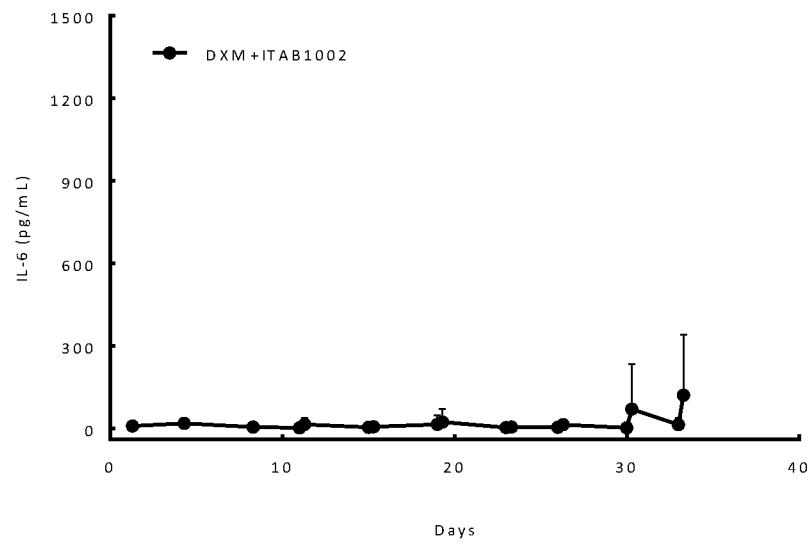


FIG. 21A

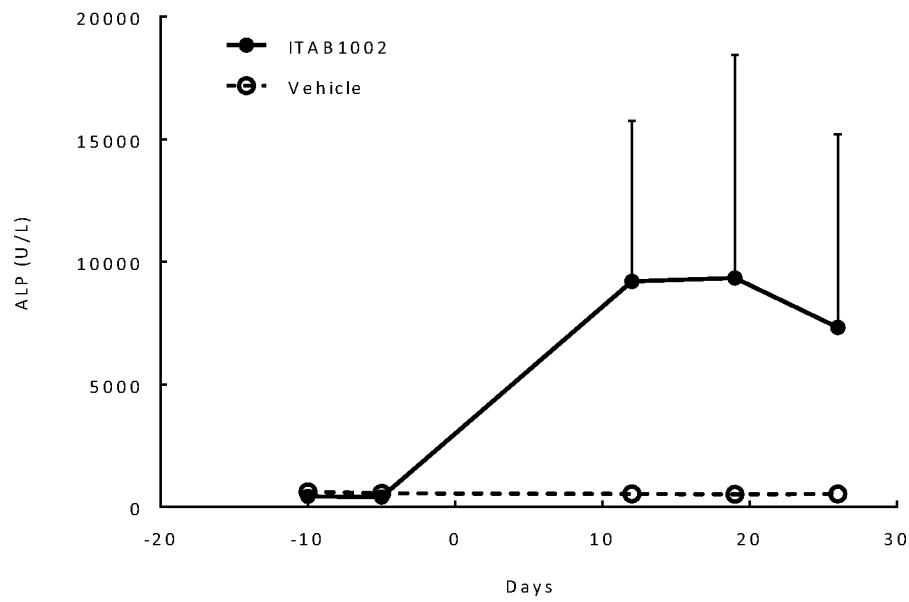
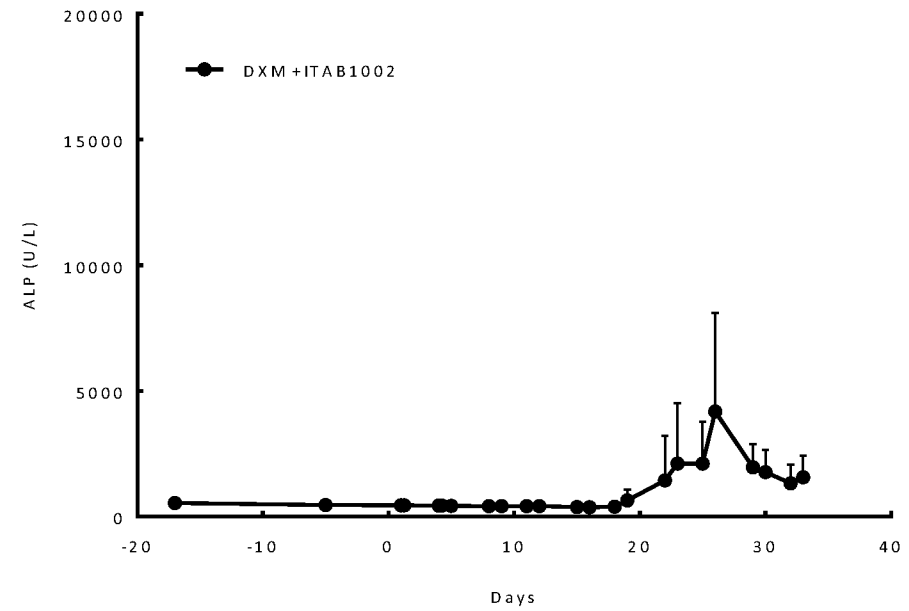


FIG. 21B



SEQUENCE LISTING

<110> GENERON (SHANGHAI) CORPORATION LTD.

<120> MULTISPECIFIC FAB FUSION PROTEINS AND
USE THEREOF

<130> 720622000841

<140> Not Yet Assigned

<141> Concurrently Herewith

<150> CN 201610147227.8

<151> 2016-03-15

<160> 47

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> CD3 HVR-H1

<400> 1

Thr Tyr Ala Met Asn
1 5

<210> 2

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> CD3 HVR-H2

<400> 2

Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser
1 5 10 15
Val Lys Asp

<210> 3

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> CD3 HVR-H3

<400> 3

His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr
1 5 10

<210> 4

<211> 14

<212> PRT

<213> Arti fi ci al Sequence

<220>

<223> CD3 HVR-L1

<400> 4

Arg	Ser	Ser	Thr	Gly	Ala	Val	Thr	Thr	Ser	Asn	Tyr	Ala	Asn
1				5					10				

<210> 5

<211> 7

<212> PRT

<213> Arti fi ci al Sequence

<220>

<223> CD3 HVR-L2

<400> 5

Gly	Thr	Asn	Lys	Arg	Ala	Pro
1				5		

<210> 6

<211> 9

<212> PRT

<213> Arti fi ci al Sequence

<220>

<223> CD3 HVR-L3

<400> 6

Ala	Leu	Trp	Tyr	Ser	Asn	Leu	Trp	Val
1				5				

<210> 7

<211> 125

<212> PRT

<213> Arti fi ci al Sequence

<220>

<223> CD3 VH

<400> 7

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asn	Thr	Tyr
			20					25					30		
Ala	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Ala	Arg	Ile	Arg	Ser	Lys	Tyr	Asn	Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala	Asp
	50				55					60					
Ser	Val	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	Lys	Asn	Thr
65				70					75					80	
Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr
			85					90					95		
Tyr	Cys	Val	Arg	His	Gly	Asn	Phe	Gly	Asn	Ser	Tyr	Val	Ser	Trp	Phe
			100					105					110		
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Met	Val	Thr	Val	Ser	Ser			
		115					120					125			

F17W0335-seql . txt

<210> 8
 <211> 109
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> CD3 VL

<400> 8
 Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
 1 5 10 15
 Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
 20 25 30
 Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
 35 40 45
 Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe
 50 55 60
 Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val
 65 70 75 80
 Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
 85 90 95
 Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
 100 105

<210> 9
 <211> 102
 <212> PRT
 <213> Homo sapi ens

<400> 9
 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
 1 5 10 15
 Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30
 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
 50 55 60
 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 65 70 75 80
 Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95
 Lys Val Glu Pro Lys Ser
 100

<210> 10
 <211> 104
 <212> PRT
 <213> Homo sapi ens

<400> 10
 Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
 1 5 10 15
 Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
 20 25 30
 Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro
 35 40 45
 Val Lys Ala Gly Val Glu Thr Thr Pro Ser Lys Gln Ser Asn Asn
 50 55 60
 Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys

F17W0335-seql . txt

65					70					75				80
Ser	His	Arg	Ser	Tyr	Ser	Cys	Gln	Val	Thr	His	Glu	Gly	Ser	Thr
				85					90					95
Glu	Lys	Thr	Val	Ala	Pro	Thr	Glu							
			100											

<210> 11
 <211> 227
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> CD3 Heavy chain

<400> 11														
Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly
1				5				10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asn	Thr
			20					25					30	
Ala	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp
		35					40					45		
Ala	Arg	Ile	Arg	Ser	Lys	Tyr	Asn	Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala
	50					55					60			
Ser	Val	Lys	Asp	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	Lys	Asn
65					70					75				80
Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val
				85					90					95
Tyr	Cys	Val	Arg	His	Gly	Asn	Phe	Gly	Asn	Ser	Tyr	Val	Ser	Trp
			100					105					110	
Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Met	Val	Thr	Val	Ser	Ser	Ala	Ser
		115					120					125		
Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr
	130					135					140			
Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro
145					150					155				160
Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val
				165					170					175
Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser
			180					185					190	
Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile
		195					200					205		
Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val
	210					215					220			
Pro	Lys	Ser												
225														

<210> 12
 <211> 213
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> CD3 Light chain

<400> 12														
Gln	Ala	Val	Val	Thr	Gln	Glu	Pro	Ser	Leu	Thr	Val	Ser	Pro	Gly
1				5					10					15
Thr	Val	Thr	Leu	Thr	Cys	Arg	Ser	Ser	Thr	Gly	Ala	Val	Thr	Thr
			20					25					30	
Asn	Tyr	Ala	Asn	Trp	Val	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg
		35					40					45		

F17W0335-seq1.txt

```

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe
 50      55      60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val
65      70      75      80
Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
      85      90      95
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro
      100      105      110
Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu
      115      120      125
Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro
      130      135      140
Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala
145      150      155      160
Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala
      165      170      175
Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg
      180      185      190
Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr
      195      200      205
Val Ala Pro Thr Glu
      210

```

<210> 13
 <211> 5
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> EpCAM HVR-H1

<400> 13
 Asn Tyr Trp Met Ser
 1 5

<210> 14
 <211> 17
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> EpCAM HVR-H2

<400> 14
 Asn Ile Lys Gln Asp Gly Ser Glu Lys Phe Tyr Ala Asp Ser Val Lys
 1 5 10 15
 Gly

<210> 15
 <211> 9
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> EpCAM HVR-H3

<400> 15
 Val Gly Pro Ser Trp Glu Gln Asp Tyr
 1 5

<210> 16
 <211> 14
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> EpCAM HVR-L1

<400> 16
 Thr Gly Ser Ser Ser Asn Ile Gly Ser Tyr Tyr Gly Val His
 1 5 10

<210> 17
 <211> 7
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> EpCAM HVR-L2

<400> 17
 Ser Asp Thr Asn Arg Pro Ser
 1 5

<210> 18
 <211> 11
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> EpCAM HVR-L3

<400> 18
 Gln Ser Tyr Asp Lys Gly Phe Gly His Arg Val
 1 5 10

<210> 19
 <211> 118
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> EpCAM VH

<400> 19
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Phe Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Val Gly Pro Ser Trp Glu Gln Asp Tyr Trp Gly Gln Gly Thr

Leu Val Thr Val Ser Ala
 100
 115

105 110

<210> 20
 <211> 113
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> EpCAM VL

<400> 20
 Gly Ala Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro
 1 5 10 15
 Gly Gln Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly
 20 25 30
 Ser Tyr Tyr Gly Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro
 35 40 45
 Lys Leu Leu Ile Tyr Ser Asp Thr Asn Arg Pro Ser Gly Val Pro Asp
 50 55 60
 Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr
 65 70 75 80
 Gly Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp
 85 90 95
 Lys Gly Phe Gly His Arg Val Phe Gly Gly Gly Thr Lys Leu Thr Val
 100 105 110
 Leu

<210> 21
 <211> 246
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> EpCAM scFv

<400> 21
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Phe Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Val Gly Pro Ser Trp Glu Gln Asp Tyr Trp Gly Gln Gly Thr
 100 105 110
 Leu Val Thr Val Ser Ala Gly Gly Gly Ser Gly Gly Gly Ser
 115 120 125
 Gly Gly Gly Gly Ser Gly Ala Gln Ser Val Leu Thr Gln Pro Pro Ser
 130 135 140
 Val Ser Gly Ala Pro Gly Gln Arg Val Thr Ile Ser Cys Thr Gly Ser
 145 150 155 160
 Ser Ser Asn Ile Gly Ser Tyr Tyr Gly Val His Trp Tyr Gln Gln Leu
 165 170 175

F17W0335-seql . txt

Pro	Gly	Thr	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Ser	Asp	Thr	Asn	Arg	Pro
			180					185					190		
Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala
		195					200					205			
Ser	Leu	Ala	Ile	Thr	Gly	Leu	Gln	Ala	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr
	210					215					220				
Cys	Gln	Ser	Tyr	Asp	Lys	Gly	Phe	Gly	His	Arg	Val	Phe	Gly	Gly	Gly
225					230					235					240
Thr	Lys	Leu	Thr	Val	Leu										
				245											

<210> 22

<211> 480

<212> PRT

<213> Artificial Sequence

<220>

<223> ITAB1002 HC

<400> 22

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5				10						15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Asn	Tyr
			20					25					30		
Trp	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Ala	Asn	Ile	Lys	Gln	Asp	Gly	Ser	Glu	Lys	Phe	Tyr	Ala	Asp	Ser	Val
	50					55					60				
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr
65					70					75					80
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85						90					95	
Ala	Arg	Val	Gly	Pro	Ser	Trp	Glu	Gln	Asp	Tyr	Trp	Gly	Gln	Gly	Thr
			100					105					110		
Leu	Val	Thr	Val	Ser	Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
		115					120					125			
Gly	Gly	Gly	Gly	Ser	Gly	Ala	Gln	Ser	Val	Leu	Thr	Gln	Pro	Pro	Ser
	130					135					140				
Val	Ser	Gly	Ala	Pro	Gly	Gln	Arg	Val	Thr	Ile	Ser	Cys	Thr	Gly	Ser
145					150					155					160
Ser	Ser	Asn	Ile	Gly	Ser	Tyr	Tyr	Gly	Val	His	Trp	Tyr	Gln	Gln	Leu
				165					170					175	
Pro	Gly	Thr	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Ser	Asp	Thr	Asn	Arg	Pro
			180					185					190		
Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala
		195					200					205			
Ser	Leu	Ala	Ile	Thr	Gly	Leu	Gln	Ala	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr
	210					215					220				
Cys	Gln	Ser	Tyr	Asp	Lys	Gly	Phe	Gly	His	Arg	Val	Phe	Gly	Gly	Gly
225					230					235					240
Thr	Lys	Leu	Thr	Val	Leu	Gly	Gly	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly
				245					250					255	
Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala
			260					265					270		
Ser	Gly	Phe	Thr	Phe	Asn	Thr	Tyr	Ala	Met	Asn	Trp	Val	Arg	Gln	Ala
		275					280						285		
Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	Ala	Arg	Ile	Arg	Ser	Lys	Tyr	Asn
	290					295					300				
Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala	Asp	Ser	Val	Lys	Asp	Arg	Phe	Thr	Ile
305					310					315					320
Ser	Arg	Asp	Asp	Ser	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu
				325					330					335	

F17W0335-seq1.txt

```

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg His Gly Asn Phe
340 345 350
Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Met
355 360 365
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
370 375 380
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
385 390 395 400
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
405 410 415
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
420 425 430
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
435 440 445
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
450 455 460
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Pro Pro Cys Ser
465 470 475 480

```

<210> 23

<211> 466

<212> PRT

<213> Artificial Sequence

<220>

<223> ITAB1002 LC

<400> 23

```

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Phe Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Val Gly Pro Ser Trp Glu Gln Asp Tyr Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ala Gly Gly Gly Ser Gly Gly Gly Ser
115 120 125
Gly Gly Gly Gly Ser Gly Ala Gln Ser Val Leu Thr Gln Pro Pro Ser
130 135 140
Val Ser Gly Ala Pro Gly Gln Arg Val Thr Ile Ser Cys Thr Gly Ser
145 150 155 160
Ser Ser Asn Ile Gly Ser Tyr Tyr Gly Val His Trp Tyr Gln Gln Leu
165 170 175
Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Ser Asp Thr Asn Arg Pro
180 185 190
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala
195 200 205
Ser Leu Ala Ile Thr Gly Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr
210 215 220
Cys Gln Ser Tyr Asp Lys Gly Phe Gly His Arg Val Phe Gly Gly Gly
225 230 235 240
Thr Lys Leu Thr Val Leu Gly Gly Gln Ala Val Val Thr Gln Glu Pro
245 250 255
Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Arg Ser
260 265 270

```

F17W0335-seq1.txt

Ser	Thr	Gly	Ala	Val	Thr	Thr	Ser	Asn	Tyr	Ala	Asn	Trp	Val	Gln	Gln
		275					280					285			
Lys	Pro	Gly	Gln	Ala	Pro	Arg	Gly	Leu	Ile	Gly	Gly	Thr	Asn	Lys	Arg
	290					295					300				
Ala	Pro	Gly	Thr	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Leu	Leu	Gly	Gly	Lys
305					310					315					320
Ala	Ala	Leu	Thr	Leu	Ser	Gly	Val	Gln	Pro	Glu	Asp	Glu	Ala	Glu	Tyr
				325					330					335	
Tyr	Cys	Ala	Leu	Trp	Tyr	Ser	Asn	Leu	Trp	Val	Phe	Gly	Gly	Gly	Thr
			340					345					350		
Lys	Leu	Thr	Val	Leu	Gly	Gln	Pro	Lys	Ala	Ala	Pro	Ser	Val	Thr	Leu
		355					360					365			
Phe	Pro	Pro	Ser	Ser	Glu	Glu	Leu	Gln	Ala	Asn	Lys	Ala	Thr	Leu	Val
370					375						380				
Cys	Leu	Ile	Ser	Asp	Phe	Tyr	Pro	Gly	Ala	Val	Thr	Val	Ala	Trp	Lys
385					390					395					400
Ala	Asp	Ser	Ser	Pro	Val	Lys	Ala	Gly	Val	Glu	Thr	Thr	Thr	Pro	Ser
				405					410					415	
Lys	Gln	Ser	Asn	Asn	Lys	Tyr	Ala	Ala	Ser	Ser	Tyr	Leu	Ser	Leu	Thr
			420				425						430		
Pro	Glu	Gln	Trp	Lys	Ser	His	Arg	Ser	Tyr	Ser	Cys	Gln	Val	Thr	His
		435					440					445			
Glu	Gly	Ser	Thr	Val	Glu	Lys	Thr	Val	Ala	Pro	Thr	Glu	Cys	Pro	Pro
	450					455					460				
Cys	Ser														
465															

<210> 24
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 24
 Gly Gly Ser Gly
 1

<210> 25
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 25
 Gly Gly Ser Gly Gly
 1 5

<210> 26
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 26

Gly Ser Gly Ser Gly
1 5

<210> 27
<211> 5
<212> PRT
<213> Arti fi ci al Sequence

<220>
<223> Syntheti c Construct

<400> 27
Gly Ser Gly Gly Gly
1 5

<210> 28
<211> 5
<212> PRT
<213> Arti fi ci al Sequence

<220>
<223> Syntheti c Construct

<400> 28
Gly Gly Gly Ser Gly
1 5

<210> 29
<211> 5
<212> PRT
<213> Arti fi ci al Sequence

<220>
<223> Syntheti c Construct

<400> 29
Gly Ser Ser Ser Gly
1 5

<210> 30
<211> 6
<212> PRT
<213> Arti fi ci al Sequence

<220>
<223> Syntheti c Construct

<400> 30
Pro Leu Gly Leu Ala Gly
1 5

<210> 31
<211> 1443
<212> DNA
<213> Arti fi ci al Sequence

<220>
<223> ITAB1002 HC

F17W0335-seql . txt

```
<400> 31
gagggtgcagc tgggtggagtc aggggggaggc ttggtccagc ctggggggatc cctgagactc 60
tccctgtgcag cctctggatt caccttttagt aattatttga tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtggccaac ataaagcaag atggaagtga gaaattctat 180
gcggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctactgtat 240
ctgcaaatga acagcctgag agccgaagac acggctgtct attactgtgc gagagtgggg 300
ccgtcctggg agcaggacta ctggggccag ggaaccctgg tctactgtct ggccggtggc 360
gggtggcagc gcggtggtag gtccggtggc ggcgatctg gcgcgcagtc tgtactgact 420
caaccgcccc cagtgtctgg ggccccaggg cagaggggtca ccatctcctg cactgggagc 480
agctccaaca tcgggtctta ttatggtgtg cactggtacc agcagcttcc aggaacagcc 540
cccaaactcc tcatctattc tgacactaat cgaccctcag ggttccctga ccgattctct 600
ggctccaagt ctggcacctc ggctccctg gccatcactg ggctccaggc tgaggatgag 660
gctgattatt actgccagtc gtatgacaag ggcttcgggc accgggtgtt cggcggaggg 720
accaagctga ccgtcctagg ggcgaggtg cagctgggtg agtctggggg aggcctggta 780
cagcctgggg gggtccctgag actctcctgt gcagcctctg gattcacctt taacacctac 840
gccatgaact gggtccgcca ggctccaggg aaggggctgg agtgggtcgc acgcataaga 900
agtaaatata ataattatgc aacatattat gccgattcag tgaaagaccg gttcaccatc 960
tccagagacg attccaagaa cacgctgtat ctgcaaatga acagcctgag agccgaggac 1020
acggccgtat attactgtgt gagacatggg aacttcggta atagctacgt ttcttggttt 1080
gcttactggg gccaaaggac aatggtcacc gtctcttcag ctagcaccaa gggcccatcc 1140
gtcttcccc tggcacctc ctccaagagc acctctgggg gcacagcggc cctgggctgc 1200
ctggtcaagg actacttccc cgaaccgggt acggtgtcgt ggaactcagg cgccctgacc 1260
agcggcggtg acaccttccc ggctgtccta cagtctcag gactctactc cctcagcagc 1320
gtggtgaccg tgccctccag cagcttgggc acccagacct acatctgcaa cgtgaatcac 1380
aagccagca acaccaaggt ggacaagaaa gttgagccca aatcttgtcc accgtgtc 1440
tga
```

```
<210> 32
<211> 1401
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> ITAB1002 LC
```

```
<400> 32
gagggtgcagc tgggtggagtc aggggggaggc ttggtccagc ctggggggatc cctgagactc 60
tccctgtgcag cctctggatt caccttttagt aattatttga tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtggccaac ataaagcaag atggaagtga gaaattctat 180
gcggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctactgtat 240
ctgcaaatga acagcctgag agccgaagac acggccgtct attactgtgc gagagtgggg 300
ccgtcctggg agcaggacta ctggggccag ggaaccctgg tctactgtct ggccggtggc 360
gggtggcagc gcggtggtag gtccggtggc ggcgatctg gcgcgcagtc tgtactgact 420
caaccgcccc cagtgtctgg ggccccaggg cagaggggtca ccatctcctg cactgggagc 480
agctccaaca tcgggtctta ttatggtgtg cactggtacc agcagcttcc aggaacagcc 540
cccaaactcc tcatctattc tgacactaat cgaccctcag ggttccctga ccgattctct 600
ggctccaagt ctggcacctc ggctccctg gccatcactg ggctccaggc tgaggatgag 660
gctgattatt actgccagtc gtatgacaag ggcttcgggc accgggtgtt cggcggaggg 720
accaagctga ccgtcctagg ggccaggct gtggtgactc aggagccctc actgactgtg 780
tccccaggag ggacagtcac tctcacctgt cgctcaagta ctggggctgt tacaactagt 840
aactatgcca actgggtcca gcagaaacct ggacaagcac ccagggtctt gattggtggt 900
accaacaagc gagctccagg taccctgcc cggttctcag gctccctcct tgggggcaaa 960
gctgccctga cactgtcagg tgtgcagcct gaggacgagg ctgagtatta ctgcgtcta 1020
tggtagagca acctctgggt gttcggcgga gggaccaagc tgaccgtcct aggccaaccg 1080
aaagcggcgc cctcggtcac tctgttcccg ccctcctctg aggagcttca agccaacaag 1140
gccacactgg tgtgtctcat aagtgacttc taccggggag ccgtgacagt ggccctggaag 1200
gcagatagca gccccgtcaa ggcgggagtg gagaccacca caccctcaa acaaagcaac 1260
aacaagtacg cggccagcag ctatctgagc ctgacgcctg agcagtggaa gtcccacaga 1320
agctacagct gccaggtcac gcatgaaggg agcaccgtgg agaagacagt ggccccctaca 1380
gaatgtccac cgtgctcatg a
```

```
<210> 33
```

<211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Signal peptide

<400> 33
 Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gly
 1 5 10 15
 Val His Ser

<210> 34
 <211> 57
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Signal peptide

<400> 34
 atggaatgga gctgggtctt tctcttcttc ctgtcagtaa cgactgggtg ccactcc 57

<210> 35
 <211> 480
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> ITAB1012 HC

<400> 35
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Phe Tyr Val Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Val Gly Gly Ala Trp Glu Leu Gly Tyr Trp Gly Gln Gly Thr
 100 105 110
 Leu Val Thr Val Ser Ala Gly Gly Gly Ser Gly Gly Gly Ser
 115 120 125
 Gly Gly Gly Gly Ser Gly Ala Gln Ser Val Leu Thr Gln Pro Pro Ser
 130 135 140
 Val Ser Gly Ala Pro Gly Gln Arg Val Thr Ile Ser Cys Thr Gly Ser
 145 150 155 160
 Ser Ser Asn Ile Gly Ser Tyr Tyr Gly Val His Trp Tyr Gln Gln Leu
 165 170 175
 Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Ser Asp Thr Asn Arg Pro
 180 185 190
 Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala
 195 200 205
 Ser Leu Ala Ile Thr Gly Leu Gln Ala Glu Asp Glu Ala Asp Tyr Tyr
 210 215 220
 Cys Gln Ser Tyr Asp Ser Ser Leu Ser Gly Arg Val Phe Gly Gly Gly

F17W0335-seql . txt

225	Thr	Lys	Leu	Thr	Val	230	Leu	Gly	Gly	Glu	Val	235	Gln	Leu	Val	Glu	Ser	240	Gly
					245						250						255		
	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala			
				260						265					270				
	Ser	Gly	Phe	Thr	Phe	Asn	Thr	Tyr	Ala	Met	Asn	Trp	Val	Arg	Gln	Ala			
			275					280						285					
	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	Ala	Arg	Ile	Arg	Ser	Lys	Tyr	Asn			
		290					295					300							
	Asn	Tyr	Ala	Thr	Tyr	Tyr	Ala	Asp	Ser	Val	Lys	Asp	Arg	Phe	Thr	Ile			
305					310						315					320			
	Ser	Arg	Asp	Asp	Ser	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu			
				325						330					335				
	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Val	Arg	His	Gly	Asn	Phe			
				340					345					350					
	Gly	Asn	Ser	Tyr	Val	Ser	Trp	Phe	Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Met			
			355					360					365						
	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu			
		370					375					380							
	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys			
385					390						395					400			
	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser			
				405					410						415				
	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser			
			420						425					430					
	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser			
			435					440					445						
	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn			
		450				455					460								
	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Pro	Pro	Cys	Ser			
465					470						475					480			

<210> 36

<211> 466

<212> PRT

<213> Artificial Sequence

<220>

<223> ITAB1012 LC

<400> 36

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Asn	Tyr
			20					25					30		
Trp	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
			35				40					45			
Ala	Asn	Ile	Lys	Gln	Asp	Gly	Ser	Glu	Lys	Phe	Tyr	Val	Asp	Ser	Val
			50			55					60				
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr
65					70				75					80	
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Met	Ala	Val	Tyr	Tyr	Cys
			85					90					95		
Ala	Arg	Val	Gly	Ala	Trp	Glu	Leu	Gly	Tyr	Trp	Gly	Gln	Gly	Thr	
			100				105					110			
Leu	Val	Thr	Val	Ser	Ala	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser
			115			120						125			
Gly	Gly	Gly	Gly	Ser	Gly	Ala	Gln	Ser	Val	Leu	Thr	Gln	Pro	Pro	Ser
		130				135					140				
Val	Ser	Gly	Ala	Pro	Gly	Gln	Arg	Val	Thr	Ile	Ser	Cys	Thr	Gly	Ser
145					150					155					160
Ser	Ser	Asn	Ile	Gly	Ser	Tyr	Tyr	Gly	Val	His	Trp	Tyr	Gln	Gln	Leu

F17W0335-seql . txt

Pro	Gly	Thr	Ala	165	Pro	Lys	Leu	Leu	Ile	170	Tyr	Ser	Asp	Thr	Asn	175	Arg	Pro
Ser	Gly	Val	Pro	180	Asp	Arg	Phe	Ser	Gly	185	Ser	Lys	Ser	Gly	190	Thr	Ser	Ala
Ser	Leu	Ala	Ile	195	Thr	Gly	Leu	Gln	Ala	200	Glu	Asp	Glu	Ala	205	Asp	Tyr	Tyr
Cys	Gln	Ser	Tyr	210	Asp	Ser	Ser	Leu	Ser	215	Gly	Arg	Val	Phe	Gly	Gly	Gly	Gly
Thr	Lys	Leu	Thr	225	Val	Leu	Gly	Gly	Gln	230	Ala	Val	Val	Thr	Gln	Glu	Pro	240
Ser	Leu	Thr	Val	245	Ser	Pro	Gly	Gly	Thr	250	Val	Thr	Leu	Thr	Cys	Arg	Ser	255
Ser	Thr	Gly	Ala	260	Val	Thr	Thr	Ser	Asn	265	Tyr	Ala	Asn	Trp	Val	Gln	Gln	270
Lys	Pro	Gly	Gln	275	Ala	Pro	Arg	Gly	Leu	280	Ile	Gly	Gly	Thr	Asn	Lys	Arg	285
Ala	Pro	Gly	Thr	290	Pro	Ala	Arg	Phe	Ser	295	Gly	Ser	Leu	Leu	Gly	Gly	Lys	300
Ala	Ala	Leu	Thr	305	Leu	Ser	Gly	Val	Gln	310	Pro	Glu	Asp	Glu	Ala	Glu	Tyr	315
Tyr	Cys	Ala	Leu	325	Trp	Tyr	Ser	Asn	Leu	330	Trp	Val	Phe	Gly	Gly	Gly	Thr	335
Lys	Leu	Thr	Val	340	Leu	Gly	Gln	Pro	Lys	345	Ala	Ala	Pro	Ser	Val	Thr	Leu	350
Phe	Pro	Pro	Ser	355	Ser	Glu	Glu	Leu	Gln	360	Ala	Asn	Lys	Ala	Thr	Leu	Val	365
Cys	Leu	Ile	Ser	370	Asp	Phe	Tyr	Pro	Gly	375	Ala	Val	Thr	Val	Ala	Trp	Lys	380
Ala	Asp	Ser	Ser	385	Pro	Val	Lys	Ala	Gly	390	Val	Glu	Thr	Thr	Thr	Pro	Ser	395
Lys	Gln	Ser	Asn	405	Asn	Lys	Tyr	Ala	Ala	410	Ser	Ser	Tyr	Leu	Ser	Leu	Thr	415
Pro	Glu	Gln	Trp	420	Lys	Ser	His	Arg	Ser	425	Tyr	Ser	Cys	Gln	Val	Thr	His	430
Glu	Gly	Ser	Thr	435	Val	Glu	Lys	Thr	Val	440	Ala	Pro	Thr	Glu	Cys	Pro	Pro	445
Cys	Ser			450						455								460
				465														

<210> 37
 <211> 1443
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> ITAB1012 HC

<400> 37
 gaggtgcagc tgggtggagtc aggggggaggc ttggtccagc ctggggggatc actgagactc 60
 tcctgtgcag cctctggatt cacctttagt aattattgga tgagctgggt ccgccaggct 120
 ccagggaagg ggctggagtg ggtggccaac ataaagcaag atggaagtga gaaattctat 180
 gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctactgtat 240
 ctgcaaatga acagcctgag agccgaagac atggctgtct attactgtgc gagagtgggg 300
 ggggcgtggg agctaggcta ctggggccag ggaaccctgg tctactgtct ggccggtggc 360
 ggtggcagcg gcggtggtgg gtccggtggc ggcggatctg gcgcgcagtc tgtactgact 420
 caaccgccct cagtgtctgg ggcccaggc cagagggtca ccatctcctg cactgggagc 480
 agctccaaca tcgggtctta ttatggtgtg cactggtacc agcagcttcc aggaacagcc 540
 cccaaactcc tcatctattc tgacactaat cgaccctcag ggttccctga ccgattctct 600
 ggctccaagt ctggcacctc ggctccctg gccatcactg ggctccaggc tgaggatgag 660
 gctgattatt actgccagtc gtatgacagc agcctgagtg gccgggtgtt cggcggaggg 720
 accaagctga cagtactagg tggcgagggt cagctggtgg agtctggggg aggcctggta 780

F17W0335-seq1.txt

```

cagcctgggg ggtccctgag actctcctgt gcagcctctg gattcacctt taacacctac 840
gccatgaact ggggccgcca ggctccaggg aaggggctgg agtgggtcgc acgcataaga 900
agtaaatata ataattatgc aacatattat gccgattcag tgaaagaccg gttcaccatc 960
tccagagacg attccaagaa cacgctgtat ctgcaaatga acagcctgag agccgaggac 1020
acggccgtat attactgtgt gagacatggg aacttcggta atagctacgt ttcctgggtt 1080
gcttactggg gccaagggac aatggtcacc gtctcttcag ctagcaccaa gggcccatcc 1140
gtcttccccc tggcaccctc ctccaagagc acctctgggg gcacagcggc cctgggctgc 1200
ctggtaaggg actacttccc cgaaccgggt acgggtgtcgt ggaactcagg cgccctgacc 1260
agcggcgtgc acaccttccc ggctgtccta cagtcctcag gactctactc cctcagcagc 1320
gtgggtgaccg tgccctccag cagcttgggc acccagacct acatctgcaa cgtgaatcac 1380
aagcccagca acaccaaggt ggacaagaaa gttgagccca aatcttgtcc accgtgctca 1440
tga

```

<210> 38
 <211> 1401
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> ITAB1012 LC

```

<400> 38
gaggtgcagc tgggtggagtc aggggggaggc ttgggtccagc ctggggggatc actgagactc 60
tcctgtgcag cctctggatt cacctttagt aattattgga tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtggccaac ataaagcaag atggaagtga gaaattctat 180
gtggactctg tgaagggccg attcaccatc tccagagaca acgccaagaa ctcactgtat 240
ctgcaaatga acagcctgag agccgaagac atggctgtct attactgtgc gagagtgggg 300
ggggcggtggg agctaggcta ctggggccag ggaaccctgg tcactgtctc ggccgggtggc 360
gggtggcagcg gcggtgggtg gtccggtggc ggcggtatct gcgcgcagtc tgtactgact 420
caaccgccct cagtgtctgg ggccccaggg cagaggggtca ccatctcctg cactgggagc 480
agctccaaca tcgggtctta ttatggtgtg cactggtacc agcagcttcc aggaacagcc 540
cccaaatcc tcactatc tgacactaat cgaccctcag ggttccctga ccgattctct 600
ggctccaagt ctggcacctc ggccctccctg gccatcactg ggctccaggc tgaggatgag 660
gctgattatt actgccagtc gtatgacagc agcctgagtg gccgggtgtt cggcggaggg 720
accaagctga cagtactagg tggccaggct gtggtgactc aggagccctc actgactgtg 780
tccccaggag ggacagtcac tctcacctgt cgctcatcca ctggggctgt tacaactagt 840
aactatgcca actgggtcca gcagaaacct ggacaagcac ccaggggtct gattggtgg 900
accaacaagc gagctccagg taccctgcc cgttctcag gctccctcct tgggggcaaa 960
gctgccctga cactgtcagg tgtgcagcct gaggacgagg ctgagtatta ctgcgctcta 1020
tggtagagca acctctgggt gttcggcgga gggaccaagc tgaccgtcct agccaaccg 1080
aaagcggcgc cctcggtcac tctgttcccg ccctcctctg aggagcttca agccaacaag 1140
gccacactgg tgtgtctcat aagtgacttc taccggggag ccgtgacagt ggccctggaag 1200
gcagatagca gccccgtcaa ggcgggagtg gagaccacca caccctccaa acaaagcaac 1260
aacaagtacg cgccagcagc ctatctgagc ctgacgcctg agcagtggaa gtcccacaga 1320
agctacagct gccaggtcac gcatgaaggg agcaccgtgg agaagacagt ggccccctaca 1380
gaatgtccac cgtgctcatg a

```

<210> 39
 <211> 125
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> CD3 1VH

```

<400> 39
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
20          25          30
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35          40          45
Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp

```

	50					55				60					
Ser 65	Val	Lys	Asp	Arg	Phe 70	Thr	Ile	Ser	Arg	Asp 75	Asp	Ser	Lys	Asn	Thr 80
Leu	Tyr	Leu	Gln	Met 85	Asn	Ser	Leu	Arg	Ala 90	Glu	Asp	Thr	Ala	Val 95	Tyr
Tyr	Cys	Ala	Arg 100	His	Gly	Asn	Phe	Gly 105	Asn	Ser	Tyr	Val	Ser 110	Trp	Phe
Ala	Tyr	Trp 115	Gly	Gln	Gly	Thr	Met 120	Val	Thr	Val	Ser	Ser 125			

<220>
<223> CD3 2VH

<210> 41
<211> 125
<212> PRT
<213> Artificial Sequence

<400> 41															
Gl u	Val	Gl n	Leu	Val	Gl u	Ser	Gl y	Gl y	Gl y	Leu	Val	Gl n	Pro	Gl y	Gl y
1				5					10					15	
Ser	Leu	Arg	Leu	Ser	Cys	Al a	Al a	Ser	Gl y	Phe	Thr	Phe	Ser	Thr	Tyr
			20					25					30		
Al a	Met	Asn	Trp	Val	Arg	Gl n	Al a	Pro	Gl y	Lys	Gl y	Leu	Gl u	Trp	Val
		35					40					45			
Al a	Arg	I le	Arg	Ser	Lys	Tyr	Asn	Asn	Tyr	Al a	Thr	Tyr	Tyr	Al a	Asp
	50					55					60				
Ser	Val	Lys	Asp	Arg	Phe	Thr	I le	Ser	Arg	Asp	Asp	Ser	Lys	Asn	Thr
65					70					75					80
Leu	Tyr	Leu	Gl n	Met	Asn	Ser	Leu	Arg	Al a	Gl u	Asp	Thr	Al a	Val	Tyr
				85					90					95	
Tyr	Cys	Al a	Arg	Hi s	Gl y	Asn	Phe	Gl y	Asn	Ser	Tyr	Val	Ser	Trp	Phe
			100					105					110		
Al a	Tyr	Trp	Gl y	Gl n	Gl y	Thr	Met	Val	Thr	Val	Ser	Ser			
		115					120					125			

F17W0335-seql . txt

<210> 42
 <211> 125
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> CD3 4VH

<400> 42
 Gl u Val Gl n Leu Val Gl u Ser Gly Gly Gly Leu Val Gl n Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Al a Al a Ser Gly Phe Thr Phe Ser Thr Tyr
 20 25 30
 Al a Met Asn Trp Val Arg Gl n Al a Pro Gly Lys Gly Leu Gl u Trp Val
 35 40 45
 Al a Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Al a Thr Tyr Tyr Al a Asp
 50 55 60
 Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
 65 70 75 80
 Leu Tyr Leu Gl n Met Asn Ser Leu Arg Al a Gl u Asp Thr Al a Val Tyr
 85 90 95
 Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
 100 105 110
 Al a Tyr Trp Gly Gl n Gly Thr Met Val Thr Val Ser Ser
 115 120 125

<210> 43
 <211> 125
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> CD3 11VH

<400> 43
 Gl u Val Gl n Leu Val Gl u Ser Gly Gly Gly Leu Val Gl n Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Leu Ser Cys Al a Al a Ser Gly Phe Thr Phe Ser Thr Tyr
 20 25 30
 Al a Met Asn Trp Val Arg Gl n Al a Ser Gly Lys Gly Leu Gl u Trp Val
 35 40 45
 Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Al a Thr Tyr Tyr Al a Asp
 50 55 60
 Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
 65 70 75 80
 Al a Tyr Leu Gl n Met Asn Ser Leu Lys Thr Gl u Asp Thr Al a Val Tyr
 85 90 95
 Tyr Cys Thr Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
 100 105 110
 Al a Tyr Trp Gly Gl n Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

<210> 44
 <211> 109
 <212> PRT
 <213> Arti fi ci al Sequence

<220>
 <223> CD3 2VL

F17W0335-seql . txt

<400> 44

```

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
 1          5          10          15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
          20          25          30
Asn Tyr Ala Asn Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
          35          40          45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe
          50          55          60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val
65          70          75          80
Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
          85          90          95
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
          100          105

```

<210> 45

<211> 109

<212> PRT

<213> Arti fi ci al Sequence

<220>

<223> CD3 3VL

<400> 45

```

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
 1          5          10          15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
          20          25          30
Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
          35          40          45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Trp Thr Pro Ala Arg Phe
          50          55          60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val
65          70          75          80
Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
          85          90          95
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
          100          105

```

<210> 46

<211> 109

<212> PRT

<213> Arti fi ci al Sequence

<220>

<223> CD3 4VL

<400> 46

```

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
 1          5          10          15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
          20          25          30
Asn Tyr Ala Asn Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
          35          40          45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Trp Thr Pro Ala Arg Phe
          50          55          60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val
65          70          75          80
Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn

```

F17W0335-seq1.txt

Leu Trp Val Phe 85 Gly Gly Gly Thr Lys 90 Leu Thr Val Leu 95
100 105

<210> 47
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> CD3 5VL

<400> 47
Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1 5 10 15
Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30
Asn Tyr Ala Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
35 40 45
Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Trp Thr Pro Ala Arg Phe
50 55 60
Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val
65 70 75 80
Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
85 90 95
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105