ANTIBODIES TARGETING HUMAN BASAL CELL ADHESION MOLECULE

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims the benefit of U.S. Provisional Application No. 63/571,595, filed March 29, 2024, the entire contents of which is incorporated herein by reference.

FIELD

[2] This disclosure relates to antibodies that specifically bind to human Basal Cell Adhesion Molecule (BCAM).

SEQUENCE LISTING

[3] The instant application contains a Sequence Listing which has been submitted electronically in .xml format and is hereby incorporated by reference in its entirety. The Sequence Listing, created on 13 February 2024, is named "3320.W01WO_Sequence Listing.xml" and is 134 kilobytes in size.

BACKGROUND

[4] High-grade serous ovarian cancer (HGSOC) is the most common and lethal type of ovarian cancer. The vast majority of women diagnosed are at an advanced stage of the disease. Current treatment options are limited to a combination of surgery and chemotherapy, and no matter the treatment course, the cancer typically recurs. 70% of patients die within 5 years of being diagnosed with HGSOC.

[5] Human Basal Cell Adhesion Molecule (BCAM) is a heavily glycosylated transmembrane protein that acts as a receptor for Laminin alpha-5. BCAM is highly expressed in a variety of tumors, with relatively low expression in most healthy tissue. 35%-40% of primary HGSOC tumors show high expression of BCAM. However, it remains unclear whether Laminin alpha-5 is the primary ligand for BCAM in ovarian cancer. Further unclear is the nature of the inhibitory effect of targeting BCAM.

[6] The current arsenal of potent anti-BCAM antibodies suitable for therapeutic use is limited. Therefore, a need remains to identify antibodies that target BCAM and that can be used in treating cancers such as HGSOC.

SUMMARY

[7] The present disclosure relates to antibodies that specifically bind human basal cell adhesion molecule (BCAM).

[8] There is provided herein an antibody that specifically binds human BCAM, wherein the antibody comprises a variable region and a constant region, wherein the variable region comprises a framework region and a complementarity determining means for binding human BCAM.

[9] In particular, there is provided herein an antibody that specifically binds human BCAM, comprising:

a) a heavy chain complementarity determining region (HCDR) 1 comprising an amino acid sequence of DYYVH (SEQ ID NO: 2), an HCDR2 comprising an amino acid sequence of IINPFGGSATYAPKFQG (SEQ ID NO: 3), an HCDR3 comprising an amino acid sequence of GIYGHFDY (SEQ ID NO: 4); and

b) a light chain complementarity determining region (LCDR) 1 comprising an amino acid sequence of KSSQSVLSASNNQNYLA (SEQ ID NO: 5), an LCDR2 comprising an amino acid sequence of WASTRES (SEQ ID NO: 6), and an LCDR3 comprising an amino acid sequence of QQYYSSPYT (SEQ ID NO: 7).

[10] There is also provided herein an antibody that specifically binds human BCAM, comprising:

a) an HCDR1 comprising an amino acid sequence of SYAFS (SEQ ID NO: 8), an HCDR2 comprising an amino acid sequence of GIIPFSGTTNYAQKFQG (SEQ ID NO: 9), and HCDR3 comprising an amino acid sequence of DPILSFWSGYYYYYGMDV (SEQ ID NO: 10); and

b) an LCDR1 comprising an amino acid sequence of RASQSISSWLA (SEQ ID NO: 11), an LCDR2 comprising an amino acid sequence of DASNLET (SEQ ID NO: 12), an LCDR3 comprising an amino acid sequence of QQSYSTPPT (SEQ ID NO: 13).

[11] There is also provided herein an antibody that specifically binds human BCAM and competes for binding to human BCAM with an antibody comprising:

a) an immunoglobulin heavy chain variable (VH) region comprising the amino acid sequence

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQG
LEWMGIINPFGGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDT
AVYYCARGIYGHFDYWGQGTLVTVSS (SEQ ID NO: 14), and
an immunoglobulin light chain variable (VL) region comprising the amino acid sequence
DIVMTQSPDSLAVSLGERATINCKSSQSVLSASNNQNYLAWYQQKP
GQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC
QQYYSSPYTFGQGTKLEIK (SEQ ID NO: 15); or

b) an immunoglobulin heavy chain variable (VH) region comprising the amino acid sequence
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLE
WMGGIIPFSGTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAV
YYCTTDPILSFWSGYYYYYGMDVWGQGTTVTVSS (SEQ ID NO: 16), and
an immunoglobulin light chain variable (VL) region comprising the amino acid sequence
DIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLI
YDASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPP
TFGQGTRLEIK (SEQ ID NO: 17).

[12] There is also provided herein an antibody that specifically binds human BCAM, comprising:

a) an HCDR1 comprising an amino acid sequence of SYYMH (SEQ ID NO: 18), an HCDR2 comprising an amino acid sequence of WMNPNSGDSGYAQKFQG (SEQ ID NO: 19), an HCDR3 comprising an amino acid sequence of TYSSSWIFDY (SEQ ID NO: 20); and

b) an LCDR1 comprising an amino acid sequence of KSSQSVLYSSNNKNYLA (SEQ ID NO: 21), an LCDR2 comprising an amino acid sequence of WASTRES (SEQ ID NO: 22), an LCDR3 comprising an amino acid sequence of QQYYSIPIT (SEQ ID NO: 23).

[13] There is also provided herein an antibody that specifically binds human BCAM and competes for binding to human BCAM with an antibody comprising:

a) an immunoglobulin heavy chain variable (VH) region comprising the amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWMNPNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTYSSSWIFDYWGQGTLVTVSS (SEQ ID NO: 24), and

b) an immunoglobulin light chain variable (VL) region comprising the amino acid sequence DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSIPITFGQGTRLEIK (SEQ ID NO: 25).

[14] Furthermore, there is provided herein an antibody drug conjugate (ADC) comprising means for killing a tumor cell conjugated via a linking means to an antibody that specifically binds human BCAM, wherein the antibody comprises a variable region and a constant region, wherein the variable region comprises a framework region and a complementarity determining means for binding human BCAM. The ADC may comprise:

a) an anti-BCAM antibody;

b) an anti-tumor drug; and

c) a linker connecting the anti-BCAM antibody with the anti-tumor drug.

[15] There is also provided a biparatopic antibody comprising at least one antibody or an ADC as described in any one of the paragraphs.

[16] There is also provided herein a bispecific T cell engager (BiTE) comprising an antibody means for binding human BCAM, an antibody means for binding CD3, and a linking means to link the antibody means for binding human BCAM to an antibody means for binding CD3. The BiTE may comprise:

a) an anti-BCAM antibody;

b) an anti-CD3 antibody;

c) a linker connecting the anti-BCAM antibody with the anti-CD3 antibody; and optionally

d) a means for extending half-life (e.g., an Fc region).

[17] There is also provided a pharmaceutical composition comprising an antibody, ADC, or BiTE as described herein and one or more pharmaceutically acceptable excipient(s).

[18] Also provided are methods of treating cancer in subject in need thereof comprising administering a therapeutically effective amount of an antibody, ADC or BiTE described herein to the subject.

DEFINITIONS

[19] *A or An*: The articles "a" and "an" refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[20] *Affinity*: The term "affinity" refers to the characteristics of a binding interaction between a binding means (e.g., an antigen binding region, e.g., a variable domain comprising light and heavy chain variable regions, and/or Fc receptor binding means, e.g., an Fc region) and a target (e.g., an antigen such as BCAM or an Fc receptor (FcR) such as neonatal FcR (FcRn)) and indicates the strength of the binding interaction. The measure of affinity may be expressed as a dissociation constant (K_D). A binding means may have a high affinity for a target (e.g., a K_D in the nanomolar or picomolar range). A binding means may have a high affinity for a target at a first pH (e.g., pH 7-7.4) and lower affinity (e.g., 10-folder lower, or 100-fold lower) for the target (e.g., higher than about 100 nM, higher than about 1 μM, higher than about 10 μM, or higher than about 100 μM) at a second pH (e.g., pH 4.5-5).

[21] *Approximately or about:* The term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. The term "approximately" or "about" may refer to a range of values that fall within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, greater than or less than of the stated reference value (e.g., ±5% or ±1%) unless otherwise stated or otherwise evident from the context.

[22] *Antibody*: The term "antibody" refers to an antigen binding polypeptide that includes six complementarity determining regions (CDRs). Exemplary antigen-binding polypeptides comprised by the term "antibody" include, but are not limited to, IgA antibodies, IgG antibodies, IgE antibodies, IgM antibodies, bi- or multi- specific antibodies (including bispecific T cell engagers (BiTEs) and biparatopic antibodies), Fab fragments, Fab' fragments, F(ab')2 fragments, Fd' fragments, Fd fragments, and single-chain variable fragments (scFvs). An antibody typically includes at least a heavy (H) chain variable region (abbreviated herein as VH), and at least a light (L) chain variable region (abbreviated herein as VL). An antibody may include two heavy (H) chain variable regions and two light (L) chain variable regions. The term "antibody" encompasses antigen-binding fragments of

antibodies (e.g., Fab, F(ab')$_2$, Fd, and Fv fragments), single chain antibodies (e.g., single-chain Fvs), as well as full-length antibodies, e.g., intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof). A full-length antibody typically includes a constant region. A full-length antibody may be an IgG, e.g., IgG1, IgG3, or IgG4. The light chains of the immunoglobulin can be of types kappa or lambda. An "antibody" may be an immunoglobulin molecule comprising two heavy chains (HCs) and two light chains (LCs) interconnected by disulfide bonds, wherein the amino terminal portion of each LC and HC includes a variable region of about 100-120 amino acids primarily responsible for antigen recognition via the CDRs contained therein. The CDRs are interspersed with regions that are well-known and generally conserved among and between species (e.g., mouse and human), which are termed framework regions (FRs).

[23] *Binding Means*: A "binding means" is any molecule or part of a molecule (e.g., an antibody or an antigen binding region or fragment of an antibody) capable of specifically binding a target, e.g., a target of interest (e.g., an antigen such as BCAM).

[24] *Antigen-binding fragment or antigen-binding region* refers to a portion of an intact antibody that binds to an antigen (e.g., BCAM). An antigen-binding fragment can contain the antigenic determining variable regions of an intact antibody. Examples of antibody fragments include, but are not limited to Fab, Fab', F(ab')2, and Fv fragments. Antigen-binding fragments or regions also comprise single chain antibodies, such as single-chain Fvs (scFvs).

[25] *Antibody means for binding*: The term "*antibody means for binding*" describes an antibody that specifically binds a target of interest (e.g., an antigen such as BCAM). For example, an antibody means for binding human basal cell adhesion molecule (BCAM) is an antibody that specifically binds human BCAM. Anti-BCAM antibodies are described herein and include the antibodies in Table 1, in particular MAbs 452, 477, and 929. Equivalent antibodies differ in their amino acid sequence but perform the same function of specifically binding BCAM through CDR-target interaction and achieve a comparable result of inhibiting tumor growth alone or as an ADC or BiTE. Equivalent CDRs may also share at least 85% sequence identity and/or compete with the disclosed antibodies for binding of human BCAM, as determined by a cell-based assay using Fluorescence-Activated Cell Sorting (FACS, see, e.g., example 4).

[26] *Complementarity determining means for binding:* The term "*complementarity determining means for binding*" describes the complementarity determining region (CDR) that makes up the antigen (BCAM) binding site of an antibody. Specific CDRs can be found in Table 1. Equivalent CDRs differ in their amino acid sequence but perform the same function of specifically binding BCAM through CDR-target interaction and achieve a comparable result of inhibiting tumor growth alone or as an ADC or BiTE. Equivalent CDRs may also share at least 85% sequence identity to and/or compete with the present anti-BCAM antibodies for binding of human BCAM, as determined by a cell-based assay using Fluorescence-Activated Cell Sorting (FACS, see, e.g., example 4).

[27] *Complementarity Determining Region (CDR)*: A "complementarity determining region" or "CDR" are amino acid residues within a variable region that are identified herein in accordance with the definition by Kabat et al. (see, e.g., Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C). Other CDR boundary definitions may not strictly follow Kabat's approach, but will nonetheless overlap with at least a portion of the Kabat CDRs. For example, they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues do not significantly impact antigen binding.

[28] *Constant region*: The term "constant region" refers to a polypeptide that corresponds to, or is derived from, one or more constant region immunoglobulin domain(s) of an antibody. A constant region can include the following immunoglobulin domains: a C_H1 domain, a hinge region, a C_H2 domain, a C_H3 domain (derived from an IgA, IgD, IgG, IgE, or IgM), and a C_H4 domain (derived from an IgE or IgM). Typically, a constant region includes a C_H1 domain, a hinge region, a C_H2 domain, a C_H3 domain (derived from, e.g., IgG1 or IgG4).

[29] *Epitope*: An "epitope" is a term known in the art and refers to a localized region of an antigen to which an antibody can specifically bind. An epitope can be, for example, contiguous amino acids of a polypeptide (linear or contiguous epitope) or an epitope can, for example, come together from two or more non-contiguous regions or residues of a polypeptide or polypeptides (conformational, non-linear, discontinuous, or non-contiguous epitope).

[30] *Fc region*: The term "Fc region" refers to a dimer of two "Fc polypeptides", each "Fc polypeptide" comprising the constant region of an antibody excluding the first

constant region immunoglobulin domain. An "Fc region" may include two Fc polypeptides linked by one or more disulfide bonds, chemical linkers, or peptide linkers. "Fc polypeptide" refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and may also include part or all of the flexible hinge N-terminal to these domains. For IgG, an "Fc polypeptide" comprises immunoglobulin domains Cgamma2 (Cγ2) and Cgamma3 (Cγ3) and the lower part of the hinge between Cgamma1 (Cγ1) and Cγ2. Although the boundaries of the Fc polypeptide may vary, the human IgG heavy chain Fc polypeptide is usually defined to comprise residues starting at T223 or C226 or P230, to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat et al. (1991, NIH Publication 91-3242, National Technical Information Services, Springfield, VA). For IgA, an Fc polypeptide comprises immunoglobulin domains Calpha2 (Cα2) and Calpha3 (Cα3) and the lower part of the hinge between Calpha1 (Cα1) and Cα2.

[31] *K_a*: "K_a" refers to an association rate of a particular binding means and a target to form a binding means/target complex.

[32] *K_d*: "K_d" refers to a dissociation rate of a particular binding means/target complex.

[33] *K_D*: "K_D" refers to a dissociation constant, which is obtained from the ratio of K_d to K_a (i.e., K_d/K_a) and is expressed as a molar concentration (M). K_D values can be determined using methods well established in the art, e.g., by using surface plasmon resonance, e.g., using a BIACORE® system, or cell-based methods (using human BCAM-expressing cells and flow cytometry, as, e.g., described in example 1). K_D values can also be determined with a biolayer interferometry using a dip and read biosensor such as a Sartorius Octet RH16 or Octet N1 (as, e.g., described in example 5).

[34] *Killing means or means for killing a tumor cell*: The term "*killing means*" or "*means for killing a tumor cell*" refers to any compound that can be used to kill a cell and especially a tumor cell. This includes, e.g., cytotoxic drugs as well as radionuclides as described herein. The term "means for killing a tumor cell" may be used interchangeably with the terms "anti-tumor drug" or "anti-cancer drug". Anti-tumor drugs and anti-cancer drugs include well-known classes of cytotoxic agents such as tubulin inhibitors or a DNA damaging agents including, e.g., DNA alkylating agents, topoisomerase inhibitors, and DNA crosslinking agents.

[35] *Means for extending half-life*: The term "*means for extending half-life*" refers well-known structural modifications of antibodies that prolong half-life in human serum. These include PEGylation as well as fusion to human serum albumin (HSA) or a human serum albumin-binding means (e.g., an anti-HSA antibody). In some instances, the term may refer to an Fc region, e.g., an Fc region comprising well-known structural modifications such as amino acid substitutions that extend serum half-life of an antibody. Such amino acid substitutions include, e.g., M252Y/S254T/T256E (YTE mutant), L309D/Q311H/N434S (DHS mutant), V259I/V308F/M428L (IFL mutant), H433K/N434F (KF mutant), and M428L/N434S (LS mutant), which exhibit improved serum half-life relative to, *e.g.*, a native IgG1 Fc region.

[36] *Linking means or linker*: The term "linking means" or "linker" refers to well-known structures to link an antibody to a drug conjugate or two binding domains in bi-specific T-cell engagers (BiTEs). Exemplary linking means or linkers (including cleavable linkers) are described herein.

[37] *Specific binding*: "Specific binding" or "specifically binds" refers, with respect to a binding means and a target, to a preferential association of a binding means to a target and not to an entity that is not the target. A certain degree of non-specific binding may occur between a binding means and a non-target. A binding means may specifically bind a target if binding between the binding means and the target is greater than 2-fold, greater than 5-fold, greater than 10-fold, or greater than 100-fold as compared with binding of the binding means and a non-target. Specific binding to human BCAM can be determined as described in example 1.

[38] *Single-chain variable fragment (scFv):* The term "single-chain variable fragment" or "scFv" refers to a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of an immunoglobulin (e.g., a mouse or human immunoglobulin) covalently linked to form a VH::VL heterodimer. The heavy (VH) and light chains (VL) are either joined directly or joined by a peptide-based linker (e.g., 10, 15, 20, 25 amino acids), which connects the N-terminus of the VH with the C-terminus of the VL, or the C-terminus of the VH with the N-terminus of the VL. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility. The linker can link the heavy chain variable region and the light chain variable region of an antibody or an antigen-binding fragment thereof. Non-limiting examples of linkers are disclosed in Shen et al., Anal. Chem.

80(6):1910-1917 (2008) and WO 2014/087010. Exemplary peptide-based linkers include GGGG (SEQ ID NO: 26) and GGGGS (SEQ ID NO: 27).

[39] *Subject*: The term "subject" means any subject for whom the therapy is desired. Typically, the subject is a human subject.

[40] *Therapeutically effective amount:* The term "therapeutically effective amount" refers to an amount of a therapeutic molecule (e.g., an anti-BCAM antibody described herein) which confers a therapeutic effect on a treated subject, at a reasonable benefit/risk ratio applicable to any medical treatment. Therapeutic effect may be objective (i.e., measurable by some test or marker) or subjective (i.e., subject gives an indication of or feels an effect). In particular, the "therapeutically effective amount" refers to an amount of a therapeutic molecule or composition effective to treat, ameliorate, or prevent a particular disease or condition, or to exhibit a detectable therapeutic or preventative effect, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset of the disease, and/or also lessening the severity or frequency of symptoms of the disease.

[41] *Treatment*: The term "treatment" (also "treat" or "treating") refers to any administration of a therapeutic molecule (e.g., an anti-BCAM antibody described herein) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Such treatment may be of a subject who does not (yet) exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. More commonly, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition (e.g., cancer).

BRIEF DESCRIPTION OF DRAWINGS

[42] Drawings are for illustration purposes only.

[43] FIG. 1 shows a schematic of BCAM, MCAM, and chimeric constructs comprising domains of both proteins that were used to narrow down the location of the epitope bound by anti-BCAM antibodies disclosed herein.

[44] FIG. 2 shows representative histograms that illustrate the capability of anti-BCAM antibodies of the invention to bind certain BCAM/MCAM chimeric constructs shown in FIG. 1. FIG. 2A shows representative histograms obtained using the anti-BCAM antibody

929. FIG. 2B shows representative histograms obtained using the anti-BCAM antibody 477. FIG. 2C shows representative histograms obtained using the anti-BCAM antibody 452.

[45] FIG. 3 shows representative graphs depicting the ADCC activity of anti-BCAM antibodies 228, 232, 236, 330, 452, 477, 606, 929, and 937 when provided to cells at various concentrations. FIG. 3A shows ADCC activity of exemplary anti-BCAM antibodies against OVSAHO cells. FIG. 3B shows ADCC activity of exemplary anti-BCAM antibodies against OAW28 cells. FIG. 3C shows ADCC activity of exemplary anti-BCAM antibodies against SKOV3-BCAM cells.

[46] FIG. 4 shows a line graph demonstrating the concentration-dependent effect of anti-BCAM antibodies 228, 232, 236, 330, 452, 477, 606, 929, and 937 on the interaction between human BCAM and its ligand Laminin alpha-5.

[47] FIG. 5 shows representative histograms that illustrate binding competition between anti-BCAM antibodies 228, 232, 236, 452, 477, 606, 929, and 937 to human BCAM. FIG. 5A shows representative histograms from an experiment in which the anti-BCAM antibodies were added to a preformed complex of antibody 452 and human BCAM. FIG. 5B shows representative histograms from an experiment in which the anti-BCAM antibodies were added to a preformed complex of antibody 929 and human BCAM. FIG. 5C shows representative histograms from an experiment in which the anti-BCAM antibodies were added to a preformed complex of antibody 477 and human BCAM.

[48] FIG. 6 shows representative histograms that illustrate how the presence of point mutations in BCAM affects the binding of anti-BCAM antibodies of the invention. FIG. 6A shows representative histograms depicting the effect of point mutations on the capability of antibody 477 to bind human BCAM. FIG. 6B shows representative histograms depicting the effect of point mutations on the capability of antibody 452 to bind human BCAM. FIG. 6C shows representative histograms depicting the effect of point mutations on the capability of antibody 929 to bind human BCAM.

[49] FIG. 7A shows an alignment of human, cynomolgus, and mouse BCAM amino acid sequences. The sequence of human BCAM used was residues 1 to 516 of SEQ ID NO: 1. The sequence of cynomolgus BCAM used was SEQ ID NO: 28, which corresponds to residues 32 to 547 of UniProtKB ID: A0A2K5TW87_MACFA. The sequence of mouse BCAM used was SEQ ID NO: 29, which corresponds to residues 26 to 541 of UniProtKB ID: Q9R069.

[50] FIG. 7B shows representative histograms depicting the effect of point mutations in human BCAM on the capability of antibody 929 to bind human BCAM.

[51] FIG. 7C shows representative histograms depicting the effect of point mutations in human BCAM on the capability of antibody 477 to bind human BCAM.

[52] FIG. 8 shows representative histograms illustrating the effect of various polymorphisms of human BCAM on the binding of the anti-BCAM antibodies 452, 477, and 929.

[53] FIG. 9 shows representative biolayer interferometry (BLI) curves of anti-BCAM antibodies. FIG. 9A shows representative BLI curves for anti-BCAM antibody 452. FIG. 9B shows representative BLI curves for anti-BCAM antibody 477. FIG. 9C shows representative BLI curves for anti-BCAM antibody 929.

[54] FIG. 10A shows a schematic of a bi-specific T-cell engager (BiTE) construct comprising an anti-BCAM binding means (i.e., single chain variable fragment (scFv) that specifically binds BCAM) linked to an anti-CD3 binding means (i.e., a scFv that specifically binds CD3). FIG. 10B shows a schematic of a BiTE construct of FIG. 10A, further comprising a means for extending half-life (i.e., a human IgG1-Fc with LALA-PG mutations).

[55] FIG. 11A shows representative graphs demonstrating the effect of anti-BCAM BiTE constructs on T-cell activation at various concentrations. FIG. 11B shows representative graphs illustrating the percentage of activated T cells after incubation of donor PBMCs with a 477-based BiTE construct. The normalized percentage of target cells lysed as a result of the T cell activation after incubation of donor PBMCs with a 477-based BiTE construct are correspondingly shown in FIG. 11C.

[56] FIG. 12A shows a representative graph demonstrating that a 477-based anti-BCAM BiTE-Fc construct described herein activates T cells that are incubated in the presence of SKOV3 wild-type (WT) target cells. The percentage of target SKOV3 WT cells that are lysed as a result of the T cell activation with this BCAM BiTE-Fc construct is correspondingly shown in FIG. 12B. FIG. 12C shows a representative graph demonstrating that a 477-based anti-BCAM BiTE-Fc construct described herein activates T cells that are incubated in the SKOV3 cells modified to express recombinant human BCAM. The percentage of the modified SKOV3 cells that are lysed as a result of the T cell activation with this BCAM BiTE-Fc construct is correspondingly shown in FIG. 12D.

[57] FIG. 13A shows a representative graph demonstrating that a 477-based anti-BCAM BiTE-Fc construct described herein activates T cells that are incubated in the presence of OVSAHO target cells. The percentage of OVSAHO cells that were lysed as a result of the T cell activation anti-BCAM BiTE-Fc construct is correspondingly shown in FIG. 13B.

[58] FIG. 14 shows a representative graph demonstrating that a 477-based anti-BCAM BiTE-Fc was effective in preventing growth of tumors established in mice using SKOV3-BCAM cells.

[59] FIG. 15A shows a representative graph demonstrating internalization of each of anti-BCAM antibodies 452, 477, and 929, respectively, into BCAM-expressing cells.

[60] FIG. 15B shows a representative confocal microscopy image illustrating that OVSAHO cells can internalize antibody-drug conjugates (ADCs) comprising anti-BCAM antibody 452, 477, or 929 conjugated to deruxtecan (DXd). ADCs internalized into low pH environments within the cell, i.e., lysosomes and endosomes, show as white spots. Arrows point to internalized ADCs.

[61] FIG. 16 shows a representative graph demonstrating the ability of an ADC comprising an anti-BCAM antibody of the invention to bind to human BCAM at drug-antibody ratios of 4 (DAR4) or 8 (DAR8). The drug was deruxtecan (DXd).

[62] FIG. 17 shows a representative graph demonstrating that picomolar concentrations of ADCs based on anti-BCAM antibodies 477, 452 or 929 with a drug-antibody ratio of 8 (DAR8) were able to reduce the viability of OVCAR8 (OV8) cells.

[63] FIG. 18 shows representative graphs demonstrating that picomolar concentrations of ADCs comprising an anti-BCAM antibody of the invention were able to comparably reduce the viability of OVCAR8 (OV8) cells at antibody-drug ratios of 4 (DAR4) or 8 (DAR8). FIG. 18A shows cell viability after treatment with an anti-BCAM antibody 929-based ADC. FIG. 18B shows cell viability after treatment with an anti-BCAM antibody 452-based ADC. FIG. 18C shows cell viability after treatment with an anti-BCAM antibody 477-based ADC.

[64] FIG. 19 shows representative graphs demonstrating that cell viability of various cancer cell lines is reduced after administration of an exemplary anti-BCAM ADC described herein. The anti-BCAM antibody was antibody 477 and the drug was deruxtecan

(DXd). FIG. 19A shows the reduction of viability of OVSAHO cells. FIG. 19B shows the reduction of viability of Kuramochi cells. FIG. 19C shows the reduction of viability of OVCAR3 cells. FIG. 19D shows the reduction of viability of OVCAR8 (OV8) cells. FIG. 19E shows the reduction of viability of MFE-280 cells.

[65] FIG. 20 shows a representative graph demonstrating a sustained reduction of tumor volume in NSG mice treated with an anti-BCAM antibody 477-based ADC comprising Dxd at an antibody-drug ratio of 4 (DAR4), compared to treatment with an anti-RSV ADC (including Dxd at DAR4) and vehicle only (negative controls).

[66] FIG. 21 shows a representative ADC comprising a biparatopic anti-BCAM antibody. This biparatopic anti-BCAM ADC comprises (1) a first full-length anti-BCAM antibody comprising an IgG Fc region, and (2) a second anti-BCAM single-chain variable fragment (scFv) of which two copies are fused to the C-termini of the IgG Fc region. The full-length anti-BCAM antibody is labelled as "α-BCAM IgG (1)" and shown in black and light grey. The scFvs are labelled as "α-BCAM scFv (2)" and shown in dark grey and white. Two drug moieties linked to the IgG Fc region are represented by the vertical-striped ovals.

[67] FIG. 22 shows representative graphs illustrating that picomolar concentrations of biparatopic anti-BCAM ADCs in the format shown in FIG. 21 and comprising MMAE as the drug were able to reduce the viability of OVCAR3 cells at antibody-drug ratios of 2 (DAR2). FIG. 22A shows cell viability after treatment with a biparatopic anti-BCAM ADC comprising a 929-based IgG1 and a 477-based scFv ("929-477scFv ADC"). FIG. 22B shows cell viability after treatment with a biparatopic anti-BCAM ADC comprising a 452-based IgG1 and a 477-based scFv ("452-477scFv ADC"). FIG. 22C shows cell viability after treatment with a biparatopic anti-BCAM ADC comprising a 452-based IgG1 and a 929-based scFv ("452-929scFv ADC"). ADCs of the component antibodies of the tested biparatopic constructs were included as control. The control ADCs were formatted as full-length IgG antibodies. The nomenclature of FIG. 21 is used to indicate whether the control ADC formed the α-BCAM IgG (1) or the α-BCAM scFv (2). An IgG comprising MMAE ("IgG-oYo") was included as a negative control.

DETAILED DESCRIPTION

[68] Provided herein are antibodies that specifically bind to BCAM, in particular human BCAM. Also provided are methods of treating cancer using the present anti-BCAM antibodies.

Basal cell adhesion molecule (BCAM)

[69] BCAM, also referred to in the art as Lutheran antigen and CD239, is a glycoprotein that has been described as a receptor for Laminin alpha-5.

[70] BCAM contains five immunoglobulin superfamily extracellular domains, a single pass transmembrane domain, and a short C-terminal cytoplasmic tail. The five extracellular domains comprise, from N-terminus to C-terminus, V1, V2, C1, C2, and C3 (correspondingly referred to as D1, D2, D3, D4, and D5, respectively). The Laminin alpha-5 binding is expected to involve residues in the V2 and/or C1 domains, as well as in the region linking these two domains. In particular, residues E309, D310 and D312 of the amino acid sequence of human BCAM as set forth in SEQ ID NO: 1 have been shown to be important for Laminin alpha-5 binding (Mankelow et al. 2007, *Blood* 110(9):3398-3406).

[71] Two transcript variants of human BCAM have been identified in the art (the sequences of which have been previously deposited as NCBI reference numbers NM_005581.5 and NM_001013257.2). The difference between these two transcript variants is the length of the C-terminal cytoplasmic tail. The shorter of the two isoforms lacks C terminal residues 558 to 597 of SEQ ID NO: 1. The N-terminal region of each sequence comprises a signal peptide which is cleaved, such that BCAM at the cell surface typically does not comprise the N-terminal signal peptide.

[72] The amino acid sequence of human BCAM is shown below. This sequence does not include the first 31 amino acids comprising the N-terminal signal peptide sequence. The residue numbering used herein begins with the first residue shown below, E. SEQ ID NO: 1 corresponds to residues 32 to 628 of UniProtKB ID: P50895.

EVRLSVPPLVEVMRGKSVILDCTPTGTHDHYMLEWFLTDRSGARPRLASAEMQGS
ELQVTMHDTRGRSPPYQLDSQGRLVLAEAQVGDERDYVCVVRAGAAGTAEATAR
LNVFAKPEATEVSPNKGTLSVMEDSAQEIATCNSRNGNPAPKITWYRNGQRLEVPV
EMNPEGYMTSRTVREASGLLSLTSTLYLRLRKDDRDASFHCAAHYSLPEGRHGRL
DSPTFHLTLHYPTEHVQFWVGSPSTPAGWVREGDTVQLLCRGDGSPSPEYTLFRLQ

DEQEEVLNVNLEGNLTLEGVTRGQSGTYGCRVEDYDAADDVQLSKTLELRVAYL
DPLELSEGKVLSLPLNSSAVVNCSVHGLPTPALRWTKDSTPLGDGPMLSLSSITFDS
NGTYVCEASLPTVPVLSRTQNFTLLVQGSPELKTAEIEPKADGSWREGDEVTLICSA
RGHPDPKLSWSQLGGSPAEPIPGRQGWVSSSLTLKVTSALSRDGISCEASNPHGNK
RHVFHFGTVSPQTSQAGVAVMAVAVSVGLLLLVVAVFYCVRRKGGPCCRQRREK
GAPPPGEPGLSHSGSEQPEQTGLLMGGASGGARGGSGGFGDEC (SEQ ID NO: 1).

Anti-BCAM antibody

[73] The present anti-BCAM antibodies are capable of specifically binding BCAM, in particular human BCAM. Typically, such antibodies are provided as isolated antibodies, in particular isolated monoclonal antibodies ("MAbs"). MAbs are antibodies derived from a single copy or clone including, for example, any eukaryotic, prokaryotic or phage clone. The amino acid sequence of illustrative anti-BCAM antibodies of the present disclosure and their CDR and variable chain sequences are shown in Table 1.

Table 1. Anti-BCAM antibody amino acid sequences

Antibody	Heavy chain SEQ ID NO					Light chain SEQ ID NO				
	CDR1	CDR2	CDR3	VH	HC	CDR1	CDR2	CDR3	VL	LC
228	30	31	32	36	38	33	34	35	37	39
232	40	41	42	46	48	43	44	45	47	49
452	18	19	20	24	50	21	22	23	25	51
477	2	3	4	14	52	5	6	7	15	53
606	54	55	56	60	62	57	58	59	61	63
929	8	9	10	16	64	11	12	13	17	65
937	66	67	68	72	74	69	70	71	73	75

[74] The present anti-BCAM antibodies have been selected, among other parameters, for their high affinity and specificity for human BCAM and their capability of binding human BCAM polymorphisms. For example, MAbs 228, 232, 452, 477, 606, 929, and 937 each are capable of binding at least eight different human BCAM polymorphisms. Accordingly, the anti-BCAM antibody may be capable of binding at least nine different human BCAM polymorphisms, e.g. MAbs 228, 232, 452, 477, 606, and 929. For example, the anti-BCAM antibody may be capable of binding nine or more of Lu21, Lu20, Lu17, Lu16, Lu13, Lu12, Lu8, Lu6, Lu5, Lu4, and Lu2. Other characteristics of present anti-BCAM antibodies may include the capability of the antibody to be effectively internalized into cells upon binding of human BCAM and the absence of potential sequence liabilities.

[75] For example, the present anti-BCAM antibody may meet at least the first three requirements set out in Table 2. In some instances, the present anti-BCAM antibody may meet at least the first three requirements and the last requirement set out in Table 2. Alternatively, the present anti-BCAM antibody may meet all the requirements set out in Table 2.

Table 2: Binding characteristics

Parameter	**Requirement**
Binding to BCAM knock out cells	< 2.5% binding
Cross-reactivity to homologs RAGE, MCAM, and ALCAM	< 5% binding
K_D (as determined by cell-based assay)	< 5 nM
K_D (as determined by BLI)	< 1 nM
K_D (as determined by ELISA)	< 6 nM
No. of BCAM polymorphisms bound	≥ 9

[76] Antibodies can be produced, for example, by hybridoma technologies, recombinant technologies, phage display technologies, synthetic technologies (e.g., CDR or specificity-determining residue, SDR, grafting), or combinations of such or other technologies known in the art. The use of hybridoma technologies typically involves immunization of an animal (e.g., a mouse) with the extracellular portion of human BCAM. A variety of well-known methods and tools can be used for producing and purifying the present anti-BCAM antibodies, including vectors, for example, plasmids, viruses, or other vehicles for polynucleotide insertion or expression, and hosts, for example, microbial, yeast, insect, and mammalian organisms (see, e.g., Process Scale Purification of Antibodies, Uwe Gottschalk, ed., 2d ed. 2017).

Modulating binding to Laminin alpha-5

[77] The present anti-BCAM antibodies may be capable of modulating the binding of human BCAM to Laminin alpha-5 (e.g., MAbs 232, 452, 477, 929, and 937). For example, the present anti-BCAM antibody (e.g., MAbs 232, 477, 929, and 937) may be capable of reducing, attenuating, or inhibiting BCAM binding to Laminin alpha-5. Alternatively, the present anti-BCAM antibodies (e.g., MAb 452) may be capable of increasing BCAM binding to Laminin alpha-5. As demonstrated herein, such agonist and antagonist antibodies are both capable of specifically binding to human BCAM on cancer cells and reducing the viability of BCAM-expressing cancer cells. In some instances, the present anti-BCAM antibodies may not modulate the binding of human BCAM to Laminin alpha-5 (e.g., MAbs 228 and 606).

[78] The anti-BCAM antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence about 85% or more (e.g., about 90%, about 95% identical), or identical, to:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLEWMGIINPF GGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGIYGHFDYWG QGTLVTVSS (SEQ ID NO: 14);

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGGIIPFS GTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTDPILSFWSGYYYY YGMDVWGQGTTVTVSS (SEQ ID NO: 16);

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWMN PNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTYSSSWIFD YWGQGTLVTVSS (SEQ ID NO: 24);

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWINP NSGDTNYAQEFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARKRDGGGPFD YWGQGTLVTVSS (SEQ ID NO: 46); or

EVQLLESGGGLVQPGGSLRLSCAASGFAFDDYAMHWVRQAPGKGLEWLAVISYD GSHMSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGQLVWHPPD YWGQGTLVTVSS (SEQ ID NO: 72).

[79] The anti-BCAM antibody may comprise an immunoglobulin light chain variable (VL) region comprising an amino acid sequence about 85% or more (e.g., about 90%, about 95% identical), or identical, to:

DIVMTQSPDSLAVSLGERATINCKSSQSVLSASNNQNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPYTFGQGTKLEIK (SEQ ID NO: 15);

DIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASNLETG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTRLEIK (SEQ ID NO: 17);

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSIPITFGQGTRLEIK (SEQ ID NO: 25);

DIVMTQSPDSLAVSLGERATINCKSSQSVLSRSNNKNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPFTFGPGTKVDIK R (SEQ ID NO: 47); or

DIQMTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASYLQSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYRTPFTFGQGTKVDIK (SEQ ID NO: 73).

[80] The anti-BCAM antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence about 85%, about 90%, about 95% or more (e.g., at least 85%) identical to, or identical to QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLEWMGIINPF GGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGIYGHFDYWG QGTLVTVSS (SEQ ID NO: 14), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence about 85%, about 90%, about 95% or more (e.g., at least 85%) identical to, or identical to DIVMTQSPDSLAVSLGERATINCKSSQSVLSASNNQNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPYTFGQGTKLEIK (SEQ ID NO: 15).

[81] The anti-BCAM antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence about 85%, about 90%, about 95% or more (e.g., at least 85%) identical to, or identical to QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGGIIPFS GTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTDPILSFWSGYYYY YGMDVWGQGTTVTVSS (SEQ ID NO: 16), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence about 85%, about 90%, about 95% or more (e.g., at least 85%) identical to, or identical to DIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASNLETG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTRLEIK (SEQ ID NO: 17).

[82] The anti-BCAM antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence about 85%, about 90%, about 95% or more (e.g., at least 85%) identical to, or identical to QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWMN

PNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTYSSSWIFD YWGQGTLVTVSS (SEQ ID NO: 24), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence about 85%, about 90%, about 95% or more (e.g., at least 85%) identical to, or identical to DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSIPITFGQGTRLEIK (SEQ ID NO: 25).

[83] The anti-BCAM antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence about 85%, about 90%, about 95% or more (e.g., at least 85%) identical to, or identical to QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWINP NSGDTNYAQEFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARKRDGGGPFD YWGQGTLVTVSS (SEQ ID NO: 46), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence about 85%, about 90%, about 95% or more (e.g., at least 85%) identical to, or identical to

DIVMTQSPDSLAVSLGERATINCKSSQSVLSRSNNKNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPFTFGPGTKVDIK R (SEQ ID NO: 47).

[84] The anti-BCAM antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence about 85%, about 90%, about 95% or more (e.g., at least 85%) identical to, or identical to EVQLLESGGGLVQPGGSLRLSCAASGFAFDDYAMHWVRQAPGKGLEWLAVISYD GSHMSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGQLVWHPPD YWGQGTLVTVSS (SEQ ID NO: 72), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence about 85%, about 90%, about 95% or more (e.g., at least 85%) identical to, or identical to

DIQMTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASYLQSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYRTPFTFGQGTKVDIK (SEQ ID NO: 73).

[85] The anti-BCAM antibody may have a disassociation constant (K_D) in the nanomolar range. Alternatively, the anti-BCAM antibody may have a K_D in the picomolar

range. For example, the antibody may have a K_D of about 100 nM or less, e.g., about 100 nM or less, e.g., from about 0.001 nM to about 100 nM, or from about 0.01 nM to about 10 nM. Or, the antibody may have a K_D of about 10 nM or less, e.g., from about 0.01 nM to about 10 nM.

[86] The K_D may be determined using a cell-based assay, e.g., as demonstrated in Example 1. Accordingly, the anti-BCAM antibody may have a K_D of about 10 nM or less, e.g., from about 0.01 nM to about 10 nM, as determined using a cell-based assay. The anti-BCAM antibody may have a K_D of about 5 nM or less, e.g., from about 0.01 nM to about 5 nM, as determined using a cell-based assay. The anti-BCAM antibody may have a K_D of about 5 nM, about 4 nM, about 3 nM, about 2 nM, or about 1 nM, as determined using a cell-based assay. The anti-BCAM antibody may have a K_D of about 1 nM or less, e.g., from about 0.1 nM to about 1 nM, as determined using a cell-based assay. For example, the anti-BCAM antibody may have a K_D of about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.3 nM, about 0.2 nM, or about 0.1 nM, as determined using a cell-based assay.

[87] The K_D may be determined using an Enzyme Linked Immunosorbent Assay (ELISA), e.g., as demonstrated in Example 1. Accordingly, the anti-BCAM antibody may have a K_D of less than 10 nM, typically less than 6 nM, e.g., from about 0.01 nM to about 5 nM, as determined by ELISA. The anti-BCAM antibody may have a K_D of about 5 nM, about 4 nM, about 3 nM, about 2 nM, or about 1 nM, as determined by ELISA. The anti-BCAM antibody may have a K_D of about 1 nM or less, e.g., from about 0.1 nM to about 1 nM, as determined by ELISA. For example, the anti-BCAM antibody may have a K_D of about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.3 nM, about 0.2 nM, or about 0.1 nM, as determined by ELISA.

[88] The K_D may be determined using Biolayer interferometry (BLI), e.g., as demonstrated in Example 5. Accordingly, the anti-BCAM antibody may have a K_D of about 1 nM or less, e.g., from about 0.01 nM to about 1 nM, as determined by BLI. The anti-BCAM antibody may have a K_D of about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.3 nM, about 0.2 nM, or about 0.1 nM, as determined by BLI. The anti-BCAM antibody may have a K_D of about 0.1 nM or less, e.g., from about 0.01 nM to about 0.1 nM, as determined by BLI. For example, the anti-BCAM antibody may have a K_D of about 0.1 nM, about 0.09 nM, about 0.08 nM, about 0.07 nM, about 0.06 nM, or about

0.05 nM, about 0.04 nM, about 0.03 nM, about 0.02 nM, or about 0.01 nM, as determined by BLI.

Antagonist antibodies

[89] The present anti-BCAM antibody may be capable of inhibiting BCAM binding to Laminin alpha-5. Such an antibody may specifically bind an epitope (e.g., the non-contiguous epitope) that comprises one or more (e.g., two, three, four, five, six, seven, eight, nine or ten) amino acids selected from L9, K16, S17, I19, T25, G26, T27, H28, D29, T38, R40, S41, P45, A50, M52, L57, Q58, V59, M61, D63, T64, R65, S68, Q72, L73, Q76, L81, E83, A84, E89, R97, A104, A108, R109, L110, N111, K115, A118, E132, D133, S134, N142, R144, A149, K151, V165, M167, P169, E170, M173, S175, E180, L185, R196, S203, S211, E214, R216, Q237, P223, and H226 of human BCAM comprising the amino acid sequence of SEQ ID NO: 1.

[90] The epitope (e.g., the non-contiguous epitope) may comprise one or more (e.g., two, three, four, five, or six) amino acids selected from K16, R40, S68, E83, K115, N142, R144, E180, and P223 of human BCAM. For example, the epitope (e.g., the non-contiguous epitope) may comprise amino acid R40 of human BCAM. Or, the epitope (e.g., the non-contiguous epitope) may comprise amino acid E180 of human BCAM. Alternatively, the epitope (e.g., the non-contiguous epitope) may comprise amino acid P223 of human BCAM.

[91] The binding of an anti-BCAM antibody to an epitope (e.g., a non-continuous epitope) as defined above may be determined by contacting the antibody with a human BCAM variant in which one or more residues at positions 16, 40, 68, 83, 115, 142, 144, 180, and 223 has been substituted with a different amino acid such as alanine (e.g., E180A). The human BCAM variant may comprise one or more substitutions selected from K16E, R40E, S68R, E83K, K115D, N142S, R144N, and P223H. For example, an anti-BCAM antibody capable of inhibiting BCAM binding to Laminin alpha-5 may not bind to a variant of human BCAM in which the residue at position 180 of SEQ ID NO: 1 is substituted with alanine. An anti-BCAM antibody capable of inhibiting BCAM binding to Laminin alpha-5 may not bind to a variant of human BCAM in which the residue at position 223 of SEQ ID NO: 1 is substituted with histidine. An anti-BCAM antibody capable of inhibiting BCAM binding to Laminin alpha-5 may not bind to (i) a variant of human BCAM in which the residue at position 180 of SEQ ID NO: 1 is substituted with alanine, and (ii) a variant of human BCAM

in which the residue at position 223 of SEQ ID NO: 1 is substituted with histidine. An anti-BCAM antibody capable of inhibiting BCAM binding to Laminin alpha-5 may not bind to a variant of human BCAM in which the residue at position 40 of SEQ ID NO: 1 is substituted with glutamic acid.

[92] An anti-BCAM antibody is determined to bind to an epitope as defined above if binding to the human BCAM variant is reduced by at least 80% (e.g., 90% or 95%) relative to wild-type human BCAM. For example, binding can be determined by flow cytometry, e.g., as described in example 5, using cells (e.g., 293 cells) expressing human BCAM or a human BCAM variant, respectively. Anti-BCAM antibodies (e.g., monoclonal antibodies) comprising complementarity determining regions capable of specifically binding such epitopes are provided herein.

[93] An antibody capable of inhibiting BCAM binding to Laminin alpha-5 may comprise a heavy chain complementarity determining region (HCDR) 1 comprising an amino acid sequence of DYYVH (SEQ ID NO: 2), an HCDR2 comprising an amino acid sequence of IINPFGGSATYAPKFQG (SEQ ID NO: 3), an HCDR3 comprising an amino acid sequence of GIYGHFDY (SEQ ID NO: 4); and a light chain complementarity determining region (LCDR) 1 comprising an amino acid sequence of KSSQSVLSASNNQNYLA (SEQ ID NO: 5), an LCDR2 comprising an amino acid sequence of WASTRES (SEQ ID NO: 6), and an LCDR3 comprising an amino acid sequence of QQYYSSPYT (SEQ ID NO: 7).

[94] The antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence at least 85% identical (e.g., at least 95% identical) to

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLEWMGIINPF GGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGIYGHFDYWG QGTLVTVSS (SEQ ID NO: 14), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence at least 85% identical (e.g., at least 95% identical) to

DIVMTQSPDSLAVSLGERATINCKSSQSVLSASNNQNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPYTFGQGTKLEIK (SEQ ID NO: 15).

[95] An antibody capable of inhibiting BCAM binding to Laminin alpha-5 may comprise a single-chain variable fragment (scFv) comprising a sequence having at least about 85% (e.g., 90% or 95%) identity to:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLEWMGIINPF GGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGIYGHFDYWG QGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSVLSA SNNQNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAED VAVYYCQQYYSSPYTFGQGTKLEIK (SEQ ID NO: 78).

[96] Antibodies comprising such CDRs or heavy and light chain variable regions may not bind to a variant of human BCAM in which the residue at position 180 of SEQ ID NO: 1 is substituted with alanine, and/or a variant of human BCAM in which the residue at position 223 of SEQ ID NO: 1 is substituted with histidine.

[97] The antibody may have a K_D of about 10 nM or less, e.g., from about 0.01 nM to about 10 nM. The antibody may have a K_D of about 1 nM or less, from about 0.01 nM to about 1 nM, e.g., about 1 nM, about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.4 nM, about 0.2 nM, or about 0.1 nM.

[98] The antibody may have a K_D of about 0.7 nM, as determined using a cell-based assay. The antibody may have a K_D of about 0.5 nM, as determined using BLI. The antibody may have a K_D of about 0.4 nM, as determined using ELISA.

[99] The antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence identical to
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLEWMGIINPF GGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGIYGHFDYWG QGTLVTVSS (SEQ ID NO: 14), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence identical to
DIVMTQSPDSLAVSLGERATINCKSSQSVLSASNNQNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPYTFGQGTKLEIK (SEQ ID NO: 15).

[100] The antibody may comprise a single-chain variable fragment (scFv) comprising a sequence identical to:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLEWMGIINPF GGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGIYGHFDYWG QGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSVLSA SNNQNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAED VAVYYCQQYYSSPYTFGQGTKLEIK (SEQ ID NO: 78).

[101] Alternatively, an antibody capable of inhibiting BCAM binding to Laminin alpha-5 may comprise an HCDR1 comprising an amino acid sequence of SYAFS (SEQ ID NO: 8), an HCDR2 comprising an amino acid sequence of GIIPFSGTTNYAQKFQG (SEQ ID NO: 9), and HCDR3 comprising an amino acid sequence of DPILSFWSGYYYYYGMDV (SEQ ID NO: 10); and an LCDR1 comprising an amino acid sequence of RASQSISSWLA (SEQ ID NO: 11), an LCDR2 comprising an amino acid sequence of DASNLET (SEQ ID NO: 12), an LCDR3 comprising an amino acid sequence of QQSYSTPPT (SEQ ID NO: 13).

[102] The antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence at least 85% identical (e.g., at least 95% identical) to

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGGIIPFS GTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTDPILSFWSGYYYY YGMDVWGQGTTVTVSS (SEQ ID NO: 16), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence at least 85% identical (e.g., at least 95% identical) to

DIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASNLETG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTRLEIK (SEQ ID NO: 17).

[103] An antibody capable of inhibiting BCAM binding to Laminin alpha-5 may comprise a single-chain variable fragment (scFv) comprising a sequence having at least about 85% (e.g., 90% or 95%) identity to:

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGGIIPFS GTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTDPILSFWSGYYYY YGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCR ASQSISSWLAWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQSYSTPPTFGQGTRLEIK (SEQ ID NO: 79).

[104] Antibodies comprising such CDRs or VH and VL regions may not bind to a variant of human BCAM in which the residue at position 40 of SEQ ID NO: 1 is substituted with glutamic acid.

[105] The antibody may have a K_D of about 10 nM or less, e.g., from about 0.01 nM to about 10 nM. The antibody may have a K_D of about 2 nM or less, e.g., from about 0.01 nM to about 2 nM, e.g., about 1 nM, about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.4 nM, about 0.2 nM, or about 0.1 nM. The antibody may have a K_D of about 0.1 nM or less, e.g., about 0.1 nM, about 0.09 nM, about 0.08 nM, about 0.07 nM, about 0.06 nM, about 0.05 nM, about 0.04 nM, about 0.03 nM, about 0.02 nM, or about 0.01 nM.

[106] The antibody may have a K_D of about 1.2 nM, as determined using a cell-based assay. The antibody may have a K_D of about 0.06 nM, as determined using BLI. The antibody may have a K_D of about 0.7 nM, as determined using ELISA.

[107] The antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence identical to
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGGIIPFS
GTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTDPILSFWSGYYYY
YGMDVWGQGTTVTVSS (SEQ ID NO: 16), and

an immunoglobulin light chain variable (VL) region comprising an amino acid identical to

DIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASNLETG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTRLEIK (SEQ ID NO: 17).

[108] The antibody may comprise a single-chain variable fragment (scFv) comprising a sequence identical to:

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGGIIPFS
GTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTDPILSFWSGYYYY
YGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCR
ASQSISSWLAWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTLTISSLQPE
DFATYYCQQSYSTPPTFGQGTRLEIK (SEQ ID NO: 79).

[109] Alternatively, an antibody capable of inhibiting BCAM binding to Laminin alpha-5 may comprise a heavy chain complementarity determining region (HCDR) 1

comprising an amino acid sequence of SYYMH (SEQ ID NO: 40), an HCDR2 comprising an amino acid sequence of WINPNSGDTNYAQEFQG (SEQ ID NO: 41), an HCDR3 comprising an amino acid sequence of KRDGGGPFDY (SEQ ID NO: 42); and a light chain complementarity determining region (LCDR) 1 comprising an amino acid sequence of KSSQSVLSRSNNKNYLA (SEQ ID NO: 43), an LCDR2 comprising an amino acid sequence of WASTRES (SEQ ID NO: 44), and an LCDR3 comprising an amino acid sequence of QQYYSTPFT (SEQ ID NO: 45).

[110] The antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence at least 85% identical (e.g., at least 95% identical) to

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWINP NSGDTNYAQEFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARKRDGGGPFD YWGQGTLVTVSS (SEQ ID NO: 46), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence at least 85% identical (e.g., at least 95% identical) to

DIVMTQSPDSLAVSLGERATINCKSSQSVLSRSNNKNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPFTFGPGTKVDIK R (SEQ ID NO: 47).

[111] An antibody may be capable of inhibiting BCAM binding to Laminin alpha-5 comprises a single-chain variable fragment (scFv) comprising a sequence having at least about 85% (e.g., 90% or 95%) identity to:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWINP NSGDTNYAQEFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARKRDGGGPFD YWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQS VLSRSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSL QAEDVAVYYCQQYYSTPFTFGPGTKVDIK (SEQ ID NO: 80).

[112] The antibody may have a K_D of about 10 nM or less, e.g., from about 0.01 nM to about 10 nM. The antibody may have a K_D of about 5 nM or less, e.g., from about 0.1 nM to about 5 nM. The antibody may have a K_D of about 4 nM, about 3 nM, about 2 nM, or about 1 nM. The antibody may have a K_D of about 1 nM or less, e.g., from about 0.1 nM to about 1 nM, e.g., about 1 nM, about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.4 nM, about 0.2 nM, or about 0.1 nM.

[113] The antibody may have a K_D of about 2 nM, as determined using a cell-based assay. The antibody may have a K_D of about 0.6 nM, as determined using ELISA.

[114] Alternatively, an antibody capable of inhibiting BCAM binding to Laminin alpha-5 may comprise an HCDR1 comprising an amino acid sequence of DYAMH (SEQ ID NO: 66), an HCDR2 comprising an amino acid sequence of VISYDGSHMSYADSVKG (SEQ ID NO: 67), and HCDR3 comprising an amino acid sequence of GGQLVWHPPDY (SEQ ID NO: 68); and an LCDR1 comprising an amino acid sequence of RASQGISSYLA (SEQ ID NO: 69), an LCDR2 comprising an amino acid sequence of AASYLQS (SEQ ID NO: 70), an LCDR3 comprising an amino acid sequence of QQSYRTPFT (SEQ ID NO: 71).

[115] The antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence at least 85% identical (e.g., at least 95% identical) to

EVQLLESGGGLVQPGGSLRLSCAASGFAFDDYAMHWVRQAPGKGLEWLAVISYD GSHMSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGQLVWHPPD YWGQGTLVTVSS (SEQ ID NO: 72), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence least 85% identical (e.g., at least 95% identical) to

DIQMTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASYLQSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYRTPFTFGQGTKVDIK (SEQ ID NO: 73).

[116] An antibody capable of inhibiting BCAM binding to Laminin alpha-5 may comprise a single-chain variable fragment (scFv) comprising a sequence having at least about 85% (e.g., 90% or 95%) identity to:

EVQLLESGGGLVQPGGSLRLSCAASGFAFDDYAMHWVRQAPGKGLEWLAVISYD GSHMSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGQLVWHPPD YWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQGI SSYLAWYQQKPGKAPKLLIYAASYLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYRTPFTFGQGTKVDIK (SEQ ID NO: 81).

[117] The antibody may have a K_D of about 10 nM or less, e.g., from about 0.01 nM to about 10 nM. The antibody may have a K_D of about 5 nM or less, e.g., from about 0.1 nM to about 5 nM. The antibody may have a K_D of about 4 nM, about 3 nM, about 2 nM, or about 1 nM.

[118] The antibody may have a K_D of about 1.8 nM, as determined using a cell-based assay.

[119] The antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence identical to

EVQLLESGGGLVQPGGSLRLSCAASGFAFDDYAMHWVRQAPGKGLEWLAVISYD GSHMSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGQLVWHPPD YWGQGTLVTVSS (SEQ ID NO: 72), and

an immunoglobulin light chain variable (VL) region comprising an amino acid identical to

DIQMTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASYLQSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYRTPFTFGQGTKVDIK (SEQ ID NO: 73).

[120] The antibody may comprise a single-chain variable fragment (scFv) comprising a sequence identical to:

EVQLLESGGGLVQPGGSLRLSCAASGFAFDDYAMHWVRQAPGKGLEWLAVISYD GSHMSYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGQLVWHPPD YWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQGI SSYLAWYQQKPGKAPKLLIYAASYLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYRTPFTFGQGTKVDIK (SEQ ID NO: 81).

[121] In some instances, an optimized (e.g., affinity-matured) variant anti-BCAM antibody is provided that may comprise one or more substitutions in one or more CDR or framework residues without negatively affecting (e.g., without diminishing or altering) the binding properties of the parent antibody as defined in the preceding paragraphs. Alternatively or additionally, the variant anti-BCAM antibody may be modified to remove one or more sequence liabilities.

Agonist antibodies

[122] Also provided are anti-BCAM antibodies that are capable of increasing BCAM binding to Laminin alpha-5.

[123] Such an antibody may comprise an HCDR1 comprising an amino acid sequence of SYYMH (SEQ ID NO: 18), an HCDR2 comprising an amino acid sequence of WMNPNSGDSGYAQKFQG (SEQ ID NO: 19), an HCDR3 comprising an amino acid sequence of TYSSSWIFDY (SEQ ID NO: 20); and an LCDR1 comprising an amino acid

sequence of KSSQSVLYSSNNKNYLA (SEQ ID NO: 21), an LCDR2 comprising an amino acid sequence of WASTRES (SEQ ID NO: 22), an LCDR3 comprising an amino acid sequence of QQYYSIPIT (SEQ ID NO: 23).

[124] The antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence at least 85% identical (e.g., at least 95% identical) to

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWMN PNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTYSSSWIFD YWGQGTLVTVSS (SEQ ID NO: 24), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence at least 85% identical (e.g., at least 95% identical) to

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSIPITFGQGTRLEIK (SEQ ID NO: 25).

[125] An antibody capable of increasing BCAM binding to Laminin alpha-5 may comprise a single-chain variable fragment (scFv) comprising a sequence having at least about 85% (e.g., 90% or 95%) identity to:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWMN PNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTYSSSWIFD YWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQS VLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSL QAEDVAVYYCQQYYSIPITFGQGTRLEIK (SEQ ID NO: 82).

[126] The antibody may have a K_D of about 10 nM or less, e.g., from about 0.01 nM to about 10 nM. In some embodiments, the antibody has a K_D of less than 6 nM, e.g., from about 0.01 nM to about 5 nM. The antibody may have a K_D of about 5 nM, about 4 nM, about 3 nM, about 2 nM, or about 1 nM. The antibody may have a K_D of about 1 nM or less, e.g., from about 0.1 nM to about 1 nM, e.g., about 1 nM, about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.4 nM, about 0.2 nM, or about 0.1 nM.

[127] The antibody may have a K_D of about 0.3 nM, as determined using a cell-based assay. The antibody may have a K_D of about 5 nM, as determined using ELISA. The antibody may have a K_D of about 0.8 nM, as determined using BLI.

[128] The antibody may comprise an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence identical to
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWMN
PNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTYSSSWIFD
YWGQGTLVTVSS (SEQ ID NO: 24), and

an immunoglobulin light chain variable (VL) region comprising an amino acid sequence identical to

DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYW
ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSIPITFGQGTRLEIK
(SEQ ID NO: 25).

[129] The antibody may comprise a single-chain variable fragment (scFv) comprising a sequence identical to:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWMN
PNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTYSSSWIFD
YWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQS
VLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSL
QAEDVAVYYCQQYYSIPITFGQGTRLEIK (SEQ ID NO: 82).

[130] In some instances, an optimized (e.g., affinity-matured) variant anti-BCAM antibody may be provided that comprises one or more substitutions in one or more CDR or framework residues without negatively affecting (e.g., without diminishing or altering) the binding properties of the parent antibody. Alternatively or additionally, the variant anti-BCAM antibody may be modified to remove one or more sequence liabilities.

Further antibodies

[131] Further antibodies (e.g., further human antibodies) having a complementarity determining means for binding human BCAM equivalent to the antibodies exemplified herein (e.g., Mabs 452, 477, and 929) can be prepared or identified by well-known methods, such as immunization of transgenic animals and isolation of hybridomas, and screening of phage or yeast display libraries (see, *e.g.,* Monoclonal Antibodies: Methods and Protocols,

Vincent Ossipow and Nicolas Fischer, eds., 2d ed. 2014). In addition, a variant antibody can be provided that comprises one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) substitutions in one or more CDR(s) or framework residue(s) without negatively affecting (e.g., without diminishing) the binding properties of the parent antibody.

[132] Antibody binding means or complementarity determining means for binding human BCAM (e.g., antibodies) equivalent to the present anti-BCAM antibodies differ in their amino acid sequence but perform the same function of binding the target through CDR-target interaction and act in the same manner (e.g., as an agonist or an antagonist) and/or achieve a comparable result (such as inhibiting tumor growth). For example, complementarity determining means for binding human BCAM are antibodies having 85% identity to an anti-BCAM antibody disclosed herein and inhibit tumor growth alone or as an ADC or BiTE.

[133] The complementarity determining means for binding human BCAM may function through binding an epitope that is identical to, or overlaps with, an epitope on human BCAM bound by an antibody described herein. Antibodies with such complementarity determining means for binding human BCAM can be identified by their capability of competing with an anti-BCAM antibody described herein (e.g., MAbs 477 and 929) for binding of human BCAM. Such binding competition can be determined by an assay in which an anti-BCAM antibody of the invention provided herein prevents, inhibits, or reduces specific binding of a test antibody, and vice versa, as demonstrated, e.g., in example 4.

[134] A test antibody is determined to be in competition with an anti-BCAM antibody of the invention if binding of the anti-BCAM antibody of the invention to human BCAM is reduced by at least 10% or more (e.g., 20% or more, 30% or more, or 40% or more) relative to binding of the anti-BCAM antibody of the invention to human BCAM alone. In particular, a test antibody may be determined to be in competition with an anti-BCAM antibody of the invention if binding of the anti-BCAM antibody of the invention to human BCAM is reduced by at least 50% or more (e.g., 60% or more, 70% or more, or 80% or more) relative to binding of the anti-BCAM antibody of the invention to human BCAM alone.

[135] Antibodies that bind an epitope that is identical to the epitope of an anti-BCAM antibody of the invention compete with said antibody for binding of human BCAM. Similarly, as demonstrated in the examples provided herein, antibodies that bind an epitope that overlaps with, or is adjacent to, an epitope bound by an anti-BCAM antibody of the

invention compete with said antibody for binding of human BCAM. Typically, an overlapping epitope comprises at least one residue that is identical to at least one residue of an epitope of interest.

[136] Typically, an adjacent epitope does not have any residues in common with an epitope of interest, but binding of a further antibody to the adjacent epitope reduces binding of an anti-BCAM antibody of the invention to human BCAM by at least 10% or more (e.g., 20% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70% or more, or 80% or more) relative to binding of the anti-BCAM antibody of the invention to human BCAM alone.

[137] Numerous types of competitive binding assays can be used to determine if a test antibody competes with an anti-BCAM antibody of the invention for binding of human BCAM. Examples of assays that can be employed include solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see, e.g., Stahli et al. (1983) *Methods in Enzymology* 9:242-253), solid phase direct biotin-avidin EIA (see, e.g., Kirkland et al., (1986) *J. Immunol.* 137:3614-9), solid phase direct labeled assay, solid phase direct labeled sandwich assay, Luminex (Jia *et al.* "A novel method of Multiplexed Competitive Antibody Binning for the characterization of monoclonal antibodies" *J. Immunological Methods* (2004) 288, 91–98), and surface plasmon resonance (Song *et al.* "Epitope Mapping of Ibalizumab, a Humanized Anti-CD4 Monoclonal Antibody with Anti-HIV-1 Activity in Infected Patients" *J. Virol.* (2010) 84**,** 6935-42).

[138] Competitive binding may be determined by flow cytometry, e.g., as demonstrated in example 4, using cells (e.g., 293 cells) expressing human BCAM. Accordingly, human BCAM-expressing cells may be incubated with an unlabeled anti-BCAM antibody of the invention at a saturating concentration (e.g., 20 μg/ml) for a period sufficient to allow the anti-BCAM antibody of the invention to bind surface-expressed human BCAM (e.g., 15-60 minutes). Control cells may be incubated under the same conditions without an antibody or with a control antibody that does not bind human BCAM. At the end of the incubation period, the cells may be washed to remove any unbound antibody. A test antibody labelled with a detectable moiety (e.g., a fluorescent moiety) may be added to the cells incubated with the anti-BCAM antibody of the invention and the control cells for a period sufficient to allow the test antibody to bind surface-expressed human BCAM (e.g., 15-60 minutes). Subsequently, the cells may be washed, placed in a suitable medium (e.g.,

PBS with 5% BSA), and analyzed by flow cytometry to quantify the number of labelled cells incubated with the anti-BCAM antibody of the invention and the number of labelled control cells. A test antibody may be considered to compete with the anti-BCAM antibody of the invention if at least 10% (e.g., at least 50%) of the cells incubated with the unlabeled anti-BCAM antibody of the invention are labelled with the test antibody as compared to the control cells.

[139] For example, a further antibody may be provided that specifically binds human BCAM and competes for binding to human BCAM with an antibody comprising an HCDR1 comprising an amino acid sequence of SEQ ID NO: 2, an HCDR2 comprising an amino acid sequence of SEQ ID NO: 3, an HCDR3 comprising an amino acid sequence of SEQ ID NO: 4; and a light chain complementarity determining region (LCDR) 1 comprising an amino acid sequence of SEQ ID NO: 5, an LCDR2 comprising an amino acid sequence of SEQ ID NO: 6, and an LCDR3 comprising an amino acid sequence of SEQ ID NO: 7 (e.g., MAb 477).

[140] Alternatively, a further antibody may be provided that specifically binds human BCAM and competes for binding to human BCAM with an antibody comprising an HCDR1 comprising an amino acid sequence of SEQ ID NO: 8, an HCDR2 comprising an amino acid sequence of SEQ ID NO: 9, an HCDR3 comprising an amino acid sequence of SEQ ID NO: 10; and a light chain complementarity determining region (LCDR) 1 comprising an amino acid sequence of SEQ ID NO: 11, an LCDR2 comprising an amino acid sequence of SEQ ID NO: 12, and an LCDR3 comprising an amino acid sequence of SEQ ID NO: 13 (e.g., MAb 929).

[141] Or, a further antibody may be provided that specifically binds human BCAM and competes for binding to human BCAM with an antibody comprising an HCDR1 comprising an amino acid sequence of SEQ ID NO: 18, an HCDR2 comprising an amino acid sequence of SEQ ID NO: 19, an HCDR3 comprising an amino acid sequence of SEQ ID NO: 20; and a light chain complementarity determining region (LCDR) 1 comprising an amino acid sequence of SEQ ID NO: 21, an LCDR2 comprising an amino acid sequence of SEQ ID NO: 22, and an LCDR3 comprising an amino acid sequence of SEQ ID NO: 23 (e.g., MAb 452).

[142] In some instances, further antibodies (e.g., further human antibodies) may be provided that specifically bind human BCAM and compete for binding to human BCAM with an antibody comprising:

(A) an immunoglobulin heavy chain variable (VH) region comprising the amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLEWMGIINPFGGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGIYGHFDYWGQGTLVTVSS (SEQ ID NO: 14), and

an immunoglobulin light chain variable (VL) region comprising the amino acid sequence DIVMTQSPDSLAVSLGERATINCKSSQSVLSASNNQNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPYTFGQGTKLEIK (SEQ ID NO: 15) (i.e., the VH and VL regions of MAb 477); and/or

(B) an immunoglobulin heavy chain variable (VH) region comprising the amino acid sequence QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGGIIPFSGTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTDPILSFWSGYYYYYGMDVWGQGTTVTVSS (SEQ ID NO: 16), and

an immunoglobulin light chain variable (VL) region comprising the amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTRLEIK (SEQ ID NO: 17) (i.e., the VH and VL regions of MAb 929).

[143] Further antibodies may also be provided that specifically bind human BCAM and compete for binding to human BCAM with an antibody comprising:

an immunoglobulin heavy chain variable (VH) region comprising the amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWMNPNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTYSSSWIFDYWGQGTLVTVSS (SEQ ID NO: 24), and

an immunoglobulin light chain variable (VL) region comprising the amino acid sequence DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYW

ASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSIPITFGQGTRLEIK (SEQ ID NO: 25) (i.e., the VH and VL regions of MAb 452).

Fc region

[144] A present anti-BCAM antibody may comprise an Fc region. An anti-BCAM antibody described herein may be provided with an Fc region for various reasons. For example, an Fc region may provide one or more effector function(s). Alternatively or additionally, an Fc region may be included to extend the half-life of an antibody in circulation.

[145] Suitable Fc regions for use with the present anti-BCAM antibodies include Fc regions of an IgG subclass antibody (referred herein as "IgG Fc region"). The IgG Fc region may be human. The IgG Fc region may be humanized.

[146] The IgG Fc region may be of the IgG1 subclass. The IgG1 may be human. The IgG Fc region may be of the IgG3 subclass. The IgG3 may be human.

[147] An exemplary human IgG1 Fc comprises the amino acid sequence ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 76).

[148] The Fc region may be modified to increase one or more effector function(s). For example, the Fc region may be modified to be afucosylated.

[149] The Fc region may be modified to reduce or eliminate one or more effector functions. Effector functions may not be desirable in applications in which an anti-BCAM antibody is primarily used for targeting a payload to a BCAM-expressing cancer cell (e.g., when used in an antibody-drug conjugate) or to activate the inherent cytolytic potential of T cells against a BCAM-expressing cancer cell (e.g., when used in a BiTE).

[150] Mutations in the Fc region that result in reduced or eliminated effector function include LALA mutations (typically referred to as L234A/L235A, optionally further comprising K322A). Accordingly, the Fc region may comprise LALA mutations. Effector

functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) are induced through binding of an antibody to one or more Fc receptors (FcRs) such as FcRI, FcRII, and/or FcRIIIa. PG mutations (typically referred to as P329G) can reduce or eliminate binding to FcR. Accordingly, in some embodiments, the Fc region comprises a PG mutation.

[151] The LALA and PG mutations may be combined, i.e., the Fc region comprises the LALA mutations and the PG mutation. An exemplary IgG1 Fc region comprising LALA and PG mutations is as follows, where the LALA and PG mutations are shown in bold:

DKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL**G** APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNRFTQKSL SLSPG (SEQ ID NO: 77).

Effector function

[152] An anti-BCAM antibody described herein may comprise a functional Fc region, i.e., an Fc region that provides one or more effector function(s) such as antibody-dependent cell-mediated cytotoxicity (ADCC).

Antibody-dependent cell-mediated cytotoxicity (ADCC)

[153] ADCC is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies.

[154] When provided with a functional Fc region (e.g., as full-length antibodies), the present anti-BCAM antibodies (e.g., MAbs 232, 477 and 452) are capable of inducing measurable ADCC at nanomolar concentrations. ADCC can be assessed as described in the examples provided herein, e.g., by incubating BCAM-expressing cancer cells (e.g., OVSAHO or OAW28 cells) in the presence or absence of an antibody described herein and determining the viability of the cells over a period of time in the presence or absence of the antibody. As demonstrated herein, an antibody that is capable of inducing ADCC may have utility in anti-tumor therapy. Accordingly, the present anti-BCAM antibodies may be capable of inducing ADCC.

[155] ADCC is mediated through the binding of IgG to Fc receptors (FcRs) on the effector cells of the immune system, including natural killer (NK) cells, monocytes,

macrophages, and eosinophils. Fc of IgG binds to FcRI, FcRII, and (predominantly) FcRIIIa. For example, ADCC can be triggered by antibodies comprising an IgG1 or IgG3 Fc region.

[156] Accordingly, an anti-BCAM antibody described herein may comprise a functional Fc region, e.g., an IgG Fc region. Such an antibody is capable of inducing ADCC. The IgG may be of the IgG1 subclass. The IgG1 may be human (or humanized). Alternatively, the IgG may be of the IgG3 subclass. The IgG3 may be human (or humanized).

[157] The Fc region of antibodies of the IgG isotype possesses a conserved glycosylation site at N297 in each of the C_H2 domains. The N-linked oligosaccharides expressed at this site have a significant effect on the effector functions of antibodies of the IgG isotype. For example, the presence of fucose residues can lead to severely reduced ADCC efficiency. The removal of the core fucose residue from the N-glycans of IgG-Fc results in dramatic enhancement (>50-fold) of antibody-dependent cellular cytotoxicity (ADCC) through improved IgG binding to FcR receptor IIIa (FcRIIIa) (Yamane-Ohnuki et al., 2004; Iida et al., 2009). Accordingly, the Fc region of an anti-BCAM antibody described herein may be modified to be afucosylated.

[158] An anti-BCAM antibody provided herein may be afucosylated. Afucosylated antibodies can be produced in cell lines lacking a functional *FUT8* gene coding for the enzyme α-1,6-fucosyltransferase, or over-expressing a recombinant β-1,4-N-acetylglucosaminyl-transferase III. Antibodies produced in such cell lines are enriched in bisected and non-fucosylated oligosaccharides.

Other antibody properties

[159] The present anti-BCAM antibodies (e.g., MAbs 929, 477 and 452) have excellent thermostability, for example when provided as full-length antibodies (e.g., in an IgG format). Moreover, they show minimal polyreactivity. They also show minimal aggregation.

[160] Thermostability can be examined using full IgG antibodies. It is desirable that an antibody displays a Fab stability of >68°C during thermostability testing.

[161] Polyreactivity can be assessed by incubating an antibody with KLH, LPS and/or human insulin, respectively. It is desirable that an antibody described herein has minimal polyreactivity, e.g., does not react with keyhole limpet hemocyanin (KLH), lipopolysaccharide (LPS) and/or human insulin.

[162] Moreover, the present anti-BCAM antibodies (e.g., MAbs 929, 477 and 452) are free of sequence liabilities including those that may be involved in the formation of aggregates. For example, two or more consecutive positively charged residues (e.g., RR or RKR) can contribute to the "stickiness" of antibodies, rendering them less desirable for therapeutic applications.

[163] The formation of aggregates can be assessed by incubating an antibody in a suitable storage buffer (e.g., PBS), for 2-4 weeks (e.g., 4 weeks) at 4°C. Aggregation can be considered minimal when, e.g., 10% or less of the antibody is present as high molecular weight aggregates at the end of the incubation period as, assessed, e.g., by mass photometry.

[164] Other sequence liabilities include amino acid residues that may be subjected to post-translational modifications (PTMs). PTMs can affect affinity, stability, potency, and/or homogeneity of an antibody. PTMs can include deamidation, isomerization, oxidation, N-linked glycosylation, free thiol, pyro-Glutamate, and C-terminal lysine.

[165] An anti-BCAM antibody may be free from sequence liabilities such as two or more consecutive positively charged residues or N-linked glycosylation that interferes with affinity, stability, potency, and/or homogeneity of the anti-BCAM antibody.

[166] A present anti-BCAM antibody may meet at least the first two requirements set out in Table 3. A present anti-BCAM antibody may meet at least the first three requirements set out in Table 3. In particular, a present anti-BCAM antibody may meet all the requirements set out in Table 3.

Table 3.

Parameter	**Requirement**
Aggregation	Forms less than 10% aggregates as assessed by mass photometry after 4 weeks of storage in PBS
Thermostability	Has a Fab stability of ≥ 68°C
Polyreactivity	Does not react with KLH, LPS, and human insulin
Sequence liabilities	Does not comprise two or more consecutive positively charged residues or N-linked glycosylation that interferes with affinity and/or stability

Exemplary antibodies

[167] A first human or humanized IgG antibody is provided that specifically binds human BCAM and comprises:

a) a HCDR1 comprising an amino acid sequence of DYYVH (SEQ ID NO: 2), an HCDR2 comprising an amino acid sequence of IINPFGGSATYAPKFQG (SEQ ID NO: 3), an HCDR3 comprising an amino acid sequence of GIYGHFDY (SEQ ID NO: 4); and

b) a LCDR1 comprising an amino acid sequence of KSSQSVLSASNNQNYLA (SEQ ID NO: 5), an LCDR2 comprising an amino acid sequence of WASTRES (SEQ ID NO: 6), and an LCDR3 comprising an amino acid sequence of QQYYSSPYT (SEQ ID NO: 7).

[168] A second human or humanized IgG antibody is provided that specifically binds human BCAM and comprises:

a) an HCDR1 comprising an amino acid sequence of SYYMH (SEQ ID NO: 18), an HCDR2 comprising an amino acid sequence of WMNPNSGDSGYAQKFQG (SEQ ID NO: 19), an HCDR3 comprising an amino acid sequence of TYSSSWIFDY (SEQ ID NO: 20); and

b) an LCDR1 comprising an amino acid sequence of KSSQSVLYSSNNKNYLA (SEQ ID NO: 21), an LCDR2 comprising an amino acid sequence of WASTRES (SEQ ID NO: 22), an LCDR3 comprising an amino acid sequence of QQYYSIPIT (SEQ ID NO: 23).

[169] A third human or humanized IgG antibody is provided that specifically binds human BCAM and comprises:

a) an HCDR1 comprising an amino acid sequence of SYAFS (SEQ ID NO: 8), an HCDR2 comprising an amino acid sequence of GIIPFSGTTNYAQKFQG (SEQ ID NO: 9), and HCDR3 comprising an amino acid sequence of DPILSFWSGYYYYYGMDV (SEQ ID NO: 10); and

b) an LCDR1 comprising an amino acid sequence of RASQSISSWLA (SEQ ID NO: 11), an LCDR2 comprising an amino acid sequence of DASNLET (SEQ ID NO: 12), an LCDR3 comprising an amino acid sequence of QQSYSTPPT (SEQ ID NO: 13).

[170] A fourth human or humanized IgG antibody is provided that specifically binds human BCAM and comprises:

a) an HCDR1 comprising an amino acid sequence of SYYMH (SEQ ID NO: 40), an HCDR2 comprising an amino acid sequence of WINPNSGDTNYAQEFQG (SEQ

ID NO: 41), an HCDR3 comprising an amino acid sequence of KRDGGGPFDY (SEQ ID NO: 42);

b) an LCDR1 comprising an amino acid sequence of KSSQSVLSRSNNKNYLA (SEQ ID NO: 43), an LCDR2 comprising an amino acid sequence of WASTRES (SEQ ID NO: 44), an LCDR3 comprising an amino acid sequence of QQYYSTPFT (SEQ ID NO: 45).

[171] The human or humanized IgG antibody may meet at least the first three requirements set out in Table 2. In some instances, the human or humanized IgG antibody may meet at least the first three requirements and the last requirement set out in Table 2. In particular, the human or humanized IgG antibody may meet all the requirements set out in Table 2.

[172] The human or humanized IgG antibody may meet at least the first two requirements set out in Table 3. In some instances, the human or humanized IgG antibody may meet at least the first three requirements set out in Table 3. In particular, the human or humanized IgG antibody may meet all the requirements set out in Table 3.

[173] A human or humanized IgG antibody is considered particularly suitable for therapeutic applications if it meets each of the requirements set out in Tables 2 and 3.

[174] The human or humanized IgG antibody may comprise an Fc region that is capable of inducing antibody-dependent cell-mediated cytotoxicity (ADCC) in a human BCAM-expressing cell (e.g., IgG1 or IgG3). The Fc region may comprise the amino acid sequence of SEQ ID NO: 76. In some instances, the Fc region does not include the C-terminal K of the amino acid sequence of SEQ ID NO: 76.

[175] Exemplary antibodies that may find utility in the therapeutic applications disclosed herein are the human IgG antibodies listed in Table 1, particularly the antibodies comprising (i) a HC comprising the amino acid sequence of SEQ ID NO: 52 and a LC comprising the amino acid sequence of SEQ ID NO: 53; (ii) a HC comprising the amino acid sequence of SEQ ID NO: 50 and a LC comprising the amino acid sequence of SEQ ID NO: 51; and (iii) a HC comprising the amino acid sequence of SEQ ID NO: 64 and a LC comprising the amino acid sequence of SEQ ID NO: 65. The human IgG antibodies may comprise one or more amino acid(s) (e.g., one, two, three, four, five, six, seven, eight, nine or ten) substitutions that do not affect the binding properties of the parent antibody. For example, such antibodies may be modified to remove one or more sequence liabilities (e.g.,

two or more consecutive positively charged residues such as RR or RKR). The Fc region of such antibodies may be modified to increase or decrease one or more effector function(s), depending on the desired therapeutic application. For example, the Fc region may be modified to be afucosylated. Alternatively or additionally, the Fc region may be modified to comprise the LALA mutations and the PG mutation.

Bispecific antibodies

[176] The present anti-BCAM antibodies may be comprised in a bispecific antibody. Bispecific antibodies are recombinant protein constructs comprising a first antibody binding means capable of specifically binding a first antigen (e.g., a tumor-associated surface antigen such as human BCAM) and a second antibody binding means capable of specifically binding a second antigen (e.g., a second tumor-associated surface antigen other than human BCAM). The first and second antibody binding means are typically connected via a linking means or linker (e.g., a (flexible) peptide linker). In some instances, the bispecific antibody may comprise:

a. an antibody means for binding human BCAM;
b. an antibody means for binding a second antigen other than human BCAM;
c. a linking means to link the antibody means for binding human BCAM to the antibody means for binding the other tumor-associated antigen; and optionally
d. a means for extending half-life.

[177] The present anti-BCAM antibodies may be utilized in the bispecific antibodies. Such antibodies include the agonist and antagonist antibodies described above as well as antigen-binding fragments derived therefrom, including suitable formatted single-chain antibodies.

[178] The second antigen may be a tumor associated surface antigen. Exemplary tumor-associated surface antigens other than human BCAM that the antibody binding means may bind to include folate receptor-α (FR-α), PSA (prostate specific antigen), HER2 (human epidermal growth factor receptor 2), MAGE (melanoma-associated antigen), CD33, CD30, CD22, CD79b, Nectin-4, TROP2, tissue factor (TF), CEA, immature laminin receptor, TAG-72, EpCAM, EphA3, mesothelin, SAP-1, gp100, TRP1, TRP2, MC1R, MUC1, EGFR, GD2, CD171, ErbB, PSMA, CDH6, Claudin6, transferrin receptor, PTK7, B7-H4, NaPI2b, MSLN,

AXL, and ROR1. For example, overexpression of FR-α can be observed in over 80% of ovarian cancers.

[179] Various technologies for making bispecific antibodies are known to persons of skill in the art. For example, knobs-into-holes technology comprises engineering the CH3 domains of two different heavy chains to promote heterodimer formation. One CH3 domain is engineered with a "knob" mutation (typically substituting an existing amino acid residue with an amino acid comprising a bulky side chain, e.g., T366W), while the other CH3 domain is engineered with a complementary "hole" mutation (typically substituting existing amino acid residues with amino acids comprising a small side chain, e.g., T336S, L368A, Y407V). This sterically favors the pairing of the two different heavy chains, resulting in a bispecific antibody. The knobs-into-holes technology allows for the production of full-length IgG-like bispecific antibodies with natural architecture and Fc-mediated effector functions.

[180] Another example is CrossMab technology which addresses challenges associated with light chain mispairing in bispecific antibody production. The CH1 domain of one heavy chain is exchanged with the constant light (CL) chain domain of its corresponding light chain. This domain exchange ensures that each light chain pairs with its cognate heavy chain, thus preventing light chain mispairing. The resulting bispecific antibody maintains an IgG-like structure with correct heavy and light chain associations. CrossMab technology can be combined with knobs-into-holes or other Fc engineering approaches to further enhance bispecific antibody formation.

[181] DuoBody technology involves separately producing two IgG1 antibodies, each containing single matched substitutions in the CH3 domain (K409R and F405L), using recombinant mammalian expression systems. After production and purification, the two antibodies are mixed under mild reducing conditions to undergo Fab arm exchange, resulting in bispecific antibodies. DuoBody technology allows for the efficient production of bispecific antibodies that closely resemble natural IgG1 molecules in structure and function.

[182] Bispecific antibodies may also be prepared in a straightforward manner by joining (i) a first antibody that specifically binds a first epitope on a first protein and (ii) a second antibody that specifically binds a second epitope on the first protein or on a second (different) protein. The first and second antibodies may be connected via one or more linking means or linkers. At least one of the antibodies may comprise an Fc region. For instance, one of the antibodies may be connected to an Fc region via a linking means or linker.

[183] In some instances, the first antibody and/or the second antibody may be reformatted for inclusion in a bispecific antibody. For example, a first full-length antibody binding to a first epitope may be reformatted as single-chain antibody, e.g., a scFv, and connected to the Fc region of a second full-length antibody binding to a second epitope via a linking means or linker. For instance, Coloma & Morrison (*Nat Biotechnol* **15**, 159–163 (1997)) describe the design and production of tetravalent bispecific antibodies in this manner. Alternatively, first and second single domain antibodies may be connected via linking means or linkers to each other and to an Fc region to form a bispecific antibody. Many variations that combine first and second antibodies in various formats to form a bispecific antibody are known to the skilled person.

Biparatopic antibodies

[184] Bispecific antibodies are capable of specifically binding to a first epitope and a second (different) epitope. The first and second epitopes may be on different proteins. Alternatively, the first and second epitopes may be different epitopes on the same protein, e.g., on human BCAM. In some instances, the different epitopes are non-overlapping. Accordingly, a bispecific antibody may be a biparatopic anti-BCAM antibody. In some instances, the biparatopic antibody may comprise:

a. a first antibody means for binding a first epitope on human BCAM;
b. a second antibody means for binding a second epitope on human BCAM;
c. a linking means to link the first antibody means to the second antibody binding means; and optionally
d. a means for extending half-life.

[185] The linked first and second antibody means may be connected to a half-life extender molecule via a peptide linker. The half-life extender molecule may comprise or consist of an Fc region. Or, the half-life extender molecule may be a serum protein (e.g., human serum albumin).

[186] The first and second antibody binding means may be the present anti-BCAM antibodies. Such antibodies include the agonist and antagonist antibodies described above as well as antigen-binding fragments derived therefrom, including suitable formatted single-chain antibodies.

[187] For example, a biparatopic anti-BCAM antibody may comprise a first antibody comprising a VH region comprising the amino acid sequence of SEQ ID NO: 16 and a VL region comprising the amino acid sequence of SEQ ID NO: 17 (i.e., the VH and VL regions of MAb 929); and a second antibody comprising a VH region comprising the amino acid sequence of SEQ ID NO: 14 and a VL region comprising the amino acid sequence of SEQ ID NO: 15 (i.e., the VH and VL regions of MAb 477).

[188] In another example, a biparatopic anti-BCAM antibody may comprise a first antibody comprising a VH region comprising the amino acid sequence of SEQ ID NO: 24 and a VL region comprising the amino acid sequence of SEQ ID NO: 25 (i.e., the VH and VL regions of MAb 452); and a second antibody comprising the amino acid sequence of SEQ ID NO: 14 and a VL region comprising the amino acid sequence of SEQ ID NO: 15 (i.e., the VH and VL regions of MAb 477).

[189] In a further example, a biparatopic anti-BCAM antibody may comprise a first antibody comprising a VH region comprising the amino acid sequence of SEQ ID NO: 24 and a VL region comprising the amino acid sequence of SEQ ID NO: 25 (i.e., the VH and VL regions of MAb 452); and a second antibody comprising a VH region comprising the amino acid sequence of SEQ ID NO: 16 and a VL region comprising the amino acid sequence of SEQ ID NO: 17 (i.e., the VH and VL regions of MAb 929).

[190] The biparatopic anti-BCAM antibody may further comprise an IgG Fc region, e.g., a human IgG1 Fc comprising the amino acid sequence of SEQ ID NO: 76. In some instances, the IgG Fc region may be modified to comprise one or more (e.g., two, three, or four) mutations (e.g., one or more deletions or substitutions). For example, the Fc region may comprise one or more amino acid substitutions to promote heterodimer formation.

[191] For example, the biparatopic anti-BCAM antibody may comprise: (i) a HC connected to a scFv via a linker comprising the amino acid sequence of SEQ ID NO: 115 and an LC comprising the amino acid sequence of SEQ ID NO: 65; (ii) a HC connected to scFv via a linker comprising the amino acid sequence of SEQ ID NO: 116 and an LC comprising the amino acid sequence of SEQ ID NO: 51; or (iii) a HC connected to a scFv via a linker comprising the amino acid sequence of SEQ ID NO: 117 and an LC comprising the amino acid sequence of SEQ ID NO: 51.

Bi-specific T-cell engagers (BiTEs)

[192] In some aspects, bi-specific T-cell engagers (BiTEs) are provided. BiTEs are recombinant protein constructs comprising a first antibody binding means capable of specifically binding a selected tumor-associated surface antigen (e.g., human BCAM) and a second antibody binding means for a surface-expressed subunit of the T cell receptor complex (e.g., CD3). The first and second antibody binding means are typically connected via a linking means or linker (e.g., a (flexible) peptide linker). BiTEs are designed to transiently connect T cells with target cells (e.g., cancer cells). This connection can activate the inherent cytolytic potential of T cells against the target cells.

[193] The bispecific T cell engager (BiTE) may comprise:

a) an antibody means for binding human basal cell adhesion molecule (BCAM);
b) an antibody means for binding CD3;
c) a linking means to link the antibody means for binding human BCAM to the antibody means for binding CD3; and optionally;
d) a means for extending half-life.

[194] The antibody means for binding human BCAM may comprise an anti-BCAM antibody. For example, the present anti-BCAM antibodies may be utilized in BiTEs. Such antibodies include the agonist and antagonist antibodies described above as well as antigen-binding fragments derived therefrom, including suitable formatted single-chain antibodies.

[195] Antibody means for binding CD3 (such as anti-CD3-antibodies or antigen-binding portions thereof) are well-known and include those described in, e.g., US 2014/0302037, US 2014/0308285, WO 2008/119567, WO 2014/144722, WO 2014/151910, WO 2015/048272, WO 2017/021354 and WO 2019/224717. Blinatumomab is an approved BiTE comprising an anti-CD3 single-chain variable fragment (scFv) that is suitable for use as an antibody means for binding CD3.

[196] A BiTE may comprise two antibody-derived antigen-binding regions connected via a (flexible) peptide linker. The first antigen-binding region (e.g., a first antigen-binding fragments such as a first scFv) may be capable of specifically binding a selected tumor-associated surface antigen (e.g., human BCAM) on a target cell (e.g., a BCAM-expressing cancer cell). The second antigen-binding region (e.g., a second antigen-binding

fragment such as a second scFv) is capable of specifically binding CD3 on the surface of a T cell.

[197] The BiTE may further comprise a half-life extender molecule. The linked first and second antibody-derived antigen-binding regions may be connected to a half-life extender molecule via a peptide linker. The half-life extender molecule may comprise or consist of an Fc region. Or, the half-life extender molecule may be a serum protein (e.g., human serum albumin).

[198] A first antigen-binding region targeting human BCAM may be derived from any anti-BCAM antibody described herein. Such antibodies include the agonist and antagonist antibodies described above as well as antigen-binding fragments derived therefrom, including suitable formatted single domain and single-chain antibodies.

[199] Exemplary first antigen-binding regions, derived from MAbs 477, 929, and 452, respectively, include one of the following anti-BCAM scFvs:

(A) QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLEWMGIINPFGGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGIYGHFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSVLSASNNQNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPYTFGQGTKLEIK (SEQ ID NO: 78);

(B) QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGGIIPFSGTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTDPILSFWSGYYYYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTRLEIK (SEQ ID NO: 79); and

(C) QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWMNPNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTYSSSWIFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSIPITFGQGTRLEIK (SEQ ID NO: 82).

[200] Exemplary second antigen-binding regions capable of specifically binding CD3 on the surface of a T cell include one of the following anti-CD3 scFvs:

(A) DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIG YINPSRGYTNYADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCARYYD DHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSP GERATLSCRASQSVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSG SGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK (SEQ ID NO: 83); and

(B) DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGY INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYD DHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGSGGVDDIQLTQSPAIMSASP GEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSG SGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELK (SEQ ID NO: 111).

[201] An exemplary half-life extender molecule is the following IgG Fc region comprising LALA and PG mutations:

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALG APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNRFTQKSL SLSPG (SEQ ID NO: 77).

[202] A suitable peptide linker for use in a BiTE described herein comprises, or consists of, the amino acid sequence GGGG (SEQ ID NO: 26) and GGGGS (SEQ ID NO: 27).

[203] A suitable signal peptide for use in a BiTE described herein comprises, or consists of, the amino acid sequence METDTLLLWVLLLWVPGSTG (SEQ ID NO: 84).

Chimeric antigen receptors

[204] In some aspects, chimeric antigen receptors (CARs) are provided. A CAR typically comprises a means for specifically binding a tumor-associated surface antigen (e.g., human BCAM) on a target cell (e.g., a BCAM-expressing cancer cell), a transmembrane

domain, and a cytoplasmic domain comprising a signaling domain and optionally at least one costimulatory signaling domain.

[205] The binding means may be an antibody (e.g., an antigen-binding fragment such as a scFv) that is capable of specifically binding the tumor-associated surface antigen (e.g., human BCAM) on the target cell (e.g., a BCAM-expressing cancer cell). The binding means may be derived from any anti-BCAM antibody described herein. Such antibodies include the agonist and antagonist antibodies described above as well as antigen-binding fragments derived therefrom, including suitable formatted single domain and single-chain antibodies.

[206] Exemplary binding means, derived from MAbs 477, 929, and 452, respectively, include the following anti-BCAM scFvs:

(A) QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLEWMGIINPFGGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGIYGHFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSVLSASNNQNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPYTFGQGTKLEIK (SEQ ID NO: 78);

(B) QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGGIIPFSGTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTDPILSFWSGYYYYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTRLEIK (SEQ ID NO: 79); and

(C) QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGWMNPNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTYSSSWIFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSIPITFGQGTRLEIK (SEQ ID NO: 82).

[207] The transmembrane domain may be from (e.g., comprises at least the transmembrane region(s) of) the α, β or ζ chain of the T-cell receptor (TCR), NKG2D, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD8α, CD9, CD16, CD22, CD33, CD37, CD40,

CD64, CD80, CD86, CD134, CD137, or CD154. In some instances, the transmembrane domain may be synthetic and comprise predominantly hydrophobic residues such as leucine and valine. For example, a triplet of phenylalanine, tryptophan and/or valine can be found at each end of a synthetic transmembrane domain. In other instances, the transmembrane domain may be derived from CD8 or CD28.

[208] Upon binding of the tumor-associated surface antigen, the signaling domain comprised in the cytoplasmic domain of a CAR activates one or more effector functions of an immune effector cell (e.g., T cell) in which the CAR is expressed. The effector function of a T-cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

[209] The signaling domain may be from (e.g., comprises at least the signaling domain(s) of) DAP10, DAP12, Fc epsilon receptor I γ chain (FCER1G), FcR β, NKG2D, CD3δ, CD3ε, CD3γ, CD3ζ, CD5, CD22, CD226, CD66d, CD79A, or CD79B. For example, the cytoplasmic domain may comprise a CD3ζ signaling domain.

[210] The cytoplasmic domain may further comprise one or more co-stimulatory signaling domains. The one or more co-stimulatory signaling domains may be from (e.g., comprise(s) at least the signaling domain(s) of) CD28, 41BB, IL2Rb, CD40, OX40 (CD134), CD80, CD86, CD27, ICOS, NKG2D, DAP10, DAP12, 2B4 (CD244), BTLA, CD30, GITR, CD226, CD79A, or HVEM. For example, the co-stimulatory signaling domain may be from CD28.

[211] Cells, including immune effector cells, capable of expressing a CAR as described herein are also provided. The term "immune effector cell" as used herein refers to a cell that has differentiated into a form capable of modulating or effecting a specific immune response. Such cells may include mature lymphocytes suitable for therapy, including, but not limited to, cytotoxic T cells, helper T cells, natural killer cells (NK cells), and tumor-infiltrating lymphocytes (TILs), and may also include dendritic cells or macrophages. For example, the immune effector cell may be a T cell. Alternatively, the immune effector cell may be a NK cell.

Drug conjugate

[212] In some aspects, the antibody is conjugated to a drug. Typically, drug conjugates are provided that comprise a binding means (e.g., a recombinant protein) capable

of specifically binding a selected tumor-associated surface antigen (e.g., human BCAM) connected via a linker to means for killing a tumor cell (e.g., a cytotoxic drug).

[213] An Antibody Drug Conjugate (ADC) is provided that may comprise an antibody (e.g., a full-length antibody and/or an antigen-binding fragment such as a scFv) connected via linker to a drug (also referred to as the "payload"). Typically, the drug is a cytotoxic drug, e.g., to treat cancer. The present anti-BCAM antibodies can be utilized in ADCs. For example, an ADC may comprise a bispecific antibody described herein. The bispecific antibody may be a biparatopic anti-BCAM antibody described herein.

[214] In the development of ADC-based anti-tumor therapies, an anti-cancer drug (e.g., a cytotoxin) is coupled to an antibody that specifically targets and binds to surface-expressed tumor marker such as BCAM on cancer cells. This complex is internalized via receptor-mediated endocytosis, and the cytotoxic drug is released and kills the cancer cells. The release may be due to proteolytic degradation of the antibody, e.g., in the lysosome, or due to cleavage of the linker.

[215] By combining the targeting capabilities of antibodies with the cancer-killing ability of cytotoxic drugs, ADCs allow discrimination between healthy and diseased tissue. In contrast to administering a cytotoxic drug on its own, an ADC targets cancer cells so that healthy cells are less severely affected, reducing side effects and providing a wider therapeutic window.

Linkers

[216] Both cleavable and non-cleavable linkers have been proven to be safe in preclinical and clinical trials. For example, brentuximab vedotin (ADCETRIS) includes an enzyme-sensitive cleavable linker that delivers the potent and highly toxic antimicrotubule agent monomethyl auristatin E (MMAE), a synthetic antineoplastic agent, to CD30-positive tumor cells. Because of its high toxicity, MMAE, which inhibits cell division by blocking the polymerization of tubulin, cannot be used on its own as chemotherapeutic drug. ADCs in which MMAE is linked to an anti-CD30 monoclonal antibody have proven to be stable in extracellular fluids and thus safe for therapy. Trastuzumab deruxtecan (ENHERTU) comprises the topoisomerase I inhibitor deruxtecan linked to trastuzumab via a cathepsin-cleavable GGFG (SEQ ID NO: 85) tetrapeptidyl-aminomethoxy linker. Trastuzumab emtansine (KADCYLA), another approved ADC, is a combination of the microtubule-

formation inhibitor mertansine (DM-1), a derivative of maytansine, and the antibody trastuzumab conjugated via a non-cleavable linker.

[217] Linkers may include disulfides, hydrazones, thioethers, or peptides. Peptide-based linkers can be rendered cleavable by the incorporation of a site that is cleaved by an enzyme present in the endosome or lysosome (e.g., cathepsin). A well-established cleavable linker is valine-citrulline-p-aminobenzyl carbamate. Linking the cytotoxic agent to the antibody prevents cytotoxic activity outside of target cells, thereby reducing off-target effects. A cytotoxic payload delivered via a cleavable linker can escape from the targeted cell and, in a process called "bystander killing" destroy neighboring cancer cells.

[218] Accordingly, the drug may be conjugated to the antibody via a cleavable linker. The cleavable linker may be an enzyme cleavable linker. The enzyme cleavable linker may be a peptide-based linker. The enzyme cleavable linker may comprise a protease cleavage site. The protease may be a lysosomal enzyme (e.g., cathepsin). For example, the cleavable peptide-based linker may comprise the amino acid sequence GGFG (SEQ ID NO: 85). In particular, a cleavable GGFG (SEQ ID NO: 85) tetrapeptidyl-aminomethoxy linker may be used in the ADCs described herein.

[219] Alternatively, the drug may be conjugated to the antibody via a non-cleavable linker.

Drug

[220] Typically, the drug in an ADC is an anti-cancer drug that reduces or attenuates viability of the cancer cell in which it is internalized.

[221] The anti-cancer drug may be a cytotoxic agent. The cytotoxic agent may be a tubulin inhibitor or a DNA damaging agent. The tubulin inhibitor may be an auristatin (e.g., MMAE), a maytansinoid (e.g., maytansine or DM-1), or a tubulysin. The DNA damaging agent may be an agent that induces DNA double-strand breaks, a DNA alkylating agent, a topoisomerase inhibitor, or a DNA crosslinking agent. The agent that induces DNA double-strand breaks may be a calicheamicin. The DNA alkylating agent may be a duocarmycin. The topoisomerase inhibitor may be an exatecan (e.g., deruxtecan). The DNA crosslinking agent may be a pyrrolobenzodiazepine.

[222] The anti-cancer drug may be an immunomodulatory agent. The immunomodulatory agent may be a TLR agonist or a STING agonist.

[223] The anti-cancer drug may be a toxin. The toxin may be selected from ricin, abrin, alpha toxin, saporin, a ribonuclease (Rnase), Dnase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, *Pseudomonas* exotoxin, and *Pseudomonas* endotoxin.

[224] The anti-cancer drug may be a radionuclide. The radionuclide may be an α-particle-emitting radionuclide. Exemplary α-particle-emitting radionuclides comprise ^{211}At, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{223}Ra, ^{225}Ac, and ^{227}Th. Radionuclides can be linked to an antibody via a chelate-comprising linker (e.g., derivatives of DTPA or DOTA).

[225] The ADC may comprise one drug. Alternatively, the ADC may comprise more than one drug, e.g., two different drugs. The two or more different drugs may have distinct modes of action. Alternatively, the two or more different drugs may have the same mode of action. ADCs comprising two or more drugs may be particularly efficacious in cancer therapy (see, e.g., Yamazaki et al. 2021, *Nat. Comms.* **12**(3528).

Drug antibody ratio (DAR)

[226] In an ADC, the drug-antibody ratio (DAR) typically refers to the average number of drug molecules conjugated to the antibody. The optimal DAR for a given ADC may vary, e.g., dependent on the type of antibody, drug, and/or conjugation site that is/are used.

[227] Typically, the DAR is between about 1 to about 20. The DAR may be between about 1 to about 16. The DAR may be between 1 to 10 (e.g., about 4-8). The DAR may be about 1, about 2, about 4, about 6, about 8, or about 10. As demonstrated herein, a DAR of 4 is suitable to achieve the desired therapeutic effect.

Nucleic acids, expression vectors, and host cells

[228] Nucleic acids and expression vectors encoding the present anti-BCAM antibodies, BiTEs, or CARs are provided, as are host cells comprising a nucleic acid or expression vector encoding the anti-BCAM antibodies, BiTEs, or CARs described herein. Exemplary nucleic acids encoding HC and LC regions of anti-BCAM antibodies of Table 1 are provided in Table 4.

Table 4. Anti-BCAM antibody nucleic acid sequences of HC and LC regions

Antibody	Heavy chain constant SEQ ID NO	Light chain constant SEQ ID NO
228	86	87

232	88	89
452	90	91
477	92	93
606	94	95
929	96	97
937	98	99

[229] In some instances, a single nucleic acid encoding a present anti-BCAM antibody, BiTE, or CAR is provided. An expression vector comprising the nucleic acid may also be provided.

[230] In other instances, a first nucleic acid and a second nucleic acid are provided, wherein:

a) the first nucleic acid comprises a nucleotide sequence encoding an immunoglobulin heavy chain variable (V_H) region of a present anti-BCAM antibody, and
b) the second nucleic acid comprises a nucleotide sequence encoding an immunoglobulin light chain variable (V_L) region of the same antibody.

[231] A first expression vector and a second expression vector respectively comprising the first and second nucleic acids are also provided. Alternatively, the first and second nucleic acids may be comprised in a single expression vector.

[232] A host cell comprising the nucleic acid(s) or expression vector(s) is also provided.

[233] Methods of producing the provided anti-BCAM antibody, BiTE, or CAR are also provided. The methods comprise providing a host cell comprising one or more nucleic acids (e.g., expression vectors) encoding the present anti-BCAM antibody, BiTE, or CAR. and incubating the host cells under conditions suitable for expression of the antibody, BiTE, or CAR encoded by the one or more nucleic acids. The one or more nucleic acids (or expression vectors) may comprise a first nucleic acid (or expression vector) encoding an amino acid sequence of the light chain of an antibody described herein and a second nucleic acid (or expression vector) encoding an amino acid sequence of the heavy chain of the antibody.

[234] For expression in a suitable host cell, the present anti-BCAM antibodies, BiTEs, or CARs may be provided with a suitable signal peptide. Such a signal peptide may

comprise, or consist of, the amino acid sequence METDTLLLWVLLLWVPGSTG (SEQ ID NO: 84) or MGWSCIILFLVATATGVHS (SEQ ID NO: 100).

Pharmaceutical composition

[235] Provided herein is a pharmaceutical composition comprising an antibody that specifically binds human basal cell adhesion molecule (BCAM), comprising a variable region and a constant region, wherein the variable region comprises a framework region and a complementarity determining means for binding human BCAM admixed with a pharmaceutically acceptable carrier.

[236] For example, the anti-BCAM antibodies, anti-BCAM drug conjugates (e.g., anti-BCAM ADCs), anti-BCAM BiTEs, and (immune effector) cells capable of expressing an anti-BCAM CAR described herein may be formulated into a pharmaceutical composition.

[237] The pharmaceutical composition may comprise one or more of a pharmaceutically acceptable carrier, diluent, or excipient.

[238] The pharmaceutically acceptable carrier may be conventional (e.g., as described in Remington, The Science and Practice of Pharmacy, 22nd Edition, Loyd V., ed., Pharmaceutical Press, 2012). In general, the nature of the carrier depends on the mode of administration. For instance, parenteral formulations typically comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids, such as water for injection, physiological saline, balanced salt solutions or the like as a vehicle. Pharmaceutical compositions can additionally include minor amounts of non-toxic auxiliary substances for stability (e.g., one or more buffering agent).

[239] The carrier may be sterile and/or suspended or otherwise contained in a unit dosage form including one or more measured doses of the composition suitable for administration to a subject of an effective amount of an antibody described herein. Medications for use in therapy may also be included in such embodiments. The unit dosage form may be in a sealed vial that contains sterile contents or a syringe for injection into a subject, lyophilized for subsequent solubilization and administration. The pharmaceutical composition may be provided in a solid or controlled release dosage form.

[240] A pharmaceutical composition typically contains an "effective" or "therapeutically effective" amount, as used interchangeably herein, of an antibody described herein. The dosages and dosage regimen to achieve the desired therapeutic result may depend

on the means of administration. They may vary according to factors including, but not limited to, the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of an antibody described herein are outweighed by the therapeutically beneficial effects.

Therapy

[241] Provided herein is a method of treating cancer in a human subject, the method comprising administering to the human subject an effective amount of an antibody that specifically binds human basal cell adhesion molecule (BCAM), comprising a variable region and a constant region, wherein the variable region comprises a framework region and a complementarity determining means for binding human BCAM admixed with a pharmaceutically acceptable carrier.

[242] For example, the anti-BCAM antibodies, anti-BCAM ADCs, and anti-BCAM BiTEs described herein, and pharmaceutical compositions comprising the same can find utility in therapy, in particular, in methods of treating cancer (e.g., ovarian or endometrial cancer) in a subject in thereof. Similarly, the anti-BCAM CARs and immune effector cells expressing such CARs, are useful in methods of treating cancer in a subject in need thereof. Specifically contemplated is the use of the anti-BCAM antibodies, anti-BCAM ADCs, anti-BCAM BiTEs, anti-BCAM CARs, and (immune effector) cells capable of expressing an anti-BCAM CAR described herein in the manufacture of a medicament for treating cancer.

[243] A method of treating cancer in a subject in need thereof is provided, where the method comprises administering to the subject a therapeutically effective amount of a means for specifically binding human BCAM and modulating the binding of human BCAM to Laminin alpha-5. In addition, a method of treating cancer in a subject in need thereof is provided, where the method comprises administering to the subject a therapeutically effective amount of a means for specifically binding human BCAM and inhibiting the binding of human BCAM to Laminin alpha-5. Furthermore, a method of treating cancer in a subject in need thereof is provided, where the method comprises administering to the subject a therapeutically effective amount of a means for specifically binding human BCAM and increasing the binding of human BCAM to Laminin alpha-5.

[244] Moreover, a method of treating cancer in a subject in need thereof is provided, where the method comprises administering to the subject a therapeutically effective amount

of a means for specifically binding human BCAM and selectively inducing cell death in BCAM-expressing cancer cells. Cell death may be induced, e.g., through one or more effector functions of the binding means, through killing means (e.g., one or more cytotoxic drugs) linked to the binding means, or through eliciting immune effector cells (e.g., T cells or NK cells) to kill BCAM expressing cancer cells,

[245] Also provides is a method of treating cancer in a subject in need thereof, where the method comprises administering to the subject a therapeutically effective amount of bi-specific T-cell engager (BiTEs) comprising a first binding means capable of specifically binding human BCAM and a second binding means for a surface-expressed subunit of the T cell receptor complex (e.g., CD3), wherein the first and second binding means are connected via a peptide linker. In addition, a method of treating cancer in a subject in need thereof is provided, where the method comprises administering to the subject a therapeutically effective amount of an drug conjugate (e.g., an ADC) comprising a binding means capable of specifically binding human BCAM and a means for killing a tumor cell (e.g., a cytotoxic drug), wherein the binding means and the killing means are connected via a linker (e.g., a cleavable linker).

[246] A therapeutically effective amount can be administered in a dosing regimen that may comprise multiple unit doses. A therapeutically effective amount (and/or an appropriate unit dose within an effective dosing regimen) may vary, for example, depending on route of administration, on combination with other pharmaceutical agents. Also, the specific therapeutically effective amount (and/or unit dose) for any particular subject may depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific pharmaceutical agent employed; the specific composition employed; the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and/or rate of excretion or metabolism of the specific therapeutic molecule employed; the duration of the treatment; and like factors as is well known in the medical arts.

[247] Clinical efficacy of the therapeutic treatments described herein may be determined by measuring the clinical benefit rate (CBR). The clinical benefit rate is measured by determining the sum of the percentage of patients who are in complete remission (CR), the number of patients who are in partial remission (PR) and the number of patients having stable disease (SD) at a time point at least 6 months out from the end of therapy. The CBR for a particular therapeutic regimen may be at least 25%, 30%, 35%, 40%, 45%, 50%, 55%,

60%, 65%, 70%, 75%, 80%, 85%, or more. An effective amount may also be assessed by an improvement in important cancer endpoints, Overall Survival (OS), Disease-Free Survival (DFS), Objective Response Rate (ORR), Complete Response Rate (CRR), or Progression Free Survival (PFS). See *Dept. of Health and Human Services, Food and Drug Admin, Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics: Guidance for Industry* (2018); E.A. Eisenhauer *et al.*, *New Response Evaluation Criteria in Solid Tumors: Revised RECIST Guideline (Version 1.1)*, 45 Eur. J. Cancer 228 (2009). The degree of improvement in these endpoints that is clinically meaningful will vary on the cancer to be treated and other considerations by the tending physician. The OS or PFS may be an anticipated relative improvement of at least 20% or at least 25% and an absolute increase of at least 2.5 months (e.g., 6 months) in PFS and/or OS compared with that achieved with standard-of-care treatments. See Kumar *et al.*, *An Appraisal of Clinically Meaningful Outcomes Guidelines for Oncology Clinical Trials*, JAMA Oncol. 2016 September 01; 2(9): 1238–1240; Ellis *et al.*, *American Society of Clinical Oncology perspective: Raising the bar for clinical trials by defining clinically meaningful outcomes*, J Clin Oncol. 2014 April 20; 32(12):1277-80.

[248] The cancer may be selected from the group consisting of ovarian cancer, endometrial cancer, colorectal cancer, breast cancer, prostate cancer, lung cancer, liver cancer, thyroid cancer, head and neck cancer, stomach cancer, pancreatic cancer, urothelial cancer, cervical cancer, skin cancer, and bladder cancer. In some instances, the cancer is a sarcoma, a carcinoma, or a basal cell carcinoma. In particular, the cancer may ovarian cancer or endometrial cancer. The ovarian cancer may be high grade ovarian cancer. The ovarian cancer may be serous ovarian cancer.

[249] The methods of treatments described herein may comprise administering a therapeutically effective amount of the anti-BCAM antibody, anti-BCAM bi-specific T-cell engager (BiTE), or anti-BCAM antibody-drug conjugate (ADC) (e.g., formulated as a pharmaceutical composition as described herein) to a subject in need of such treatment. The anti-BCAM antibody, BiTE, or ADC may comprise an IgG Fc region. The IgG Fc region may be human. The IgG Fc region may be humanized. The IgG Fc region may be IgG1, e.g., human IgG1.

[250] The subject may be treated with one or more additional therapies. The one or more additional therapies may comprise radiotherapy. The one or more additional therapies may comprise chemotherapy. The one or more additional therapies may comprise surgery.

Alternatively, the subject may be treatment-naïve. In some instances, the subject may be first treated with the one or more additional therapies. Alternatively, the subject may be first treated with the anti-BCAM antibody, anti-BCAM BiTE, or anti-BCAM ADC (e.g., to shrink a tumor prior to surgery).

[251] The anti-BCAM antibody, anti-BCAM BiTE, or anti-BCAM ADC may be administered to the subject sequentially with one or more additional therapeutic agents (e.g., an immune checkpoint inhibitor). The additional therapeutic agent may be administered to the subject before, during, or after administration of the anti-BCAM antibody, anti-BCAM BiTE, or anti-BCAM ADC.

EXAMPLES

[252] The following examples are for illustrative purposes only and are not meant to limit the scope of the appended claims.

Example 1. Identification and characterization of anti-BCAM antibodies

[253] This example demonstrates the identification and characterization of anti-BCAM antibodies.

[254] A fully human scFv phage display library (SuperHuman 2.0 antibody library, Distributed Bio/Charles River Labs) was screened to identify antibodies that bind human BCAM, similar to the process described in Schrofelbauer et al. (2022) *PNAS* 120(1)1-11, and in published international patent application WO2023/114543. 37 fully human antibodies were identified with specificity for human BCAM. The identified antibodies were characterized by determining their affinity to human BCAM expressed on cells, their cross-reactivity to cynomolgus monkey and mouse BCAM, their binding to BCAM knock-out cells and their cross-reactivity to homologous proteins, namely receptor for advanced glycation endproducts (RAGE), melanoma cell adhesion molecule (MCAM) and activated leukocyte cell adhesion molecule (ALCAM). The results of these studies are summarized in Table 6. In Table 6, "Nd" means "not determined".

Table 6. Characterization of anti-BCAM human antibodies.

Antibody #	**Affinity (nM)**		**Cross-reactivity**			**Binding to BCAM KO cells >2.5%**	**Cross-reactivity to homologs (RAGE, MCAM, ALCAM)**
	on cells	**ELISA**	**human**	**cyno**	**mouse**		
16	0.31	Nd	Yes	Yes	Weak	Yes	No

Antibody #	Affinity (nM)		Cross-reactivity			Binding to BCAM KO cells >2.5%	Cross-reactivity to homologs (RAGE, MCAM, ALCAM)
	on cells	ELISA	human	cyno	mouse		
48	0.53	Nd	Yes	Yes	No	Yes	No
51	0.24	Nd	Yes	Yes	Yes	Yes	No
154	1	Nd	Yes	weak	No	No	No
174	50.49	Nd	Yes	Yes	weak	No	No
216	0.3	Nd	Yes	Yes	weak	Yes	Yes
220	11.63	Nd	Yes	Yes	weak	No	No
228	1.15	Nd	Yes	Yes	No	No	No
230	16.27	Nd	Yes	Yes	No	No	No
231	0.3	Nd	Yes	Yes	Yes	Yes	Yes
232	2.02	0.57	Yes	Yes	No	No	No
233	2.54	Nd	Yes	No	No	No	No
234	6.87	Nd	Yes	No	No	No	No
311	3.42	Nd	Yes	No	No	No	No
330	1.35	Nd	Yes	Yes	No	No	No
424	8.19	Nd	Yes	Yes	Yes	No	No
477	0.76	0.41	Yes	Yes	No	No	No
600	22.75	Nd	Yes	No	No	No	No
602	0.3228	Nd	Yes	Yes	Yes	No	No
604	0.3484	Nd	Yes	Yes	Yes	No	No
606	0.4679	0.38	Yes	Yes	Yes	No	No
608	0.5713	Nd	Yes	Yes	No	Yes	Yes
609	1.158	Nd	Yes	No	No	No	No
613	1.048	Nd	Yes	No	No	No	No
837	0.581	Nd	Yes	No	No	No	No
886	3.228	Nd	Yes	Yes	Yes	No	No
902	6.088	Nd	Yes	Yes	weak	No	Yes
927	0.2799	Nd	Yes	No	No	No	Yes
929	1.207	0.75	Yes	Yes	No	No	No
935	4.303	Nd	Yes	Yes	No	No	No
936	0.3422	Nd	Yes	Yes	weak	Yes	No
937	1.83	Nd	Yes	Yes	No	No	No
942	2.881	Nd	Yes	Yes	No	No	No
236	0.4397	0.37	Yes	Yes	Yes	Yes	No
406	Nd	Nd	Yes	Nd	Nd	No	No
941	0.1803	Nd	Yes	Yes	Yes	No	No
452	0.3093	5.3	Yes	Yes	No	No	No

[255] The binding affinities of the antibodies to human BCAM expressed on cells were measured using flow cytometry. Expi293 cells transfected with human BCAM were washed with phosphate-buffered saline (PBS) and harvested using CellStripper (Thermo Fisher). Antibody staining was performed in PBS/5% bovine serum albumin (BSA) with amounts of primary antibody as indicated in the manufacturer's manual. Additional staining

with corresponding fluorescently labelled secondary antibody was performed. All wash steps were performed in PBS/5%BSA.

[256] For some antibodies, binding affinities to recombinant human BCAM were also determined, using ELISA. 20 µg/ml recombinant BCAM (Sino) was coated onto 96-well high-attachment plates and incubated at 4°C overnight. The plate was washed two times with PBS (Thermo Fisher) and blocked with PBS/10% BSA for 1 hour at room temperature. Primary antibodies in PBS/10% BSA were added in serial dilution from 10 µg/ml to 0.01 µg/ml and incubated for 1 hour at room temperature. The plate was washed three times with PBS/0.1%Tween before adding the secondary anti-human-HRP (Cell Technologies) in a 1:1000 ratio diluted in PBS/10%BSA and incubated for 30 minutes at room temperature. The samples were washed three times with PBS/0.1%Tween, and TMB substrate (Pierce) was added to the wells for 15 minutes at room temperature. 2M Sulfuric acid was added directly to the TMB substrate to stop the reaction. The absorbance was recorded at 450 nm on SpectraMax M5E (Molecular Devices).

[257] To assess cross-reactivity, Expi293 cells were transfected with human BCAM, cynomolgus monkey BCAM, mouse BCAM, RAGE, MCAM and ALCAM cDNA constructs, respectively. Overexpression of the respective proteins was verified with commercially available anti-BCAM, anti-RAGE, anti-MCAM and anti-ALCAM antibodies, respectively. Binding of test antibodies to each of the proteins was determined by flow cytometry. An antibody was categorized as being cross-reactive to cynomolgus monkey and mouse BCAM and the BCAM homologs RAGE, MCAM, and ALCAM when greater than >5% binding was observed.

[258] To assess non-specific cell binding, flow cytometry was performed after incubation of the test antibodies with a Kuramochi Cas9 sgControl cell line (positive control) and a Kuramochi Cas9-sghBCAM knock-out cell line (negative control). An antibody was characterized as binding a BCAM knock-out cell line when >2.5% binding was observed.

[259] Antibodies with cross-reactivity to one or more of RAGE, MCAM, and ALCAM were not pursued further.

Example 2. Epitope mapping of anti-BCAM antibodies

[260] This example demonstrates the further biochemical and biophysical properties of a subset of 15 of the 37 anti-BCAM antibodies identified in example 1.

[261] Human BCAM/MCAM chimeras were used to map the binding epitope of anti-BCAM antibodies. A schematic overview of the chimeras used in these experiments is shown in FIG. 1. Cells were transfected with cDNA of the respective chimera, BCAM, or MCAM. Antibody binding was measured by flow cytometry after staining with a test antibody and anti-human-APC secondary antibody. The results of these experiments are summarized in Table 7.

Table 7. Characterization of BCAM and MCAM binding.

Antibody	**Binds to**
51	BCAM, B2, B3, B4, M4(weak)
16	BCAM, M1, B3, B4, M4(weak)
48	BCAM, B2, B3, B4, M4(weak)
154	BCAM, B2, B3, B4
600	BCAM, B2, B3, B4, M4(weak)
609	BCAM, B2, B3, B4, M4(weak)
236	BCAM, B2, B3, B4
929	BCAM, M1, B2, B3, B4, M4(weak)
604	BCAM, B2, B3, B4
606	BCAM, B2, B3, B4
941	BCAM, B2, B3, B4
330	BCAM, M1, B2, B3, B4, M4(weak)
174	BCAM, B2, B3, B4, M4(weak)
424	BCAM, B2, B3, B4, M4(weak)
220	M1
452	BCAM, M1, B3, B4
477	BCAM, M1, B2, B3, B4
228	BCAM, B2, B3, B4
232	BCAM, M1, B2, B3, B4
937	BCAM, M1, B2, B3, B4

[262] Illustrative histograms from the flow cytometry assay using the antibodies 929, 477, and 452, respectively, are shown in FIG. 2A, FIG. 2B and FIG. 2C. These histograms show that antibodies 929 and 477 required BCAM V1-V2 domains for binding. In contrast, 452 required BCAM V2 and C1 domains for binding. These domains overlap with the Laminin alpha-5 binding domain. Antibodies with cross-reactivity to mouse BCAM were excluded from further investigation.

Example 3. Biophysical characterization of selected anti-BCAM antibodies

[263] Further biophysical characterization was performed on antibodies 228, 232, 236, 330, 452, 477, 606, 929, and 937.

Thermostability

[264] Thermostability of antibodies 228, 232, 236, 330, 452, 477, 606, 929, and 937 was examined using full IgG antibodies. All of antibodies 228, 232, 236, 330, 452, 477, 606, 929, and 937 displayed a Fab stability of >68°C, indicating that the antibodies are thermally stable.

Aggregation

[265] Mass photometry was used to determine whether antibodies 228, 232, 236, 330, 452, 477, 606, 929, and 937 aggregate after purification. The results of this analysis are summarized in Table 8.

Table 8. 150 kDa antibody fraction after purification.

Antibody	Fraction of ~150 kDa
228	97%
232	83%
236	96%
330	88%
452	96%
477	92%
606	82%
929	96%
937	89%

[266] All antibodies showed molecular weight distributions around 150 kDa, the native antibody weight. For most of the tested antibodies, only minimal amounts (e.g., 10% or less) of high molecular weight aggregates were detected.

[267] Among the tested antibodies, 228, 236, 452, 477 and 929 had the smallest amounts (10% or less) of high molecular weight aggregates.

Example 4. Functional characterization of selected anti-BCAM antibodies

[268] Antibodies 228, 232, 236, 330, 452, 477, 606, 929, and 937 were further characterized functionally.

Polyreactivity

[269] Using ELISA, antibodies 228, 232, 236, 452, 477, 606, 929, and 937 were also tested for their reactivity with keyhole limpet hemocyanin (KLH), lipopolysaccharide (LPS), and human insulin by ELISA. Lenzilumab was used as a positive control, as it reacts with insulin. None of the tested antibodies was reactive with KLH, LPS or human insulin.

Antibody-dependent cellular cytotoxicity

[270] Antibodies 228, 232, 236, 330, 452, 477, 606, 929, and 937 were tested for their capability to induce antibody-dependent cellular cytotoxicity (ADCC). Various concentrations of each of the nine antibodies were added to the human high-grade serous ovarian cancer cell line OVSAHO or the human ovarian serous cystadenocarcinoma cell line OAW28, both co-cultured with a Jurkat-NFAT-Luc reporter cell line. Both OVSAHO and OAW28 express high levels of human BCAM. The luciferase signal was used as a readout for ADCC activity. The fold change in ADCC activity detected in the presence of various concentrations of the test antibodies are shown in FIG. 3A and FIG. 3B, respectively. Antibodies 232, 477 and 452 were capable of inducing measurable ADCC in both OVSAHO and OAW28 cells at nanomolar concentrations, e.g., as low as 0.3 nM. Antibody 937 and 606 also showed some activity, but only at the higher end of the tested nanomolar concentrations, e.g., about 40 nM.

[271] Antibodies 452, 477, 606, 929 and 937 were further tested with a recombinant BCAM-expressing SKOV3 cell line (SKOV3-BCAM), co-cultured with the same Jurkat-NFAT-Luc reporter cell line at various antibody concentrations. SKOV3 is a human ovarian cancer cell line with epithelial-like morphology that does not normally express high levels of BCAM. As a positive control, corresponding experiments were conducted with various concentrations of the anti-Her2 antibody trastuzumab. The luciferase signal (expressed as relative light units) was used as a readout for natural killer (NK) cell activation. The results for each of the test antibodies and trastuzumab are shown in FIG. 3C. Antibodies 452 and 477 induced ADCC at a level similar to that of trastuzumab at concentrations of 1 nM or less. Antibodies 606, 929 and 937 also induced ADCC, at an antibody concentration exceeding 1 nM.

Effect on BCAM:laminin interaction

[272] An ELISA was used to test the effect of antibodies 228, 232, 236, 330, 452, 477, 606, 929, and 937 on the interaction between human BCAM and its native ligand Laminin alpha-5. The ELISA plate was coated with recombinant Laminin alpha-5. Binding of BCAM was tested in the presence or absence of each test antibody. Various antibody concentrations were tested. The results of these experiments are summarized in FIG. 4.

[273] Antibodies 228 and 606 did not affect binding of Laminin alpha-5 to BCAM. Antibodies 452 and 236 increased BCAM binding to Laminin alpha-5 (i.e., acted as agonists), while the presence of antibodies 330, 937, 929, 477, and 232 reduced binding (i.e., acted as antagonists). These results indicate that antibodies 228 and 606, antibodies 452 and 236, and antibodies 232, 330, 477, 929, and 937 bind to distinct epitope regions on human BCAM. These results also indicated that binding of an anti-BCAM antibody to each of these epitope regions affects human BCAM's capability to bind Laminin alpha-5 differently.

Competition assay

[274] Antibodies 452, 477, and 929 were tested for binding competition with each other and each of antibodies 228, 232, 236, 606, and 937.

[275] Antibodies were labelled with Alexa Fluor 488 according to the manufacturer's instructions (Thermo Fisher). For staining 3×10^5 cells were aliquoted into separate wells. For blocking, unlabeled antibodies were added to the samples at saturating amounts (20 mg/ml) for 30 minutes on ice. Control cells were incubated without antibody or with an unrelated control antibody. After washing, Alexa Fluor 488-labeled antibodies were added for 30 minutes to blocked and control samples. Upon washing cells were resuspended in PBS/5%BSA for flow analysis. Fluorescence-activated cell sorting (FACS) analysis was performed on a BD Fortessa as described in Schröfelbauer et al. (PNAS, Jan 2023). The results of these experiments are summarized in FIG. 5A, FIG. 5B and FIG. 5C.

[276] As can be seen from FIG. 5A, no competition was detected between antibody 452 and antibodies 477 and 929, indicating that their epitopes do not overlap. This observation is consistent with the finding that antibody 452 binds a distinct epitope located in domains V2 and C1 of human BCAM. Antibody 452 was also found to bind to an epitope distinct from the epitopes bound by antibodies 228, 232, 236, 606, and 937. Notably,

antibody 452 did not compete with antibody 236 for binding to human BCAM even though both antibodies increased Laminin alpha-5 binding to BCAM.

[277] As illustrated in FIG. 5B, antibodies 232 and 477 competed with antibody 929 for binding of human BCAM, consistent with the finding that all three antibodies are capable of reducing Laminin alpha-5 binding to BCAM.

[278] Similarly, antibody 477 was found to compete with antibody 232 for binding of BCAM (see FIG. 5C). Interestingly, in this experiment, no competition between antibody 477 and 929 was observed, indicating that both antibodies may bind adjacent, but non-overlapping epitopes in domains V1-V2 of human BCAM.

Example 5. Binding characteristics of anti-BCAM antibodies

[279] The binding characteristics of antibodies 452, 477 and 929 were further analyzed.

Characterization of the effect of point mutations

[280] To further map the amino acids involved in binding of these antibodies to human BCAM, each antibody was incubated with 293T cells transiently transfected with Myc-tagged BCAM point mutants in which structure-predicted surface-exposed charged residues had been replaced with alanine. The expression of all constructs in 293T cells was comparable. Antibody binding was assessed by flow cytometry. The results of these experiments are summarized in FIG. 6A, FIG. 6B and FIG. 6C.

[281] Most point mutations had no or only a minimal effect on the test antibodies' capability to bind human BCAM. As can be seen from FIG. 6A, the E180A substitution interfered with the binding of antibody 477 to human BCAM, indicating that this residue is part of the epitope recognized by this antibody. The reduced APC signals and wide binding profiles seen in FIG. 6B suggest a partial requirement of antibody 452 for charged residues around the laminin binding domain for binding of BCAM. The narrower binding profiles and higher APC signals seen in FIG. 6C indicate that the charged residues around the laminin binding domain were dispensable for the binding of antibody 929 to human BCAM. These observations provide a further indication that each of antibodies 452, 477 and 929 bind distinct epitopes on the surface of human BCAM.

Epitope characterization by sequence alignment with mouse BCAM

[282] The data presented in example 1 and Table 6 show that the 477 and 929 antibodies bind to human and cynomolgus BCAM, but not mouse BCAM. This demonstrates that the difference(s) between residues of human and cynomolgus BCAM and residues of mouse BCAM were sufficient to prevent the binding of these antibodies and, therefore, one or more of these residues are likely involved in the epitope of each antibody.

[283] To further characterize these residues, the sequences of human BCAM (i.e., residues 32 to 547 of UniProtKB ID: P50895, which corresponds to residues 1 to 516 of SEQ ID NO: 1), cynomolgus BCAM (SEQ ID NO: 28, which corresponds to residues 32 to 547 of UniProtKB ID: A0A2K5TW87_MACFA), and mouse BCAM (SEQ ID NO: 29, which corresponds to residues 26 to 541 of UniProtKB ID: Q9R069) were aligned using the MUSCLE alignment tool, as shown in FIG. 7A.

[284] The data presented in example 2 and FIG. 2B and FIG. 2A, respectively, show that antibodies 477 and 929 require human BCAM domains V1-V2 for binding. The residues present within the sequence making up this region of human BCAM that were found to be different in mouse BCAM only are as follows: L9, K16, S17, I19, T25, G26, T27, H28, D29, T38, R40, S41, P45, A50, M52, L57, Q58, V59, M61, D63, T64, R65, S68, Q72, L73, Q76, L81, E83, A84, E89, R97, A104, A108, R109, L110, N111, K115, A118, E132, D133, S134, N142, R144, A149, K151, V165, M167, P169, E170, M173, S175, E180, L185, R196, S203, S211, E214, R216, Q237, P223, and H226.

Characterization of the effect of additional point mutations

[285] Human BCAM variants were prepared in which individual structure-predicted surface-exposed charged residues identified by the MUSCLE sequence alignment were replaced amino acids with opposing characteristics to the native residue. 293T cells were transiently transfected with Myc-tagged version of these BCAM point mutants. The expression of all constructs in 293T cells was comparable in general, however a proportion of untransfected cells were observable in the cells transfected with wild-type BCAM. Antibody binding was assessed by flow cytometry. The results of these experiments are summarized in FIG. 7B and FIG. 7C.

[286] FIG. 7B shows that introduction of R40E into human BCAM resulted in loss of antibody 929 binding. Conversely, binding of antibody 929 to human BCAM was not

affected by introduction of mutations S68R or K16E. These data indicate that residue R40 of human BCAM may form part of the epitope bound by antibody 929.

[287] FIG. 7C shows that binding of antibody 477 to human BCAM was abrogated by introduction of P223H. Conversely, binding of antibody 477 to human BCAM was not affected by introduction of mutations E244K or H226R. These data indicate that residue P223 of human BCAM may form part of the epitope bound by antibody 477 (possibly in addition to E180, which was identified by alanine substitution).

Binding to BCAM polymorphisms

[288] The capability of binding to BCAM polymorphisms was examined for antibodies 228, 232, 236, 452, 477, 606, 929, and 937. Each antibody was incubated with 293T cells transiently transfected with a construct expressing a BCAM polymorphism. Specifically, the polymorphisms tested were Lu21, Lu20, Lu17, Lu16, Lu13, Lu12, Lu8, Lu6, Lu5, Lu4, and Lu2. Antibody binding was assessed by flow cytometry. No binding was detected for the Lu12 polymorphism, which is likely an artefact resulting from the low expression level and/or transfection inefficiency of this construct in 293T cells.

[289] Antibodies 452 and 477 had similar binding profiles in these experiments, as can be seen from FIG. 8. Both antibodies bound to the majority of tested BCAM polymorphisms, as summarized in Table 9.

Table 9. Binding anti-BCAM antibodies to BCAM polymorphisms.

Antibody	**Binding to # of polymorphisms**
228	10
232	10
452	10+
477	10
606	11
937	8
929	9
236	8

Binding kinetics

[290] Biolayer interferometry (BLI) was used to determine the binding kinetics of each of antibodies 452, 477, and 929 to human BCAM. The BLI curves for each antibody are shown in FIG. 9A, 9B, and 9C, respectively. All antibodies had a very slow off rate,

which is characteristic for antibody binding. The K_D values for each of antibodies 452, 477 and 929 are shown in Table 10.

Table 10. Binding kinetics of antibodies 452, 477, and 929.

Antibody	Kinetic K_D
452	0.8 nM
477	0.5 nM
929	0.06 nM

Example 6. Bispecific T-cell engager (BiTE) designs and *in vitro* testing

[291] Two bispecific T-cell engager (BiTE) designs were produced to identify potential additional therapeutic modalities of antibodies 228, 232, 236, 452, 477, 606 and 937. The designs are shown schematically in FIG. 10A and FIG. 10B.

anti-BCAM-anti-CD3-T-cell engager design

[292] Single-chain Fvs (scFvs) of antibodies 228, 232, 236, 452, 477, 606 and 937 were constructed. These scFvs were individually fused to an anti-CD3 scFv via a linker sequence as shown in FIG. 10A. The resulting bispecific T-cell engagers (BiTEs), shown in Table 11, were tested for their capability to activate T cells using a Jurkat-NFAT-Luc reporter cell line. The anti-EpCAM-anti-CD3-T-cell engager solitumab was used as a positive control. An irrelevant antibody (A19) served as a negative control. The results of this experiment are shown in FIG. 11A. The BCAM-targeting BiTEs efficiently activated T cells as shown by an increase in relative light units (RLUs). Notably, the BiTE based on an scFv of antibody 477 activated T cells at levels comparable to solitumab. Similar results were also obtained for the 452-based BiTE construct.

Table 11. BiTE constructs.

BiTe construct	SEQ ID NO	Sequence
228 BiTE	101	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYWM HWVRQAPGKGLEWVAVISPDGIDKYYADSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCARDR RGYSYGNFDYWGQGTLVTVSSGGGGSGGGGSG GGGSDIQMTQSPSSLSASVGDRVTITCRASQDIDK YLSWYQQKPGKAPKLLIYAASSLQSGVPSRFSGS GSGTDFTLTISSLQPEDFATYYCQQYNNYPLTFGP GTKVDIKGGGGSDVQLVQSGAEVKKPGASVKVS CKASGYTFTRYTMHWVRQAPGQGLEWIGYINPS RGYTNYADSVKGRFTITTDKSTSTAYMELSSLRS EDTATYYCARYYDDHYCLDYWGQGTTVTVSSG EGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGER

		ATLSCRASQSVSYMNWYQQKPGKAPKRWIYDTS KVASGVPARFSGSGSGTDYSLTINSLEAEDAATY YCQQWSSNPLTFGGGTKVEIKHHHHHH
232 BiTE	102	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYY MHWVRQAPGQGLEWMGWINPNSGDTNYAQEF QGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCA RKRDGGGPFDYWGQGTLVTVSSGGGGSGGGGS GGGGSDIVMTQSPDSLAVSLGERATINCKSSQSV LSRSNNKNYLAWYQQKPGQPPKLLIYWASTRES GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQ YYSTPFTFGPGTKVDIKGGGGSDVQLVQSGAEVK KPGASVKVSCKASGYTFTRYTMHWVRQAPGQG LEWIGYINPSRGYTNYADSVKGRFTITTDKSTSTA YMELSSLRSEDTATYYCARYYDDHYCLDYWGQ GTTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSP ATLSLSPGERATLSCRASQSVSYMNWYQQKPGK APKRWIYDTSKVASGVPARFSGSGSGTDYSLTINS LEAEDAATYYCQQWSSNPLTFGGGTKVEIKHHH HHH
236 BiTE	103	EVQLLESGGGLVQPGGSLRLSCAASGFAFSNDGM HWVRQAPGKGLEWISYISGSSSTIYYADSVKGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCARDGIA AAGTGGWYFDLWGRGTLVTVSSGGGGSGGGGS GGGGSDIQMTQSPSSLSASVGDRVTITCRASQSIS TYLSWYQQKPGKAPKLLIYAASSLQSGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQSYNTPITFGQ GTRLEIKGGGGSDVQLVQSGAEVKKPGASVKVS CKASGYTFTRYTMHWVRQAPGQGLEWIGYINPS RGYTNYADSVKGRFTITTDKSTSTAYMELSSLRS EDTATYYCARYYDDHYCLDYWGQGTTVTVSSG EGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGER ATLSCRASQSVSYMNWYQQKPGKAPKRWIYDTS KVASGVPARFSGSGSGTDYSLTINSLEAEDAATY YCQQWSSNPLTFGGGTKVEIKHHHHHH
452 BiTE	104	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYY MHWVRQAPGQGLEWMGWMNPNSGDSGYAQKF QGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCA STYSSSWIFDYWGQGTLVTVSSGGGGSGGGGSG GGGSDIVMTQSPDSLAVSLGERATINCKSSQSVL YSSNNKNYLAWYQQKPGQPPKLLIYWASTRESG VPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQY YSIPITFGQGTRLEIKGGGGSDVQLVQSGAEVKKP GASVKVSCKASGYTFTRYTMHWVRQAPGQGLE WIGYINPSRGYTNYADSVKGRFTITTDKSTSTAY MELSSLRSEDTATYYCARYYDDHYCLDYWGQG TTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPA TLSLSPGERATLSCRASQSVSYMNWYQQKPGKA PKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSL EAEDAATYYCQQWSSNPLTFGGGTKVEIKHHHH HH

477 BiTE	105	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYY VHWVRQAPGQGLEWMGIINPFGGSATYAPKFQG RVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGI YGHFDYWGQGTLVTVSSGGGGSGGGGSGGGGS DIVMTQSPDSLAVSLGERATINCKSSQSVLSASNN QNYLAWYQQKPGQPPKLLIYWASTRESGVPDRF SGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPY TFGQGTKLEIKGGGGSDVQLVQSGAEVKKPGAS VKVSCKASGYTFTRYTMHWVRQAPGQGLEWIG YINPSRGYTNYADSVKGRFTITTDKSTSTAYMELS SLRSEDTATYYCARYYDDHYCLDYWGQGTTVT VSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLS PGERATLSCRASQSVSYMNWYQQKPGKAPKRWI YDTSKVASGVPARFSGSGSGTDYSLTINSLEAED AATYYCQQWSSNPLTFGGGTKVEIKHHHHHH
606 BiTE	106	EVQLLESGGGLVQPGGSLRLSCAASGFTFGDYD MNWVRQAPGKGLEWVSGISRSGGGTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCART RGYGQLLDVWGQGTTVTVSSGGGGSGGGGSGG GGSDIQMTQSPSSLSASVGDRVTITCRASQSISMY LNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSG SGTDFTLTISSLQPEDFATYYCQQTKSIPYTFGQG TKLEIKGGGGSDVQLVQSGAEVKKPGASVKVSC KASGYTFTRYTMHWVRQAPGQGLEWIGYINPSR GYTNYADSVKGRFTITTDKSTSTAYMELSSLRSE DTATYYCARYYDDHYCLDYWGQGTTVTVSSGE GTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERA TLSCRASQSVSYMNWYQQKPGKAPKRWIYDTSK VASGVPARFSGSGSGTDYSLTINSLEAEDAATYY CQQWSSNPLTFGGGTKVEIKHHHHHH
937 BiTE	107	EVQLLESGGGLVQPGGSLRLSCAASGFAFDDYA MHWVRQAPGKGLEWLAVISYDGSHMSYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR GGQLVWHPPDYWGQGTLVTVSSGGGGSGGGGS GGGGSDIQMTQSPSSLSASVGDRVTITCRASQGIS SYLAWYQQKPGKAPKLLIYAASYLQSGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQSYRTPFTFG QGTKVDIKGGGGSDVQLVQSGAEVKKPGASVKV SCKASGYTFTRYTMHWVRQAPGQGLEWIGYINP SRGYTNYADSVKGRFTITTDKSTSTAYMELSSLR SEDTATYYCARYYDDHYCLDYWGQGTTVTVSS GEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGE RATLSCRASQSVSYMNWYQQKPGKAPKRWIYDT SKVASGVPARFSGSGSGTDYSLTINSLEAEDAAT YYCQQWSSNPLTFGGGTKVEIKHHHHHH
929 BiTE	108	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAF SWVRQAPGQGLEWMGGIIPFSGTTNYAQKFQGR VTITADESTSTAYMELSSLRSEDTAVYYCTTDPIL SFWSGYYYYYGMDVWGQGTTVTVSSGGGGSGG GGSGGGGSDIQMTQSPSSLSASVGDRVTITCRAS

		QSISSWLAWYQQKPGKAPKLLIYDASNLETGVPS RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPP TFGQGTRLEIKGGGGSDVQLVQSGAEVKKPGAS VKVSCKASGYTFTRYTMHWVRQAPGQGLEWIG YINPSRGYTNYADSVKGRFTITTDKSTSTAYMELS SLRSEDTATYYCARYYDDHYCLDYWGQGTTVT VSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLS PGERATLSCRASQSVSYMNWYQQKPGKAPKRWI YDTSKVASGVPARFSGSGSGTDYSLTINSLEAED AATYYCQQWSSNPLTFGGGTKVEIKHHHHHH

Example 7. Selection criteria

[293] Antibodies 228, 232, 236, 452, 477, 606, 929, and 937 were assessed for their suitability as therapeutic antibodies. The selection criteria are summarized in Tables 12, 13 and 14. The assessment was based on the data collected for each of the antibodies as described in the preceding examples.

Table 12. Selection criteria of binding and affinity.

	Antibody property: Binding and affinity		
Antibody	< 2.5% binding to BCAM KO cells	K_D < 6 nM measured by ELISA	K_D < 1 nM measured by BLI
228	Yes	Not determined	Not determined
232	Yes	Yes	Not determined
236	No	Yes	Not determined
452	Yes	Yes	Yes
477	Yes	Yes	Yes
606	Yes	Yes	Not determined
929	Yes	Yes	Yes
937	Yes	Not determined	Not determined

[294] Antibodies were considered suitable for use in therapy if they exhibited less than 2.5% binding to BCAM knockout cells and a K_D of less than 6 nM when measured by ELISA and/ or a K_D of less than 1 nM when measured by BLI. Antibodies 228, 232, 452, 477, 606, 929, and 937 fulfilled these criteria, as shown in Table 12.

Table 13. Selection criteria of cross-reactivity and polyreactivity.

	Antibody property: Cross-reactivity and polyreactivity		
Antibody	Binding to ≥ 9 BCAM polymorphisms	Homolog cross-reactivity	Polyreactivity
228	Yes	No	No
232	Yes	No	No
236	No	No	No
452	Yes	No	No
477	Yes	No	No

606	Yes	No	No
929	Yes	No	No
937	No	No	No

[295] Antibodies were considered suitable for use in therapy if they were capable of binding at least nine known BCAM polymorphisms. As summarized in Table 13, antibodies 228, 232, 452, 477, 606, and 929 fulfilled this requirement. In addition, antibodies were considered suitable for therapeutic use only if they exhibited no homolog cross-reactivity with RAGE, MCAM or ALCAM and no polyreactivity with KLH, LPS or human insulin. All of antibodies 228, 232, 236, 452, 477, 606, 929, and 937 met these additional selection criteria.

[296] Next, the antibodies were assessed for physical properties that could render them unsuitable for long-term storage. In addition, the antibodies were also evaluated for potential sequence liabilities that could interfere with affinity, stability, potency, and/or homogeneity of these antibodies, in particular "stickiness" due to having two or more consecutive positively charged residues, e.g., RR or RKR, and N-linked glycosylation. The selection criteria are summarized in Table 14.

Table 14. Selection criteria of aggregation, thermostability and sequence liabilities.

	Antibody property		
Antibody	< 10% aggregates	Thermostable at ≥ 68°C	Sequence liabilities
228	Yes	Yes	Yes
232	No	Yes	Yes
236	Yes	Yes	No
452	Yes	Yes	No
477	Yes	Yes	No
606	No	Yes	No
929	Yes	Yes	No
937	No	Yes	No

[297] Antibodies 228, 236, 452, 477, and 606 showed minimal aggregation. All Fab antibody fragments were thermostable at greater than or equal to 68°C. Antibodies 228 and 232 were not considered further due to having two or more consecutive positively charged residues. Such antibodies could be rendered suitable for therapeutic applications by substitution of one or more of the two or more of consecutive positively charged residues with one or more amino acids that are not positively charged.

[298] Antibodies 452, 477, and 929 met all requirements set out in Tables 12, 13 and 14.

Example 8. *In vitro* and *in vivo* testing of an anti-BCAM based BiTE construct

[299] Further experiments were performed with the 477-based BiTE construct. This BiTE construct was incubated separately with human peripheral blood mononuclear cells (PBMCs) obtained from four different donors (PBMC 409, PBMC 627, PBMC 877 and PBMC 905). Various concentrations of the 477-based BiTE construct were tested in this assay. Both T cell activation and cell lysis were determined. The results of these experiments are shown in FIG. 11B and FIG. 11C, respectively. The 477-based BiTE construct was effective in activating T cells even at picomolar concentrations (see FIG. 11B). It also induced potent target cell cytotoxicity at sub-nanomolar concentrations (see FIG. 11C).

Half-life extended T-cell engager design

[300] To extend its half-life in circulation, the 477-based BiTE was fused to a LALA-PG effector function inactivated IgG1-Fc as shown in FIG. 10B. In addition, mutations that decrease Fc-protein A binding affinity were incorporated into the design to allow more straight forward purification (as described by Tustian et al., *Mabs.* 2016; **8**(4):828-38). The resulting modified BiTE-Fc construct, shown in Table 15, bound BCAM with an affinity of ~3.5 nM, which was comparable to the conventional hIgG1 format described in the previous section.

Table 15. Modified BiTE-Fc construct.

Modified BiTE-Fc construct	**SEQ ID NO**	**Sequence**
477 based anti-BCAM-BiTE-Fc with human IgG1 Fc, comprising LALA-PG H435R Y436F	109	QVQLVQSGAEVKKPGASVKVSCKASGYTFTD YYVHWVRQAPGQGLEWMGIINPFGGSATYAP KFQGRVTMTRDTSTSTVYMELSSLRSEDTAVY YCARGIYGHFDYWGQGTLVTVSSGGGGSGGG GSGGGGSDIVMTQSPDSLAVSLGERATINCKSS QSVLSASNNQNYLAWYQQKPGQPPKLLIYWA STRESGVPDRFSGSGSGTDFTLTISSLQAEDVA VYYCQQYYSSPYTFGQGTKLEIKGGGGSDVQL VQSGAEVKKPGASVKVSCKASGYTFTRYTMH WVRQAPGQGLEWIGYINPSRGYTNYADSVKG RFTITTDKSTSTAYMELSSLRSEDTATYYCARY YDDHYCLDYWGQGTTVTVSSGEGTSTGSGGS GGSGGADDIVLTQSPATLSLSPGERATLSCRAS QSVSYMNWYQQKPGKAPKRWIYDTSKVASG VPARFSGSGSGTDYSLTINSLEAEDAATYYCQ

		QWSSNPLTFGGGTKVEIKGGGGDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALGAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNRFTQKSLSLSPG
Anti-PSMA-Fc-BiTEe, with human IgG1 Fc, comprising LALA-PG H435R Y436F	110	QVQLVESGGGLVKPGESLRLSCAASGFTFSDY YMYWVRQAPGKCLEWVAIISDGGYYTYYSDII KGRFTISRDNAKNSLYLQMNSLKAEDTAVYY CARGFPLLRHGAMDYWGQGTLVTVSSGGGGS GGGGSGGGGSDIQMTQSPSSLSASVGDRVTIT CKASQNVDTNVAWYQQKPGQAPKSLIYSASY VYWDVPSRFSGSASGTDFTLTISSVQSEDFATY YCQQYDQQLITFGCGTKLEIKGGGGSDVQLVQ SGAEVKKPGASVKVSCKASGYTFTRYTMHWV RQAPGQGLEWIGYINPSRGYTNYADSVKGRFT ITTDKSTSTAYMELSSLRSEDTATYYCARYYD DHYCLDYWGQGTTVTVSSGEGTSTGSGGSGG SGGADDIVLTQSPATLSLSPGERATLSCRASQS VSYMNWYQQKPGKAPKRWIYDTSKVASGVP ARFSGSGSGTDYSLTINSLEAEDAATYYCQQW SSNPLTFGGGTKVEIKGGGGDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LGAPIEKTISKAKGQPREPQVYTLPPSRDELTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNRFTQKSLSLSPG

[301] The BiTE-Fc construct was tested for its capability to induce T cell activation and targeted cell lysis in a BCAM expression-dependent manner. A corresponding BiTE-Fc construct in which the anti-BCAM scFv was replaced with an anti-PSMA scFv served as a negative control, also shown in Table 15.

[302] In an initial experiment, the anti-BCAM BiTE-Fc construct and the control were incubated at various concentrations with the $BCAM^{low}$-expressing SKOV3 cell line co-cultured with a T cell line. The percentage of activated T cells observed in this experiment did not exceed 60% (see FIG. 12A). Despite the low levels of BCAM expression, target cell lysis was induced already at picomolar concentrations of the anti-BCAM BiTE-Fc construct (see FIG. 12B). The experiment was then repeated with a SKOV3 cell line that was modified to express recombinant BCAM at its cell surface. With this $BCAM^{high}$-expressing cell line,

the percentage of activated T cells exceeded 80% (see FIG. 12C). Moreover, effective target cell lysis was already observed at picomolar concentrations of the anti-BCAM BiTE-Fc construct (see FIG. 12D). No T cell activation or cell lysis was observed with the control construct, i.e., the anti-PSMA BiTE-Fc construct. These results confirmed that T-cell activation and targeted cell lysis were BCAM expression-dependent. These results also confirmed that T-cell activation and targeted cell lysis were specific to an anti-BCAM BiTE-Fc construct.

[303] The experiment was then repeated with the OVSAHO cell line. BCAM expression in this cell line falls in-between the levels observed in wild-type SKOV3 cells and SKOV3 modified to express human BCAM. As expected, the observed T cell activation and cell lysis levels also fell in-between the levels observed with the two SKOV3 cell lines. The percentage of activated T cells peaked around 60% (see FIG. 13A), and targeted cell lysis was detected a picomolar concentrations of the BiTE-Fc construct (see FIG. 13B).

[304] *In vivo* efficacy of the BCAM-targeting BiTE-Fc construct was assessed in a mouse tumor model. SKOV3 cells modified to recombinantly express human BCAM were engrafted into immunodeficient NOD *scid* gamma (NSG) mice. Once tumors were established (i.e., when the tumor size was $\geq$ 200 mm^3 tumor size), human PBMCs were engrafted (day 0). Treatment with the BCAM-targeting BiTE-Fc construct at a dose of 500 μg/kg started at day 1 post engraftment and was repeated twice at the same dose, i.e., once on day 3 and once on day 5 post engraftment. Tumor volume was monitored twice weekly. Mice were sacrificed once the tumor size reached >2000 mm^3. Mice treated with a PMSA-targeting BiTE-Fc served as negative controls. Mice treated with PBMCs only, and mice treated with vehicle (PBS) only, served as additional controls. Each group included 10 mice.

[305] The results of this experiment are summarized in FIG. 14. A statistically significant difference was observed between mice treated with the BCAM-BiTE-Fc and mice treated with the PMSA-BiTE-Fc (negative control). As can be seen from FIG. 14, the BCAM-targeting BiTE-Fc construct was highly effective in preventing further tumor growth, whereas treatment with PMSA-BiTE-Fc had no effect on preventing tumor growth (the tumors of mice in the PMSA-BiTE-Fc treatment group became significantly larger over time).

Example 9. Antibody-drug conjugate (ADC) design and testing

[306] To determine their suitability for use in antibody-drug conjugates, the internalization rate of each of antibodies 452, 477, and 929 was assessed using an IncuCyte® internalization assay. Specifically, antibodies 452, 477, and 929 were labeled with a-human-pHrodo pH sensitive dye and incubated with OVCAR3 cells. Internalization was measured using IncuCyte® instrument by quantifying red fluorescent intensity over time. As shown in FIG. 15A, each of antibodies 452, 477, and 929 were internalized into OVCAR3 cells.

[307] ADCs were then prepared by crosslinking antibodies 477, 452 and 929 with deruxtecan (DXd) via a GGFG (SEQ ID NO: 85) linker through random cysteine conjugation, similar to the approved anti-Her2-ADC trastuzumab (ENHERTU), to provide antibody drug conjugates (ADCs) for testing. Effective internalization was also demonstrated for antibodies 452, 477, and 929 when they were in the ADC format. The GGFG (SEQ ID NO: 85) linker is cleaved in the lysosome following receptor mediated internalization of the ADC. DXd is highly cell-permeable topoisomerase inhibitor. Once released from the antibody is exhibits efficient bystander killing. FIG. 15B illustrates that anti-BCAM-DXd-conjugates were effectively internalized ("N" stands for "nucleus", and the arrows indicate internalized labelled antibody). ADCs internalized into low pH environments within the cell, i.e., lysosomes and endosomes, show as white spots. Antibody internalization properties of conjugated and naked antibodies were similar.

[308] Drug-to-antibody ratios (DARs) of 4 and 8 were tested. As illustrated in FIG. 16, DXd conjugation at DAR4 and DAR8 did not interfere with the antibodies' capability to bind BCAM. The non-conjugated (naked) anti-BCAM antibody and anti-RSV ADCs served as positive and negative controls, respectively. Conjugated antibodies maintained similar binding affinities to the naked antibodies.

[309] ADCs based on antibodies 477, 452 and 929 with DAR8 were incubated with OV8 cells at various concentrations to determine the reduction in cell viability following ADC internalization. An anti-RSV ADC served as a negative control. As shown in FIG. 17, the efficacy of all three ADCs was comparable. ADCs comprising antibodies 477 and 452, respectively, were slightly more effective at sub-nanomolar concentrations. The experiment was repeated with each ADC at DAR4 and DAR8, with matching anti-RSV ADCs as negative controls. The results are summarized in FIG. 18A (antibody 929), FIG. 18B (antibody 452), and FIG. 18C (antibody 477). No difference in cytotoxicity was observed between DAR4 and DAR8.

[310] Next, an anti-BCAM-DXd ADC was tested *in vitro* in a number of human BCAM-expressing ovarian (OVSAHO and Kuramochi) and endometrial (MFE-280) cancer cell lines to assess cytotoxicity. This ADC included antibody 477. There was a noticeable difference in the capability of the ADC to induce cytotoxicity in these cell lines. While sub-nanomolar concentrations of the ADC were effective in reducing the viability of OVSAHO (see FIG. 19A), only nanomolar concentrations were effective in the Kuramochi (see FIG. 19B). This is reflected in the IC50 values. The IC50 for OVSAHO was 0.06 nM, whereas the IC50 for Kuramochi was 2.26 nM. The IC50 for the human ovarian cancer cell line OVCAR3 at 0.11 nM fell in the middle range between OVSAHO and Kuramochi (see FIG. 19C). The lowest IC50 of 0.02 nM was observed with an OVCAR8 cell line that was modified to recombinantly express human BCAM (see FIG. 19D). This was the same value as the IC50 determined for the human endometrial cancer cell line MFE-280 (see FIG. 19E). The IC50 values for each of the tested cancer cell lines are summarized in Table 16 below.

Table 16. IC50 of anti-BCAM-DXd ADC in various cancer cell lines.

Cell line	IC50 (nM)
OVSAHO	0.06
OVCAR8-BCAM	0.02
OVCAR3	0.11
MFE-280	0.02
Kuramochi	2.26

[311] *In vivo* efficacy of BCAM-targeting ADCs was assessed in a mouse tumor model. Immunodeficient NOD *scid* gamma (NSG) mice were either engrafted with the human ovarian cancer cell line OVCAR3. After tumors were established at a size of about 200 mm^3 (day 0), mice were dosed twice (at day 0 and day 7) with a 4 mg/kg dose of the ADC. Mice treated with vehicle (PBS) only (referred to as "vehicle control" in FIG. 20) or with an anti-RSV ADC (referred to as "RSV-ADC" in FIG. 20) served as negative controls. Each treatment group included 10 mice. Tumor volume was monitored twice weekly. Mice were sacrificed where the tumor size exceeded 1000 mm^3.

[312] FIG. 20 shows the outcome of the above study up to day 56 using the anti-BCAM antibody 477 conjugated to DXd at a drug:antibody ratio of 4 (referred to as "BCAM-ADC" in the figure).

[313] By day 28, nine of the ten mice treated with the BCAM-targeting ADC ("BCAM-ADC") showed a complete response, i.e., absence of a measurable tumor, and the

other mouse showed a partial response, i.e., a >75% decrease in tumor volume, with a tumor volume of 4 mm^3. By day 56, the mouse that showed a partial response at day 28 also showed a complete response. In contrast, none of the mice treated with the control ADC or vehicle only showed either a partial or complete response, at any point in the study.

[314] Only the BCAM-targeting ADC resulted in a sustained reduction of tumor volume over the course of the study. Treatment with the anti-RSV ADC resulted in some initial reduction of tumor volume (presumably due to non-specific action of DXd). However, this reduction was not sustained and mean tumor volume rapidly increased thereafter.

[315] This example demonstrates that an ADC comprising an anti-BCAM antibody described herein is effective at reducing human ovarian cancer tumor volume. This example further demonstrates that this reduction in tumor volume is sustained.

Example 10. Biparatopic antibody design

[316] This example illustrates the preparation of biparatopic anti-BCAM antibodies each comprising a first antibody in an IgG1 format and a second antibody in an scFv format.

[317] In each instance, the scFv was fused to the HC of the IgG1 antibody via a linker, providing an "anti-BCAM HC-anti-BCAM scFv" construct. The anti-BCAM HC-anti-BCAM scFv construct was expressed in a mammalian cell together with an anti-BCAM LC to form a biparatopic antibody. Three biparatopic anti-BCAM antibodies were designed and their amino acid sequences are listed in Table 17.

[318] The first biparatopic anti-BCAM antibody comprised (i) a 929-based HC fused to a 477-based scFv construct ("929HC-477scFv"; SEQ ID NO: 112) and (ii) a 929-based LC (SEQ ID NO: 65). The 929-based HC comprised the 929-based VH sequence of SEQ ID NO: 16 and the human IgG1 Fc of SEQ ID NO: 76 except for the C-terminal K residue. The 477-based scFv comprised the 477-based VH sequence of SEQ ID NO: 14 and the 477-based VL sequence of SEQ ID NO: 15. The self-assembled biparatopic anti-BCAM antibody "929-477scFv" was obtained from co-expressing SEQ ID NO: 112 and SEQ ID NO: 65 in Expi293 cells.

[319] The second biparatopic anti-BCAM antibody comprised (i) a 452-based HC fused to a 477-based scFv construct ("452HC-477scFv"; SEQ ID NO: 113) and (ii) a 452-based LC (SEQ ID NO: 51). The 452-based HC comprised the 452-based VH sequence of SEQ ID NO: 24 and the human IgG1 Fc of SEQ ID NO: 76 except for the C-terminal K

residue. The 477-based scFv comprised the 477-based VH sequence of SEQ ID NO: 14 and the 477-based VL sequence of SEQ ID NO: 15. The 452-based LC comprised. The self-assembled biparatopic anti-BCAM antibody "452-477scFv" was obtained from co-expressing SEQ ID NO: 113 and SEQ ID NO: 51 in Expi293 cells.

[320] The third biparatopic anti-BCAM antibody comprised (i) a 452-based HC and a 929-based scFv construct ("452HC-929scFv"; SEQ ID NO: 114) and (ii) a 452-based LC (SEQ ID NO: 51). The 452-based HC comprised the 452-based VH sequence of SEQ ID NO: 24 and the human IgG1 Fc of SEQ ID NO: 76 except for the C-terminal K residue. The 929-based scFv comprised the 929-based VH sequence of SEQ ID NO: 16 and the 929-based VL sequence of SEQ ID NO: 17. The self-assembled biparatopic anti-BCAM antibody "452-929scFv" was obtained from co-expressing SEQ ID NO: 114 and SEQ ID NO: 51 in Expi293 cells.

[321] The signal peptide(s) used to express the constructs encoding the components of the self-assembled biparatopic anti-BCAM antibodies described above in a mammalian cell are cleaved, such that the secreted antibody in the supernatant does not comprise the signal peptide(s).

Table 17. Amino acid sequences of exemplary anti-BCAM HC-anti-BCAM scFV constructs

Biparatopic construct	**SEQ ID NO**	**Sequence***
929HC-477scFv	112	mgwsciilflvatatgvhsQVQLVQSGAEVKKPGSSVKVS CKASGGTFSSYAFSWVRQAPGQGLEWMGGIIPFS GTTNYAQKFQGRVTITADESTSTAYMELSSLRSE DTAVYYCTTDPILSFWSGYYYYYGMDVWGQGT TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGG SGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSC KASGYTFTDYYVHWVRQAPGQGLEWMGIINPFG GSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSE DTAVYYCARGIYGHFDYWGQGTLVTVSSASGGG GSGGGGSGGGGSDIVMTQSPDSLAVSLGERATIN CKSSQSVLSASNNQNYLAWYQQKPGQPPKLLIY

		WASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVA VYYCQQYYSSPYTFGQGTKLEIKR
452HC-477scFv	113	mgwsciilflvatatgvhsQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYYMHWVRQAPGQGLEWMGWMN PNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSS LRSEDTAVYYCASTYSSSWIFDYWGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGG SGGGGSQVQLVQSGAEVKKPGASVKVSCKASGY TFTDYYVHWVRQAPGQGLEWMGIINPFGGSATY APKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVY YCARGIYGHFDYWGQGTLVTVSSASGGGGSGGG GSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQS VLSASNNQNYLAWYQQKPGQPPKLLIYWASTRE SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QYYSSPYTFGQGTKLEIKR
452HC-929scFv	114	mgwsciilflvatatgvhsQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYYMHWVRQAPGQGLEWMGWMN PNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSS LRSEDTAVYYCASTYSSSWIFDYWGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGG SGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGG TFSSYAFSWVRQAPGQGLEWMGGIIPFSGTTNYA QKFQGRVTITADESTSTAYMELSSLRSEDTAVYY CTTDPILSFWSGYYYYYGMDVWGQGTTVTVSSA SGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCRASQSISSWLAWYQQKPGKAPKLLIYDAS NLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYSTPPTFGQGTRLEIKR

*Amino acids presented in lower case in the table correspond to the signal peptide presented in SEQ ID NO: 100.

Example 11. Biparatopic anti-BCAM ADC design and testing

[322] This example demonstrates that biparatopic anti-BCAM ADCs exhibit increased efficacy compared to monoparatopic anti-BCAM ADCs.

[323] Each of the biparatopic anti-BCAM antibodies 929-477scFv, 452-477scFv and 452-929scFv designed in Example 10 were purified from Expi293 supernatants using protein A beads using standard protocols. To produce biparatopic anti-BCAM ADCs, the purified biparatopic antibodies were then incubated with "oYo-Vc antimicrotubule agent monomethyl auristatin E (MMAE)" (ALPHATHERA) link reagents according to manufacturer's instructions and photo-crosslinked using an ALPHATHERA crosslinking device, see e.g., Hui et al. 2015 Bioconjugate Chemistry **26**(8). Crosslinking was validated by SDS-PAGE. A representative schematic of the biparatopic anti-BCAM ADCs is shown in FIG. 21. Hereinafter, the biparatopic anti-BCAM ADC are referred to as "929-477scFv ADC", "452-477scFv ADC" and "452-929scFv ADC", respectively.

[324] The cytotoxicity of each biparatopic anti-BCAM ADC was tested *in vitro*. Specifically, OVCAR3 cells were incubated with 929-477scFv ADC, 452-477scFv ADC, or 452-929scFv ADC or corresponding control constructs as well as a negative control at various concentrations for 72-96 hours followed by measuring cell viability using the Cell titer glow assay according to manufacturer's instructions. The controls were: (i) "BCAM-IgG-oYo (1)", i.e., a full-length 452-based or 929-based antibody cross-linked to MMAE corresponding to the IgG1 in the biparatopic antibody; (ii) "BCAM-IgG-oYo (2)", i.e., a full length 477-based or 929-based antibody cross-linked to MMAE corresponding to the scFv in the biparatopic antibody; and (iii) "IgG-oYo", i.e., an IgG control antibody cross-linked to MMAE (negative control). The results of these experiments are shown in FIG. 22A, 22B, and 22C, respectively. The results were used to calculate the IC50 value for each antibody as shown in Table 18.

Table 18. IC50 values of biparatopic anti-BCAM ADCs and their monoparatopic controls

Biparatopic construct	**BCAM-IgG-oYo (1) IC_{50} (nM)**	**BCAM-IgG-oYo (2) IC_{50} (nM)**	**Biparatopic oYo IC_{50} (nM)**
929-477scFv ADC	0.7	0.4	0.1
452-477scFv ADC	3	0.4	0.08
452-929scFv ADC	3	0.7	0.1

[325] As shown in Table 18, the biparatopic format improved the cytotoxicity of each anti-BCAM ADC. Surprisingly, each tested biparatopic anti-BCAM ADC had an IC50 of about 0.1 nM or less, which was a significant improvement over the monoparatopic control antibodies.

[326] This example demonstrates that biparatopic anti-BCAM ADCs are more effective at reducing the viability of ovarian cancer cells than monoparatopic anti-BCAM ADCs. The results in this example indicate the utility of such biparatopic antibodies in cancer therapy.

[327] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described herein.

[328] While a number of embodiments are described herein, the present disclosure and examples may be altered to provide other methods and compositions. Therefore, it will be appreciated that the scope is to be defined by the appended claims in addition to the specific embodiments that have been represented by way of example.

CLAIMS

1. An antibody that specifically binds human basal cell adhesion molecule (BCAM), wherein the antibody comprises a variable region and a constant region, wherein the variable region comprises a framework region and a complementarity determining means for binding human BCAM.

2. An antibody that specifically binds human basal cell adhesion molecule (BCAM), comprising:
    a. a heavy chain complementarity determining region (HCDR) 1 comprising an amino acid sequence of DYYVH (SEQ ID NO: 2), an HCDR2 comprising an amino acid sequence of IINPFGGSATYAPKFQG (SEQ ID NO: 3), an HCDR3 comprising an amino acid sequence of GIYGHFDY (SEQ ID NO: 4); and
    b. a light chain complementarity determining region (LCDR) 1 comprising an amino acid sequence of KSSQSVLSASNNQNYLA (SEQ ID NO: 5), an LCDR2 comprising an amino acid sequence of WASTRES (SEQ ID NO: 6), and an LCDR3 comprising an amino acid sequence of QQYYSSPYT (SEQ ID NO: 7).

3. The antibody of claim 2, comprising:
    a. an immunoglobulin heavy chain variable (V_H) region comprising an amino acid sequence that is at least about 85% identical to, or identical to, QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLE WMGIINPFGGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYY CARGIYGHFDYWGQGTLVTVSS (SEQ ID NO: 14), and
    b. an immunoglobulin light chain variable (V_L) region comprising an amino acid sequence that is at least about 85% identical to, or identical to, DIVMTQSPDSLAVSLGERATINCKSSQSVLSASNNQNYLAWYQQKPGQP PKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYS SPYTFGQGTKLEIK (SEQ ID NO: 15).

4. The antibody of claim 2 or 3, wherein the antibody is a single-chain variable fragment (scFv) comprising a sequence having at least about 85% identity to, or identical to: QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQGLEWMGI INPFGGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGIYG

HFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATI
NCKSSQSVLSASNNQNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTISSLQAEDVAVYYCQQYYSSPYTFGQGTKLEIK (SEQ ID NO: 78).

5. An antibody that specifically binds human basal cell adhesion molecule (BCAM), comprising:
   a. an HCDR1 comprising an amino acid sequence of SYAFS (SEQ ID NO: 8), an HCDR2 comprising an amino acid sequence of GIIPFSGTTNYAQKFQG (SEQ ID NO: 9), and HCDR3 comprising an amino acid sequence of DPILSFWSGYYYYYGMDV (SEQ ID NO: 10); and
   b. an LCDR1 comprising an amino acid sequence of RASQSISSWLA (SEQ ID NO: 11), an LCDR2 comprising an amino acid sequence of DASNLET (SEQ ID NO: 12), an LCDR3 comprising an amino acid sequence of QQSYSTPPT (SEQ ID NO: 13).

6. The antibody of claim 5, comprising:
   a. an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least about 85% identical to, or identical to, QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEW
MGGIIPFSGTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCT
TDPILSFWSGYYYYYGMDVWGQGTTVTVSS (SEQ ID NO: 16), and
   b. an immunoglobulin light chain variable (VL) region comprising an amino acid sequence that is at least about 85% identical to, or identical to, DIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYD
ASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQG
TRLEIK (SEQ ID NO: 17).

7. The antibody of claim 5 or 6, wherein the antibody is a single-chain variable fragment (scFv) comprising a sequence having at least about 85% identity to, or identical to:
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGGI
IPFSGTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTDPILSFW
SGYYYYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSAS

VGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGS GTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTRLEIK (SEQ ID NO: 79).

8. An antibody that specifically binds human basal cell adhesion molecule (BCAM) and competes for binding to human BCAM with an antibody comprising:
   a. an immunoglobulin heavy chain variable (V_H) region comprising the amino acid sequence
      QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYVHWVRQAPGQG LEWMGIINPFGGSATYAPKFQGRVTMTRDTSTSTVYMELSSLRSEDT AVYYCARGIYGHFDYWGQGTLVTVSS (SEQ ID NO: 14), and
      an immunoglobulin light chain variable (V_L) region comprising the amino acid sequence
      DIVMTQSPDSLAVSLGERATINCKSSQSVLSASNNQNYLAWYQQKP GQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC QQYYSSPYTFGQGTKLEIK (SEQ ID NO: 15); or
   b. an immunoglobulin heavy chain variable (VH) region comprising the amino acid sequence
      QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLE WMGGIIPFSGTTNYAQKFQGRVTITADESTSTAYMELSSLRSEDTAV YYCTTDPILSFWSGYYYYYGMDVWGQGTTVTVSS (SEQ ID NO: 16), and
      an immunoglobulin light chain variable (V_L) region comprising the amino acid sequence
      DIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLI YDASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPP TFGQGTRLEIK (SEQ ID NO: 17).

9. The antibody of any one of claims 2-8, wherein the antibody is capable of inhibiting binding of human BCAM to Laminin alpha-5.

10. An antibody that specifically binds human basal cell adhesion molecule (BCAM), comprising:

a. an HCDR1 comprising an amino acid sequence of SYYMH (SEQ ID NO: 18), an HCDR2 comprising an amino acid sequence of WMNPNSGDSGYAQKFQG (SEQ ID NO: 19), an HCDR3 comprising an amino acid sequence of TYSSSWIFDY (SEQ ID NO: 20); and
b. an LCDR1 comprising an amino acid sequence of KSSQSVLYSSNNKNYLA (SEQ ID NO: 21), an LCDR2 comprising an amino acid sequence of WASTRES (SEQ ID NO: 22), an LCDR3 comprising an amino acid sequence of QQYYSIPIT (SEQ ID NO: 23).

11. The antibody of claim 10, wherein the antibody comprises:
a. an immunoglobulin heavy chain variable (V_H) region comprising an amino acid sequence that is at least about 85% identical to, or identical to, QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLE WMGWMNPNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAV YYCASTYSSSWIFDYWGQGTLVTVSS (SEQ ID NO: 24), and
b. an immunoglobulin light chain variable (V_L) region comprising an amino acid sequence that is at least about 85% identical to, or identical to, DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQP PKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYS IPITFGQGTRLEIK (SEQ ID NO: 25).

12. The antibody of claim 10 or 11, wherein the antibody is a single-chain variable fragment (scFv) comprising a sequence having at least about 85% identity to, or identical to:
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMG WMNPNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASTY SSSWIFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGER ATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSG SGSGTDFTLTISSLQAEDVAVYYCQQYYSIPITFGQGTRLEIK (SEQ ID NO: 82).

13. An antibody that specifically binds human basal cell adhesion molecule (BCAM) and competes for binding to human BCAM with an antibody comprising:

a. an immunoglobulin heavy chain variable (V_H) region comprising the amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLE WMGWMNPNSGDSGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAV YYCASTYSSSWIFDYWGQGTLVTVSS (SEQ ID NO: 24), and

b. an immunoglobulin light chain variable (V_L) region comprising the amino acid sequence DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQP PKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYS IPITFGQGTRLEIK (SEQ ID NO: 25).

14. The antibody of any one of claims 10-13, wherein the antibody is capable of increasing binding of human BCAM to Laminin alpha-5.

15. The antibody of any one of the preceding claims, wherein the antibody binds to human BCAM with a disassociation constant (K_D) of less than about 10 nM or less than about 1 nM as determined using a cell-based assay.

16. The antibody of claim 15, wherein the K_D is between about 0.1 nM and about 5 nM as determined using a cell-based assay.

17. The antibody of any one of the preceding claims, wherein the antibody is human or humanized.

18. The antibody of any one of the preceding claims, wherein the antibody is selected from:
    a. an IgG antibody, e.g., an IgG1 or IgG3 antibody;
    b. a bi- or multi- specific antibody;
    c. a single chain antibody; or
    d. a diabody.

19. The antibody of claim 18, wherein the antibody comprises or is a Fab fragment, a Fab' fragment, a F(ab')2 fragment, an Fd' fragment, an Fd fragment, and a single-chain variable fragment (scFv).

20. The antibody of any one of the preceding claims, wherein the antibody comprises a Fc region.

21. The antibody of claim 20, wherein the Fc region comprises LALA mutations.

22. The antibody of claim 21, wherein the Fc region further comprises a PG mutation.

23. The antibody of any one of claims 1-22, wherein the antibody further comprises an antibody means for binding CD3, e.g., an anti-CD3 scFv.

24. The antibody of any one of claims 1-23, wherein the antibody is conjugated to a drug.

25. The antibody of claim 24, wherein the drug is an anti-cancer drug.

26. The antibody of claim 25, wherein the anti-cancer drug is a cytotoxic agent or an immunomodulatory agent.

27. The antibody of claim 26, wherein the cytotoxic agent is a tubulin inhibitor or a DNA damaging agent.

28. The antibody of claim 27, wherein the tubulin inhibitor comprises an auristatin, a maytansinoid, or a tubulysin.

29. The antibody of claim 28, wherein the DNA damaging agent is an agent that induces DNA double-strand breaks, a DNA alkylating agent, a topoisomerase inhibitor, or a DNA crosslinking agent.

30. The antibody of claim 29, wherein the agent that induces DNA double-strand breaks is a calicheamicin.

31. The antibody of claim 29, wherein the DNA alkylating agent is a duocarmycin.

32. The antibody of claim 29, wherein the topoisomerase inhibitor is an exatecan.

33. The antibody of claim 29, wherein the DNA crosslinking agent is a pyrrolobenzodiazepine.

34. The antibody of claim 26, wherein the immunomodulatory agent is a TLR agonist or a STING agonist.

35. The antibody of any one of claims 24-34, wherein the drug is conjugated to the antibody via a cleavable linker.

36. The antibody of claim 35, wherein the cleavable linker comprises a protease cleavage site.

37. The antibody of claim 36, wherein the protease is a lysosomal enzyme.

38. The antibody of any one of claims 24-34, wherein the drug is conjugated to the antibody via a non-cleavable linker.

39. A biparatopic antibody comprising at least one antibody according to any one of the preceding claims.

40. A bispecific T cell engager (BiTE) comprising
    a. an antibody means for binding human basal cell adhesion molecule (BCAM);
    b. an antibody means for binding CD3;
    c. a linker connecting the antibody means for binding human BCAM to an antibody means for binding CD3; and optionally
    d. a means for extending half-life.

41. The BiTE of claim 40, wherein the antibody means comprises the antibody of any one of claims 1-22.

42. A pharmaceutical composition comprising an antibody that specifically binds human basal cell adhesion molecule (BCAM), comprising a variable region and a constant

region, wherein the variable region comprises a framework region and a complementarity determining means for binding human BCAM admixed with a pharmaceutically acceptable carrier.

43. A pharmaceutical composition comprising the antibody of any one of claims 1-38, the biparatopic antibody of claim 39, or the BiTE of claim 40 or 41, and one or more pharmaceutically acceptable excipient(s).

44. A method of treating cancer in a human subject, the method comprising administering to the human subject an effective amount of an antibody that specifically binds human basal cell adhesion molecule (BCAM), comprising a variable region and a constant region, wherein the variable region comprises a framework region and a complementarity determining means for binding human BCAM admixed with a pharmaceutically acceptable carrier.

45. A method of treating cancer in subject in need thereof, wherein the method comprises administering a therapeutically effective amount of the antibody of any one of claims 1-38, the biparatopic antibody of claim 39, the BiTE of claim 40 or 41, or the pharmaceutical composition of claim 43 to the subject.

46. The method of claim 45, wherein the cancer is ovarian cancer or endometrial cancer.

47. The method of claim 46, wherein the ovarian cancer is high-grade ovarian cancer.

48. The method of claim 46 or 47, wherein the ovarian cancer is serous ovarian cancer.

49. The method of any one of claims 45-48, wherein the subject is treatment naïve.

50. The method of any one of claims 45-48, wherein the subject is treated with one or more additional therapies.

51. The method of claim 50, wherein the one or more additional therapies comprise surgery and/or chemotherapy.

52. A first nucleic acid and a second nucleic acid, wherein:

a. the first nucleic acid comprises a nucleotide sequence encoding an immunoglobulin heavy chain variable (V_H) region of the antibody of any one of claims 4, 8, or 13, and
b. the second nucleic acid comprises a nucleotide sequence encoding an immunoglobulin light chain variable (V_L) region of the same antibody.

53. A first expression vector and a second expression vector respectively comprising the first and second nucleic acids of claim 52.

54. A nucleic acid encoding the antibody of any one of claims 1-25.

55. An expression vector comprising the nucleic acid of claim 54.

56. A host cell comprising the expression vector(s) of claim 53 or 55.