

ANTI-CERAMIDE ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims priority to U.S. Provisional Application No. 62/991,232, filed March 18, 2020, the content of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

[2] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy and is hereby incorporated by reference into the specification. A computer readable format copy of the Sequence Listing: filename: CERA-012_01WO_ST25.txt, date recorded: March 17, 2021, file size 68.1 kilobytes.

FIELD

[0003] The present disclosure generally relates to anti-ceramide antibodies and methods of use thereof. In particular, the disclosure relates to humanized anti-ceramide antibodies inhibiting cell death. Such antibodies are useful for treating and preventing gastrointestinal syndrome, Graft versus Host Disease, and autoimmune disease.

BACKGROUND

[0004] Acute Radiation Syndrome (ARS) (sometimes known as radiation toxicity or radiation sickness) is an acute illness caused by irradiation of a large portion of the body by a high dose of penetrating radiation (*e.g.*, high energy X-rays, gamma rays, and neutrons) in a very short period of time. Survival is extremely unlikely with this syndrome due to the destructive and irreparable changes in the GI tract and bone marrow caused by radiation-induced cell death.

[0005] Radiation is one of the most effective tools for the treatment of cancer. Unfortunately, an adverse side effect of radiation therapy is that healthy cells of the bone marrow, hair follicle, epidermis, and gastrointestinal tract are extremely sensitive to radiation-induced cell death. Other types of cancer strategies, such as bone marrow transplantation, evoke damaging immune responses in the host, resulting in rejection of the graft or graft-versus-host disease (GvHD).

[0006] Thus, there is an urgent need for alternative strategies to reduce the incidence of cell death associated with radiation GI syndrome and GvHD.

SUMMARY

[0007] The present disclosure provides compositions of anti-ceramide antibodies and antigen-binding fragments thereof. In some embodiments, the anti-ceramide antibodies and antigen-binding fragments thereof are humanized. In other embodiments, the anti-ceramide antibodies and antigen-binding fragments thereof are scFvs. In some embodiments, the disclosure further provides methods of preventing or inhibiting cell death in a subject in need thereof comprising administering the anti-ceramide antibodies or antigen-binding fragments to the subject. In some embodiments, the subject suffers from an autoimmune disease, GI syndrome, or GvHD.

[0008] In some embodiments, the present disclosure provides an anti-ceramide antibody or antigen-binding fragment thereof comprising an immunoglobulin heavy chain variable region (VH) comprising a heavy chain complementarity determining region (CDR) 1 (HCDR1), a heavy chain CDR2 (HCDR2), and a heavy chain CDR3 (HCDR3), and an immunoglobulin light chain variable region (VL) comprising a light chain complementarity determining region (CDR) 1 (LCDR1), a light chain CDR2 (LCDR2), and a light chain CDR3 (LCDR3), wherein: (a) the HCDR1 comprises an amino acid sequence selected from SEQ ID NO: 1 and 2; (b) the HCDR2 comprises an amino acid sequence selected from SEQ ID NO: 3, 4, 5, and 6; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises the amino acid sequence of SEQ ID NO: 10.

[0009] In some embodiments, the HCDR1 comprises SEQ ID NO: 1 and the HCDR2 comprises SEQ ID NO: 3. In some embodiments, the HCDR1 comprises SEQ ID NO: 1 and the HCDR2 comprises SEQ ID NO: 4. In some embodiments, the HCDR1 comprises SEQ ID NO: 1 and the HCDR2 comprises SEQ ID NO: 5. In some embodiments, the HCDR1 comprises SEQ ID NO: 1 and the HCDR2 comprises SEQ ID NO: 6. In some embodiments, the HCDR1 comprises SEQ ID NO: 2 and the HCDR2 comprises SEQ ID NO: 3. In some embodiments, the HCDR1 comprises SEQ ID NO: 2 and the HCDR2 comprises SEQ ID NO: 4. In some embodiments, the HCDR1 comprises SEQ ID NO: 2 and the HCDR2 comprises SEQ ID NO: 5. In some embodiments, the HCDR1 comprises SEQ ID NO: 2 and the HCDR2 comprises SEQ ID NO: 6.

[0010] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 22. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO:

17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22. In some embodiments, VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0011] In some embodiments, VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 23. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0012] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 24. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0013] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 22. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0014] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 23. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0015] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 24. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0016] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 22. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0017] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 23. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0018] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 24. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0019] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 20 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 22. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO:

20 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 20 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0020] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 21 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 22. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 21 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 21 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0021] In some embodiments, the present disclosure provides an anti-ceramide antibody or antigen-binding fragment thereof comprising an immunoglobulin heavy chain variable region (VH) and an immunoglobulin light chain variable region (VL), wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to a sequence selected from SEQ ID NO: 17, 18, 19, 20, and 21; and wherein the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to a sequence selected from SEQ ID NO: 22, 23 and 24.

[0022] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 22. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0023] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 23. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0024] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 24. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0025] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 22. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0026] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 23. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0027] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 24. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0028] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 22. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL

comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0029] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 23. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0030] In some embodiments, VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 24. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0031] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 20 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 22. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 20 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 20 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0032] In some embodiments, the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 21 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 22. In some embodiments, the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 21 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22. In some embodiments, the VH amino acid sequence consists of SEQ ID NO: 21 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0033] In some embodiments, the antibody or antigen-binding fragment is humanized. In some embodiments, the antibody or antigen-binding fragment is fully human. In some embodiments, the anti-ceramide antibody comprises one or more point mutations in the Fc domain of the antibody.

[0034] In some embodiments, the antigen-binding fragment is a single chain variable fragment (scFv). In some embodiments, the scFv comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to a sequence selected from the group consisting of SEQ ID NOs: 48-61. In some embodiments, the scFv comprises an amino acid sequence that is 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 48-61. In some embodiments, the scFv consists of a sequence selected from the group consisting of SEQ ID NOs: 48-61.

[0035] In some embodiments, the light chain variable region of said scFv is carboxy-terminal to the heavy chain variable region of said scFv. In some embodiments, the light chain variable region of said scFv is amino-terminal to the heavy chain variable region of said scFv.

[0036] In some embodiments, the scFv comprises a linker polypeptide. In some embodiments, the linker polypeptide is between the light chain variable region and the heavy chain variable region of the said scFv. In some embodiments, the linker polypeptide comprises a Gly₄Ser linker. In some embodiments, the linker polypeptide comprises the formula (Gly₄Ser)_n, wherein n = 1-5.

[0037] In some embodiments, the present disclosure provides an anti-ceramide single chain variable fragment (scFv) comprising an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 97% identical to a sequence selected from the group consisting of SEQ ID NOs: 48-61. In some embodiments, the scFv comprises an amino acid sequence that is 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 48-61. In some embodiments, the scFv consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 48-61.

[0038] In some embodiments, the present disclosure provides a polynucleotide encoding the anti-ceramide antibody or antigen-binding fragment thereof described herein.

[0039] In some embodiments, the present disclosure provides an anti-ceramide single chain variable fragment (scFv) comprising an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 48 or 51. In some embodiments, the scFv comprises an amino acid sequence that is 100% identical to SEQ ID

NO: 48 or 51. In some embodiments, the scFv consists of the amino acid sequence of SEQ ID NO: 48 or 51.

[0040] In some embodiments, the present disclosure provides a polynucleotide encoding the anti-ceramide single chain variable fragments (scFvs) described herein. In some embodiments, the present disclosure provides an expression vector comprising said polynucleotide.

[0041] In some embodiments, the present disclosure provides a host cell comprising a polynucleotide or expression vector described herein.

[0042] In some embodiments, the present disclosure provides a method of manufacturing an anti-ceramide antibody or antigen-binding fragment thereof described herein or an anti-ceramide single chain variable fragment (scFv) described herein, comprising introducing the expression vector of Embodiment 98 into a host cell.

[0043] In some embodiments, the present disclosure provides a method of inhibiting apoptosis in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-ceramide antibody or antigen-binding fragment thereof described herein or an anti-ceramide single chain variable fragment (scFv) described herein. In some embodiments, the apoptosis is associated with a disease selected from the group consisting of graft versus host disease, radiation disease, GI syndrome, and autoimmune disease. In some embodiments, the disease is radiation disease or GI syndrome and the anti-ceramide antibody or antigen-binding fragment thereof is administered before the subject is exposed to radiation. In some embodiments, the disease is graft versus host disease and the anti-ceramide antibody or antigen-binding fragment thereof is administered before the subject receives a transplant. In some embodiments, the transplant is a bone marrow transplant.

[0044] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof is administered intravenously, intramuscularly, intraperitoneally, intracerebrospinally, subcutaneously, intrasynovially, intrathecally, orally, topically, or via inhalation.

[0045] In some embodiments, the present disclosure provides a method for mitigating apoptosis in a subject with GI syndrome comprising administering to the subject an effective amount of the anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 1-89 or the anti-ceramide scFv of any one of Embodiments 94-96, after the subject is exposed to penetrating radiation. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof is administered immediately after the subject is exposed to penetrating radiation. In some embodiments, the anti-ceramide antibody or antigen-binding

fragment thereof is administered within 24 hours after the subject is exposed to penetrating radiation.

[0046] In some embodiments, the present disclosure provides a method for inhibiting apoptosis in a subject with GvHD comprising administering to the subject an effective amount of anti-ceramide antibody or antigen binding fragment thereof of any one of Embodiments 1-89 or the anti-ceramide scFv of any one of Embodiments 94-96, either before the subject receives a transplant or after the subject receives a transplant prior to the onset of GvHD. In some embodiments, the transplant is a bone marrow transplant.

BRIEF DESCRIPTION OF THE DRAWINGS

[0047] The accompanying drawings illustrate one or more embodiments of the present disclosure and, together with the written description, serve to explain the principles of exemplary embodiments of the present disclosure.

[0048] **FIG. 1** shows a dose-response curve of CD28-induced ceramide-rich platform formation (CRP) in T cells with increasing concentrations of 6B5 scFvs.

[0049] **FIG. 2A** shows a dose-response curve of radiation-induced T cell apoptosis with increasing concentrations of humanized 6B5 scFvs.

[0050] **FIG. 2B** shows a dose-response curve of radiation-induced T cell apoptosis with increasing concentrations of VH4-VL1, VH5-VL1, and parental murine 6B5 scFv.

[0051] **FIG. 2C** shows a dose-response curve of radiation-induced T cell apoptosis with increasing concentrations of VH4-VL1, VH5-VL1, and parental murine 6B5 scFv.

[0052] **FIG. 2D** shows a dose-response curve of radiation-induced T cell apoptosis with increasing concentrations of VH4-VL1 and VH5-VL1.

[0053] **FIG. 2E** shows the percentage of radiation-induced apoptotic T cells with given concentrations of VH4-VL1 and VH5-VL1.

[0054] **FIG. 3A** shows the number of apoptotic endothelial cells per villus over time in mice exposed to an LD₉₀ dose of 15 gray units (Gy). The numbers adjacent to the symbols represent the number of animals measured per time point. Gy, gray unit of absorbed radiation.

[0055] **FIG. 3B** shows the number of apoptotic endothelial cells per villus over time in mice exposed to an LD₉₀ dose of 15 gray units (Gy) and left untreated or treated with VH2-VL1

(SEQ ID NO: 51) 24 hours following irradiation. The numbers adjacent to the symbols represent the number of animals measured per time point. Gy, gray unit of absorbed radiation.

[0056] FIG. 4A shows the amino acid sequence alignments of the humanized VH regions. The CDRs are underlined. CDR amino acids that differ from the parental murine CDR sequence are shown in bold. Framework region amino acids that differ from the human germline framework sequence are shown in bold.

[0057] FIG. 4B shows the amino acid sequence alignments of the humanized VL regions. The CDRs are underlined.

DETAILED DESCRIPTION

[0058] The details of the disclosure are set forth in the accompanying description below. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, illustrative methods, and materials are now described. Other features, objects, and advantages of the disclosure will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. All patents and publications cited in this specification are incorporated herein by reference in their entireties.

Definitions

[0059] The term “antibody” refers to an immunoglobulin (Ig) molecule capable of binding to a specific target, such as a carbohydrate, polynucleotide, lipid, or polypeptide, through at least one epitope recognition site located in the variable region of the Ig molecule. As used herein, the term encompasses intact polyclonal or monoclonal antibodies and antigen-binding fragments thereof. For example, a native immunoglobulin molecule is comprised of two heavy chain polypeptides and two light chain polypeptides. Each of the heavy chain polypeptides associate with a light chain polypeptide by virtue of interchain disulfide bonds between the heavy and light chain polypeptides to form two heterodimeric proteins or polypeptides (i.e., a protein comprised of two heterologous polypeptide chains). The two heterodimeric proteins then associate by virtue of additional interchain disulfide bonds between the heavy chain polypeptides to form an immunoglobulin protein or polypeptide.

[0060] The term “antigen-binding fragment” as used herein refers to a polypeptide fragment that contains at least one Complementarity-determining region (CDR) of an immunoglobulin

heavy and/or light chain that binds to at least one epitope of the antigen of interest. In this regard, an antigen-binding fragment of the herein described antibodies may comprise 1, 2, 3, 4, 5, or all 6 CDRs of a variable heavy chain (VH) and variable light chain (VL) sequence from antibodies that specifically bind ceramide. Antigen-binding fragments include proteins that comprise a portion of a full length antibody, generally the antigen binding or variable region thereof, such as Fab, F(ab')₂, Fab', Fv fragments, minibodies, diabodies, single domain antibody (dAb), single-chain variable fragments (scFv), multispecific antibodies formed from antibody fragments, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding site or fragment of the required specificity. In certain embodiments of the disclosure, an antigen-binding fragment, rather than an intact antibody, is used to increase tissue penetration or tumor penetration. In other embodiments, antigen-binding fragments are further modified to increase serum half-life.

[0061] The term “F(ab)” refers to two of the protein fragments resulting from proteolytic cleavage of IgG molecules by the enzyme papain. Each F(ab) comprises a covalent heterodimer of the VH chain and VL chain and includes an intact antigen-binding site. Each F(ab) is a monovalent antigen-binding fragment. The term “Fab” refers to a fragment derived from F(ab')₂ and may contain a small portion of Fc. Each Fab' fragment is a monovalent antigen-binding fragment.

[0062] The term “F(ab')₂” refers to a protein fragment of IgG generated by proteolytic cleavage by the enzyme pepsin. Each F(ab')₂ fragment comprises two F(ab') fragments and is therefore a bivalent antigen-binding fragment.

[0063] An “Fv fragment” refers to a non-covalent VH::VL heterodimer which includes an antigen-binding site that retains much of the antigen recognition and binding capabilities of the native antibody molecule, but lacks the CH1 and CL domains contained within a Fab. Inbar et al. (1972) Proc. Nat. Acad. Sci. USA 69:2659-2662; Hochman et al. (1976) Biochem 15:2706-2710; and Ehrlich et al. (1980) Biochem 19:4091-4096. In some embodiments, the Fv fragment can be produced by preferential proteolytic cleavage of an IgM, and on rare occasions of an IgG or IgA immunoglobulin molecule. Fv fragments are, however, more commonly derived using recombinant techniques known in the art.

[0064] Minibodies comprising a scFv joined to a CH3 domain are also included herein (S. Hu et al., Cancer Res., 56, 3055-3061, 1996). See *e.g.*, Ward, E. S. *et al.*, Nature 341, 544-546 (1989); Bird et al., Science, 242, 423-426, 1988; Huston et al., PNAS USA, 85, 5879-5883,

1988); PCT/US92/09965; WO94/13804; P. Holliger et al., *Proc. Natl. Acad. Sci. USA* 90 6444-6448, 1993; Y. Reiter et al., *Nature Biotech*, 14, 1239-1245, 1996; S. Hu et al., *Cancer Res.*, 56, 3055-3061, 1996.

[0065] The term “diabody” refers to a bispecific antibody in which VH and VL domains are expressed in a single polypeptide chain using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen-binding sites (see, *e.g.*, Holliger et al., *Proc. Natl. Acad. Sci. USA* 90:6444-48 (1993) and Poljak et al., *Structure* 2:1121-23 (1994)).

[0066] The term “nanobody” or a “single domain antibody” refers to an antigen-binding fragment consisting of a single monomeric variable antibody domain. The Nanoclone method is a method for generating Nanobodies against a desired target based on automated high-throughput selection of B-cells. (See, WO 2006/079372)

[0067] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts.

[0068] In some embodiments, the term “chimeric antibody” as used herein refers to a monoclonal antibody in which a portion of the heavy and/or light chain is identical or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

[0069] 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 immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. Huston *et al.* (1988) *Proc. Nat. Acad. Sci. USA* 85(16):5879-5883. The linker can connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. A number of methods have been described to discern chemical structures for converting the naturally aggregated—but chemically separated—light and heavy polypeptide chains from an antibody V region into an scFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See,

e.g., U.S. Pat. Nos. 5,091,513 and 5,132,405, to Huston *et al.*; and U.S. Pat. No. 4,946,778, to Ladner *et al.*

[0070] The term “antigen” refers to a molecule or a portion of a molecule capable of being bound by an antibody or an antigen-binding fragment thereof and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes. Embodiments herein contemplate the use of ceramide, or ceramide conjugated to a hapten, as an antigen.

[0071] The term “epitope” refers to a region of an antigen that is bound by an antibody. Epitope determinants can include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl and may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[0072] Herein, the term “specifically binds” refers to the ability of an antibody or antigen-binding fragment thereof to bind a target antigen with a binding affinity (K_a) of at least 10^5 M^{-1} while not significantly binding other components or antigens present in a mixture. Reference to an anti-ceramide antibody herein refers to an antibody or antigen-binding fragment thereof that specifically binds to ceramide.

[0073] Binding affinity (K_a) refers to an equilibrium association of a particular interaction expressed in the units of $1/\text{M}$ or M^{-1} . Antibodies or antigen-binding fragments thereof can be classified as “high affinity” antibodies or antigen-binding fragments thereof and “low affinity” antibodies or antigen-binding fragments thereof. “High affinity” antibodies or antigen-binding fragments thereof refer to those antibodies or antigen-binding fragments thereof with a K_a of at least 10^7 M^{-1} , at least 10^8 M^{-1} , at least 10^9 M^{-1} , at least 10^{10} M^{-1} , at least 10^{11} M^{-1} , at least 10^{12} M^{-1} , or at least 10^{13} M^{-1} . “Low affinity” antibodies or antigen-binding fragments thereof refer to those antibodies or antigen-binding fragments thereof with a K_a of up to 10^7 M^{-1} , up to 10^6 M^{-1} , up to 10^5 M^{-1} . Alternatively, affinity can be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (e.g., 10^{-5} M to 10^{-13} , or about 500 nM, about 300 nM, about 250 nM, about 200 nM, about 150 nM, about 100 nM, about 50 nM, about 25 nM, about 10 nM, or about 5 nM). Affinities of binding domain polypeptides and single chain polypeptides according to the present disclosure can be readily determined using conventional techniques (see, e.g., Scatchard et al. (1949) Ann. N.Y. Acad. Sci. 51:660; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

[0074] A “conservative substitution” is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well-known in the art (see, e.g., PCT Application Publication No. WO 97/09433, page 10, published March 13, 1997; Lehninger, Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77; Lewin, Genes IV, Oxford University Press, NY and Cell Press, Cambridge, MA (1990), p. 8).

[0075] As used herein, the term “derivative” refers to a modification of one or more amino acid residues of a peptide by chemical or biological means, either with or without an enzyme, e.g., by glycosylation, alkylation, acylation, ester formation, or amide formation.

[0076] As used herein, a polypeptide or polynucleotide from which another polypeptide or polynucleotide is derived from is referred to as the “parental” or “reference” polynucleotide or polypeptide. For example, a humanized antibody can be derived from a parental murine antibody.

[0077] The term “variant” or “variants” as used herein refers to a polynucleotide or polypeptide with a sequence differing from that of a reference polynucleotide or polypeptide, but retaining essential properties of the parental polynucleotide or polypeptide. Generally, variant polynucleotide or polypeptide sequences are overall closely similar, and, in many regions, identical to the parental polynucleotide or polypeptide. For instance, a variant polynucleotide or polypeptide may exhibit at least about 70%, at least about 80%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% at least about 99%, or at least about 99.5% sequence identity compared to the parental polynucleotide or polypeptide.

[0078] As used herein, the term “sequence identity” refers to a relationship between two or more polynucleotide sequences or between two or more polypeptide sequences. When a position in one sequence is occupied by the same nucleic acid base or amino acid residue in the corresponding position of the comparator sequence, the sequences are said to be “identical” at that position. The percentage sequence identity is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of identical positions. The number of identical positions is then divided by the total number of positions in the comparison window and multiplied by 100 to yield the percentage of sequence identity. Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window. The comparison

window for polynucleotide sequences can be, for instance, at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 or more nucleic acids in length. The comparison window for polypeptide sequences can be, for instance, at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300 or more amino acids in length. In order to optimally align sequences for comparison, the portion of a polynucleotide or polypeptide sequence in the comparison window can comprise additions or deletions termed gaps while the reference sequence is kept constant. An optimal alignment is that alignment which, even with gaps, produces the greatest possible number of “identical” positions between the reference and comparator sequences. Percentage “sequence identity” between two sequences can be determined using the version of the program “BLAST 2 Sequences” which was available from the National Center for Biotechnology Information as of September 1, 2004, which program incorporates the programs BLASTN (for nucleotide sequence comparison) and BLASTP (for polypeptide sequence comparison), which programs are based on the algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90(12):5873-5877, 1993). When utilizing “BLAST 2 Sequences,” parameters that were default parameters as of September 1, 2004, can be used for word size (3), open gap penalty (11), extension gap penalty (1), gap dropoff (50), expect value (10) and any other required parameter including but not limited to matrix option. Two nucleotide or amino acid sequences are considered to have “substantially similar sequence identity” or “substantial sequence identity” if the two sequences have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity relative to each other.

[0079] The term “substantially identical” refers to a polypeptide sequence that contains a sufficient number of identical amino acids to a second polypeptide sequence such that the first and second polypeptide sequence have similar activity. Polypeptides that are substantially identical are at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identical in amino acid sequence.

[0080] “Fc region” or “Fc domain” refers to a polypeptide sequence corresponding to or derived from the portion of an antibody that is capable of binding to Fc receptors on cells and/or the C1q component of complement, thereby mediating the effector function of an antibody. Fc stands for “fragment crystalline,” the fragment of an antibody that will readily form a protein crystal. Distinct protein fragments, which were originally described by proteolytic digestion,

can define the overall general structure of an immunoglobulin protein. As originally defined in the literature, the Fc region is a homodimeric protein comprising two polypeptides that are associated by disulfide bonds, and each comprising a hinge region, a CH2 domain, and a CH3 domain. However, more recently the term has been applied to the single chain monomer component consisting of CH3, CH2, and at least a portion of the hinge sufficient to form a disulfide-linked dimer with a second such chain. As such, and depending on the context, use of the terms “Fc region” or “Fc domain” will refer herein to either the dimeric form or the individual monomers that associate to form the dimeric protein. For a review of immunoglobulin structure and function, see Putnam, *The Plasma Proteins*, Vol. V (Academic Press, Inc., 1987), pp. 49-140; and Padlan, *Mol. Immunol.* 31:169-217, 1994. As used herein, the term Fc domain includes variants of naturally occurring sequences.

[0081] The term “immunoglobulin constant region” or “constant region” refers to a peptide or polypeptide sequence that corresponds to or is derived from part or all of one or more constant domains of an immunoglobulin (*e.g.*, CH1, CH2, CH3). In certain embodiments, the constant region does not comprise a CH1 domain. In certain embodiments, the constant domains making up the constant region are human

[0082] The terms “light chain variable region” (also referred to as “light chain variable domain” or “VL”) and “heavy chain variable region” (also referred to as “heavy chain variable domain” or “VH”) refer to the variable binding region from an antibody light and heavy chain, respectively. The variable binding regions are made up of discrete, well-defined sub-regions known as “complementarity determining regions” (CDRs) and “framework regions” (FRs).

[0083] The term “immunoglobulin light chain constant region” (also referred to as “light chain constant region” or “CL”) is a constant region from an antibody light chain.

[0084] The term “immunoglobulin heavy chain constant region” (also referred to as “heavy chain constant region” or “CH”) refers to the constant region from the antibody heavy chain. The CH is further divisible, depending on the antibody isotype into CH1, CH2, and CH3 (IgA, IgD, IgG), or CH1, CH2, CH3, and CH4 domains (IgE, IgM).

[0085] As used herein, the term “complementarity determining region” or “CDR” refer to an immunoglobulin (antibody) molecule. There are three CDRs per variable domain: CDR1, CDR2 and CDR3 in the variable domain of the light chain and CDR1, CDR2 and CDR3 in the variable domain of the heavy chain.

[0086] In some embodiments, a “hinge” or a “hinge region” refers to a polypeptide derived from an immunoglobulin hinge region and located between an antigen-binding domain (e.g., a ceramide-binding domain) and an immunoglobulin constant region in a polypeptide described herein. A “wild-type immunoglobulin hinge region” refers to a naturally occurring upper and middle hinge amino acid sequences interposed between and connecting the CH1 and CH2 domains (for IgG, IgA, and IgD) or interposed between and connecting the CH1 and CH3 domains (for IgE and IgM) found in the heavy chain of an antibody. In certain embodiments, a wild type immunoglobulin hinge region sequence is human, and can comprise a human IgG hinge region (e.g., and IgG1, IgG2, IgG3, or IgG4 hinge region).

[0087] An “altered immunoglobulin hinge region” or “variant immunoglobulin hinge region” refers to a hinge region polypeptide with one or more mutations, substitutions, insertions, or deletions compared to a corresponding parental wild-type immunoglobulin hinge region. Typically, an altered immunoglobulin hinge region that is a fragment of a wild type immunoglobulin hinge region comprises an IgG core hinge region (e.g., a polypeptide comprising the sequence C-X-X-C, wherein X is any amino acid) as disclosed in U.S. Patent Application Publication Nos. 2013/0129723 and 2013/0095097.

[0088] As used herein, the term “humanized” refers to an antibody or antigen-binding fragment thereof derived from a non-human species that retains the antigen-binding properties of the original non-human antibody. In some embodiments, the binding fragments of an antibody (e.g., light and heavy chain variable regions, Fab, scFv) are humanized. Non-human antigen-binding fragments can be humanized using techniques known as CDR grafting (Jones et al., *Nature* 321:522 (1986)) and variants thereof, including “reshaping” (Verhoeyen, et al., 1988 *Science* 239:1534-1536; Riechmann, et al., 1988 *Nature* 332:323-337; Tempest, et al., *Bio/Technol* 1991 9:266-271), “hyperchimerization” (Queen, et al., 1989 *Proc Natl Acad Sci USA* 86:10029-10033; Co, et al., 1991 *Proc Natl Acad Sci USA* 88:2869-2873; Co, et al., 1992 *J Immunol* 148:1149-1154), and “veneering” (Mark, et al., “Derivation of therapeutically active humanized and veneered anti-CD18 antibodies.” In: Metcalf BW, Dalton BJ, eds. *Cellular adhesion: molecular definition to therapeutic potential*. New York: Plenum Press, 1994: 291-312). If derived from a non-human source, other regions of the antibody, such as the hinge region and constant region domains, can also be humanized.

[0089] As used herein, the term “pharmaceutically acceptable” refers to molecular entities and compositions that do not generally produce allergic or other serious adverse reactions when administered using routes well known in the art. Molecular entities and compositions approved

by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans are considered to be “pharmaceutically acceptable.”

[0090] The term “polynucleotide” as referred to herein means single-stranded or double-stranded nucleic acid polymers. In certain embodiments, the nucleotides comprising the polynucleotide can be RNA or DNA or a modified form of either type of nucleotide, such as a modified messenger RNA. Said modifications may include, but are not limited to, base modifications such as bromouridine, ribose modifications such as arabinoside and 2',3'-dideoxyribose and internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate and phosphoroamidate. The term “polynucleotide” specifically includes single and double stranded forms of DNA.

[0091] As used herein, a “polypeptide” or “protein” refers to a single, linear, and contiguous arrangement of covalently linked amino acids. Polypeptides can form one or more intrachain disulfide bonds. The terms polypeptide and protein also encompass embodiments where two polypeptide chains link together in a non-linear fashion, such as via an interchain disulfide bond. Herein, a protein or polypeptide may be an antibody or an antigen-binding fragment of an antibody.

[0092] As used herein, the term “transformation,” “transfection,” and “transduction” refer to the transfer of a polynucleotide into a cell. As used herein, the term “genetic transformation” refers to the transfer and incorporation of DNA, especially recombinant DNA, into a cell. The transferred nucleic acid can be introduced into a cell via an expression vector.

[0093] As used herein, the terms “treatment,” “treating,” or “ameliorating” refers to either a therapeutic treatment or prophylactic/preventative treatment. A treatment is therapeutic if at least one symptom of disease in an individual receiving treatment improves or a treatment can delay worsening of a progressive disease in an individual or prevent onset of additional associated diseases.

[0094] As used herein, the term “LD90” refers to “Lethal Dose, 90%” or “median lethal dose” and is the amount of a substance required to kill 90% of a test population.

[0095] As used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural references unless the content clearly dictates otherwise.

[0096] As used in this specification, the term “and/or” is used in this disclosure to either “and” or “or” unless indicated otherwise.

[0097] Throughout this specification, unless the context requires otherwise, the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Anti-ceramide antibodies

[0098] The present disclosure provides antibodies or antigen-binding fragments thereof that specifically bind to ceramide. In some embodiments, the anti-ceramide antibody or antigen binding fragments thereof is humanized. In other embodiments, the anti-ceramide antibody is a humanized 6B5 scFv antibody.

[0099] The term “ceramide” as used herein refers to a lipid second messenger molecule comprised of sphingosine and a fatty acid. Ceramide regulates stress signaling within the cell through reorganization of the plasma membrane and formation of lateral liquid-ordered microdomains (a type of ‘raft’ termed ‘ceramide-rich platforms’). These CRPs serve as signaling platforms that cluster activated receptor molecules (*e.g.*, members of the TNF-receptor superfamily including Fas), thereby providing feed forward mechanisms that ultimately result in signaling amplification and signal transduction. CRP formation is particularly important for Fas-mediated cell death and ceramide-rich regions on the plasma membrane of target cells are critical for sensitivity to cytotoxic T lymphocyte (CTL)-induced cell death. Exemplary ceramides are glucosylceramides, galactosylceramides and gangliosides (oligosaccharide-linked ceramides).

[0100] In some embodiments, the present disclosure provides an antibody or antigen-binding fragment that specifically binds to ceramide and comprises an immunoglobulin heavy chain variable region (VH) comprising a heavy chain complementarity determining region (CDR) 1 (HCDR1), a heavy chain CDR2 (HCDR2), and a heavy chain CDR3 (HCDR3), and an immunoglobulin light chain variable region (VL) comprising a light chain complementarity determining region (CDR) 1 (LCDR1), a light chain CDR2 (LCDR2), and a light chain CDR3 (LCDR3), wherein the HCDR1 comprises an amino acid sequence selected from SEQ ID NO: 1 and 2; the HCDR2 comprises an amino acid sequence selected from SEQ ID NO: 3, 4, 5, and 6; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; the LCDR1 comprises

the amino acid sequence of SEQ ID NO: 8; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 10.

[0101] The CDRs of exemplary anti-ceramide antibodies are shown in Table 1.

Table 1: Exemplary anti-ceramide antibody sequences

Seq Desc.	Chain	Amino Acid Sequence	SEQ ID NO.
HCDR1-IH	Heavy	GYTFTDHTIH	1
HCDR1-IH-MH	Heavy	GYTFTDHTMH	2
HCDR2-DG	Heavy	YNYPRDGSTKYNEKFQG	3
HCDR2-DG-EG	Heavy	YNYPREGSTKYNEKFQG	4
HCDR2-DG-DV	Heavy	YNYPRDVSTKYNEKFQG	5
HCDR2-NE-AE	Heavy	YNYPRDGSTKYAEKFQG	6
HCDR3	Heavy	GFITTVVPSAY	7
LCDR1	Light	RASKSISKYLA	8
LCDR2	Light	SGSTLQS	9
LCDR3	Light	QQHNEYPWT	10
Parental 6B5 HCDR1	Heavy	GYTFTDHTIH	11
Parental 6B5 HCDR2	Heavy	YNYPRDGSTKYNEKFQG	12
Parental 6B5 HCDR3	Heavy	GFITTVVPSAY	13
Parental 6B5 LCDR1	Light	RASKSISKYLA	14
Parental 6B5 LCDR2	Light	SGSTLQS	15
Parental 6B5 LCDR3	Light	QQHNEYPWT	16

[0102] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof is an scFv comprising an immunoglobulin heavy chain variable region (VH) comprising a heavy chain complementarity determining region (CDR) 1 (HCDR1), a heavy chain CDR2 (HCDR2), and a heavy chain CDR3 (HCDR3), and an immunoglobulin light chain variable region (VL) comprising a light chain complementarity determining region (CDR) 1 (LCDR1), a light chain CDR2 (LCDR2), and a light chain CDR3 (LCDR3), wherein the HCDR1 comprises an amino acid sequence selected from SEQ ID NO: 1 and 2; the HCDR2 comprises an amino acid sequence selected from SEQ ID NO: 3, 4, 5, and 6; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 10.

[0103] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the

HCDR1 comprises the amino acid sequence of SEQ ID NO: 1; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 3; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0104] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 1; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 4; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0105] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 1; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 5; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0106] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 2; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 6; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0107] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 1; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 6; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the

LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0108] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 2; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 3; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0109] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 2; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 4; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0110] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 2; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 5; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0111] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 1; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 6; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0112] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the

HCDR1 comprises the amino acid sequence of SEQ ID NO: 2; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 3; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0113] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 2; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 4; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0114] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein (a) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 2; (b) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 5; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises an amino acid sequence of SEQ ID NO: 10.

[0115] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH and a VL chain, wherein the VH chain comprises or consists of an amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 17-21.

[0116] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH and a VL chain, wherein the VL chain comprises or consists of an amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-24.

[0117] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH and a VL chain, wherein the VH chain comprises or consists of an amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 17-21, and the VL chain comprises or consists of an amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-24.

[0118] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof is an scFv comprising a VH and a VL chain, wherein the VH chain comprises or consists of an amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 17-21, and the VL chain comprises or consists of an amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-24.

Table 2. Exemplary VH and VL Amino Acid Sequences

Seq Desc.	Chain	Amino Acid Sequence	SEQ ID NO.
Humanized VH1	Heavy	QVQLVQSGAEVKKPGASVKVSCKASGYTFDHTMHWVRQA PGQGLEWMGYNYPDGSTKYAEKFQGRVTMTADKSTSTVY MELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLVTVSS	17
Humanized VH2	Heavy	QVQLVQSGAEVKKPGASVKVSCKVS ~~GY~~ TFDHTIHWMRQA PGQGLEWMGYNYPDGSTKYNEKFQGRVTMTADKSTSTVY MELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLVTVSS	18
Humanized VH3	Heavy	QVQLVQSGAEVKKPGATVKISCKVSGYTFDHTIHWMQQA PGKGLEWMGYNYPDGSTKYNEKFQGRVTITADKSTSTAY MELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLVTVSS	19
Humanized VH4	Heavy	QVQLVQSGAEVKKPGASVKVSCKVS ~~GY~~ TFDHTIHWMRQA PGQGLEWMGYNYPREGSTKYNEKFQGRVTMTADKSTSTVY MELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLVTVSS	20

Seq Desc.	Chain	Amino Acid Sequence	SEQ ID NO.
Humanized VH5	Heavy	QVQLVQSGAEVKKPGASVKVSKVSGYTFDHTIHWMRQA PGQGLEWMGYNYPDRVSTKYNEKFQGRVTMTADKSTSTVY MELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLVTVSS	21
Humanized VL1	Light	DIQLTQSPSFLSASVGDRTTITCRASKSISKYLAWYQQKP GKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQP EDFATYYCQQHNEYPWTFGGGTKVEIK	22
Humanized VL2	Light	DVQITQSPSFLSASVGDRTTITCRASKSISKYLAWYQQKP GKANKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQP EDFATYYCQQHNEYPWTFGGGTKVEIK	23
Humanized VL3	Light	DVQLTQSPSSVSASVGDRTTITCRASKSISKYLAWYQQKP GKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQP EDFATYYCQQHNEYPWTFGPGTKVEIK	24

[0119] In some embodiments, the anti-ceramide antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 17 and a VL amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 17 and a VL amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 17 and a VL amino acid sequence of SEQ ID NO: 24. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 18 and a VL amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 18 and a VL amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 18 and a VL amino acid sequence of SEQ ID NO: 24. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 19 and a VL amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 19 and a VL amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 19 and a VL amino acid sequence of SEQ ID NO: 24. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 20 and a VL amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 20 and a VL amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 20 and a VL amino acid sequence of SEQ ID NO: 24. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 21 and a VL

amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 21 and a VL amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody or antigen-fragment thereof comprises a VH amino acid sequence of SEQ ID NO: 21 and a VL amino acid sequence of SEQ ID NO: 24.

[0120] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 17 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 22. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0121] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 17 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 23. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequences consisting of 17 and a VL amino acid sequence consisting of 23.

[0122] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 17 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%,

at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 24. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0123] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 18 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 22. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0124] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 18 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 23. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0125] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 18 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%,

at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 24. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0126] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 19 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 22. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0127] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 19 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 23. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0128] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 19 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%,

at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 24. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0129] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 20 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 22. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 20 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0130] In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 21 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 22. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 21 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0131] In some embodiments, the anti-ceramide antigen-binding fragment is an scFv comprising a VH amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 18 and a VL amino acid sequence that is at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%,

at least about 99%, at least about 99.5%, or 100% identical to SEQ ID NO: 22. In some embodiments, the anti-ceramide scFv comprises a VH chain and a VL chain, wherein the VH chain amino acid sequence consisting of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0132] In some embodiments, the anti-ceramide antigen-binding fragment thereof is an scFv. In some embodiments, the anti-ceramide scFv comprises an amino acid sequence that is at least about 80%, at least about 82%, at least about 85%, at least about 87%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 48-61. In some embodiments, the anti-ceramide scFv consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 48-61. In some embodiments, the anti-ceramide scFv comprises or consists of the amino acid sequence of SEQ ID NOs: 48 or 51.

[0133] In some embodiments, the anti-ceramide scFv comprises the following structure: VH-linker-VL or VL-linker-VH. As used herein, the term “linker” generally refers to a short polypeptide sequence connecting two sub-domains of a polypeptide. Non-limiting examples of linkers include flexible linkers comprising glycine-serine repeats, and linkers derived from (a) an interdomain region of a transmembrane protein (e.g., a type I transmembrane protein); (b) a stalk region of a type II C-lectin; or (c) an immunoglobulin hinge. In some embodiments, a linker provides a spacer function compatible with interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity to the same target molecule as an antibody that comprises the same light and heavy chain variable regions. In certain embodiments, a linker is comprised of five to about 35 amino acids, for instance, about 15 to about 25 amino acids. Exemplary linkers are shown in Table 3.

Table 3. Exemplary linkers

Linker	Amino Acid Sequence	SEQ ID
STD1	NYGGGSGGGSGGGSGNS	25
STD2	NYGGGSGGGSGGGSGNYGGGSGGGSGGGSGNS	26
H1	NS	27
H2	GGGSGNS	28
H3	NYGGGSGNS	29
H4	GGGSGGGSGNS	30
H5	NYGGGSGGGSGNS	31
H6	GGGSGGGSGGGSGNS	32
H7	GCPPCPNS	33
Gly ₄ Ser	GGGS	34
(G ₄ S) ₃	GGGSGGGSGGGSG	35
H105	SGGGSGGGSGGGSG	36

Linker	Amino Acid Sequence	SEQ ID
(G ₄ S) ₄	GGGGSGGGSGGGSGGGGS	37
H75 (NKG2A quadruple mutant)	QRHNSSLNTGTQMAGHSPNS	38
H83 (NKG2A derived)	SSLNTGTQMAGHSPNS	39
H106 (NKG2A derived)	QRHNSSLNTGTQMAGHS	40
H81 (NKG2D derived)	EVQIPLTESYSPNS	41
H91 (NKG2D derived)	NSLANQEVQIPLTESYSPNS	42
H94	SGGGSGGGSGGGSGGGSPNS	43
H111	SGGGSGGGSGGGSGGGSPGS	44
H113	SGGGSGGGSGGGSGGGSPAS	45
H114	SGGGSGGGSGGGSGGGSPS	46
H115	SGGGSGGGSGGGSGGGSPSS	47

[0134] In some embodiments, the anti-ceramide antibody or antigen-binding fragments thereof is produced with an N-terminal signal sequence to promote secretion of the scFv protein. In some embodiments, the signal sequence comprises SEQ ID NO: 75. One of skill in the art will understand that these signal sequences are not a part of the mature, secreted protein. Therefore, in some embodiments, the signal sequence of SEQ ID NO: 75 is cleaved from the mature anti-ceramide antibody or antigen-binding fragment protein.

[0135] In some embodiments, the anti-ceramide antibody or antigen-binding fragments thereof are produced with a C-terminal tag sequence to facilitate purification during manufacturing. In some embodiments, the C-terminal tag comprises the amino acid sequence of SEQ ID NO: 76. In some embodiments, the anti-ceramide antibody or antigen-binding fragments thereof are produced without a C-terminal tag sequence. Therefore, in some embodiments, the amino sequences of the anti-ceramide antibody or antigen-binding fragments thereof do not comprise the C-terminal tag of SEQ ID NO: 76.

[0136] Exemplary amino acid sequences for the anti-ceramide scFvs are provided in Table 4 below. The CDRs of the scFvs are indicated by underlined text. Linker sequences are indicated by italicized text. Signal sequences are indicated by bold text. C-terminal tag sequences are indicated by bold and italicized text.

Table 4. Amino Acid Sequences of Exemplary anti-ceramide scFvs

scFv	Amino Acid Sequence	SEQ ID	SEQ ID for VH CDRs	SEQ ID for VL CDRs
VH2-VL1 (with signal sequence)	MEWSWVFLFFLSVTTGVHS <i>QVQLVQSGAEVKKPGASVKV</i> SVCKV <i>SGYTFDHTIHWMRQAPGQGLEWMGYNYP</i> RDGSTKYNEKFQGR <i>VTMTADKSTSTVYME</i> LSLRSEDTAVYYCAKGFITTVVPSAYW <i>GQGT</i> LVTVSSGGGGSGGGSGGGSDIQLTQSPSFLSASV GDR <i>VTITCR</i> ASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSR <i>FSGSGSGTEFTLTIS</i> SLQPEDFATYYCQHNEYPWF GGG TKV <i>EIK</i> RAAAGGGSGGGSHHHHHHHH	48	1, 3, 7	8, 9, 10

scFv	Amino Acid Sequence	SEQ ID	SEQ ID for VH CDRs	SEQ ID for VL CDRs
VH4-VL1 (with signal sequence)	MEWSWVFLFFLSVTTGVHS QVQLVQSGAEVKKPGASVKVSCKVSGYTFDHTIHWMRQAPGQGLEWMGYNYPRDGSTKYNEKFQGRVTMTADKSTSTVYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGSGGGGSHHHHHHH	49	1, 4, 7	8, 9, 10
VH5-VL1 (with signal sequence)	MEWSWVFLFFLSVTTGVHS QVQLVQSGAEVKKPGASVKVSCKVSGYTFDHTIHWMRQAPGQGLEWMGYNYPRDVSTKYNEKFQGRVTMTADKSTSTVYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGSGGGGSHHHHHHH	50	1, 5, 7	8, 9, 10
VH2-VL1 (“scFv #4”)	QVQLVQSGAEVKKPGASVKVSCKVSGYTFDHTIHWMRQAPGQGLEWMGYNYPRDGSTKYNEKFQGRVTMTADKSTSTVYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGSGGGGSHHHHHH	51	1, 3, 7	8, 9, 10
VH4-VL1	QVQLVQSGAEVKKPGASVKVSCKVSGYTFDHTIHWMRQAPGQGLEWMGYNYPRDGSTKYNEKFQGRVTMTADKSTSTVYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGSGGGGSHHHHHH	52	1, 4, 7	8, 9, 10
VH5-VL1	QVQLVQSGAEVKKPGASVKVSCKVSGYTFDHTIHWMRQAPGQGLEWMGYNYPRDVSTKYNEKFQGRVTMTADKSTSTVYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGSGGGGSHHHHHH	53	1, 5, 7	8, 9, 10
VH1-VL1 (“scFv #1”)	QVQLVQSGAEVKKPGASVKVSCKASGYTFDHTMHVWRQAPGQGLEWMGYNYPRDGSTKYAEKFQGRVTMTADKSTSTVYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGSGGGGSHHHHHH	54	2, 6, 7	8, 9, 10
VH1-VL2	QVQLVQSGAEVKKPGASVKVSCKASGYTFDHTMHVWRQAPGQGLEWMGYNYPRDGSTKYAEKFQGRVTMTADKSTSTVYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDVQITQSPSFLSASVGDRVTITCRASKSISKYLAWYQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGSGGGGSHHHHHH	55	2, 6, 7	8, 9, 10
VH1-VL3 (“scFv #3”)	QVQLVQSGAEVKKPGASVKVSCKASGYTFDHTMHVWRQAPGQGLEWMGYNYPRDGSTKYAEKFQGRVTMTADKSTSTVYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDVQITQSPSSVSASVGDRVTITCRASKSISKYLAWYQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGSGGGGSHHHHHH	56	2, 6, 7	8, 9, 10
VH2-VL2 (“scFv #5”)	QVQLVQSGAEVKKPGASVKVSCKVSGYTFDHTIHWMRQAPGQGLEWMGYNYPRDGSTKYNEKFQGRVTMTADKSTSTVYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGSGGGGSHHHHHH	57	1, 3, 7	8, 9, 10

scFv	Amino Acid Sequence	SEQ ID	SEQ ID for VH CDRs	SEQ ID for VL CDRs
	RSEDTAVYYCAKGFITTVVPSAYWGQGLVTVSSGGGGSGGGGSGGGGSDVQITQSPSFLSASVGDRVTITCRASKSISKYLAWYQ QKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGGSGGGGSHHHHHH			
VH2-VL3	QVQLVQSGAEVKKPGASVKVSKVSGYTFTDHTIHWMRQAPGQGLEWMGYNYPRDGSTKYNEKFQGRVTMTADKSTSTVYMESSLRSEDTAVYYCAKGFITTVVPSAYWGQGLVTVSSGGGGSGGGGSGGGGSDVQITQSPSSVSASVGDRVTITCRASKSISKYLAWYQ QKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGPGTKVEIK RAAAGGGGSGGGGSHHHHHH	58	1, 3, 7	8, 9, 10
VH3-VL1 ("scFv #7")	QVQLVQSGAEVKKPGATVKISCKVSGYTFTDHTIHWMQAPGKGLEWMGYNYPRDGSTKYNEKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQ QKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGGSGGGGSHHHHHH	59	1, 3, 7	8, 9, 10
VH3-VL2 ("scFv #8")	QVQLVQSGAEVKKPGATVKISCKVSGYTFTDHTIHWMQAPGKGLEWMGYNYPRDGSTKYNEKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLVTVSSGGGGSGGGGSGGGGSDVQITQSPSFLSASVGDRVTITCRASKSISKYLAWYQ QKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGGGTKVEIK RAAAGGGGSGGGGSHHHHHH	60	1, 3, 7	8, 9, 10
VH3-VL3	QVQLVQSGAEVKKPGATVKISCKVSGYTFTDHTIHWMQAPGKGLEWMGYNYPRDGSTKYNEKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGLVTVSSGGGGSGGGGSGGGGSDVQITQSPSSVSASVGDRVTITCRASKSISKYLAWYQ QKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGPGTKVEIK RAAAGGGGSGGGGSHHHHHH	61	1, 3, 7	8, 9, 10
Mouse 6B5	MKKTAAIAVALAGFATVAQAQVQLQQSDAELVKPGASVKISCKVSGYTFTDHTIHWMKRPEQGLEWIGYNYPRDGSTKYNEKFKGKATLTADKSSSTAYMQNLSTSEDSAVYFCAKGFITTVVPSAYWGQGLVTVSAGGGGSGGGGSGGGGSDVQITQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISLEPEDFAMYYCQQHNEYPWTFGGGTKLEIKGSHHHHHH	62	1, 12, 7	8, 9, 10

[0137] In some embodiments, the anti-ceramide antibody is a full-length antibody comprising a full length antibody light chain and a full length antibody heavy chain. In some embodiments, the anti-ceramide antibody is an IgG isotype (e.g. IgG1, IgG2, IgG3, IgG4). In some embodiments, the Fc domain of the anti-ceramide antibody comprises a wild-type IgG amino acid sequence. Such sequences are known in the art, see *e.g.* Shields *et al.*, J Biol Chem, (2001) 276:9;6591-6604. In some embodiments, the CH2 or CH3 domain of the Fc domain comprises one or more amino acid mutations that alter the function and/or stability of the antibody. For example, in some embodiments, the Fc domain of an anti-ceramide antibody described herein lacks or has minimal effector functions while retaining the ability to bind some Fc receptors

such as the neonatal Fc receptor (FcRn) and retaining a relatively long half-life *in vivo*. In some embodiments, anti-ceramide antibodies described do not result in, or substantially reduce the induction of, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), complement activation, and/or complement-dependent cytotoxicity (CDC). Such mutations are well known in the art, see for example Shields *et al.*, J Biol Chem, (2001) 276:9;6591-6604; Arduin *et al.*, Mol Immunol (2015) 63:2;456-463; Vafa *et al.*, Methods (2014) 65:1;114-126.

[0138] In some embodiments, the anti-ceramide antibodies described are IgG1 isotype antibodies, wherein the IgG1 constant region has a mutation at one or more of the following positions: 228 (S228), 234 (L234), 235 (L235), 237 (G237), 297 (N297), 318 (E318), 320 (K320), 322 (K322), or any combination thereof (numbering according to EU). In some embodiments, the IgG1 Fc domain has an L234A and L235A mutation. In some embodiments, the IgG1 Fc domain has an S228P mutation.

[0139] As used herein, unless otherwise provided, a position of an amino acid residue in an immunoglobulin molecule is numbered according to EU nomenclature (Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94). Other numbering systems for amino acid positions within antibodies are known in the art. For example, the IMGT system (Brochet *et al.*, Nucl. Acids Res. (2008) 36, W503-508) and the Kabat numbering system (Kabat, Sequences of Proteins of Immunological Interest, 5th edition, Bethesda, MD: Public Health Service, National Institutes of Health (1991)). Methods and information to convert between one numbering system and the other are known in the art. See, for example, the IMGT Scientific Chart – Correspondence between C numberings, available at imgt.org.

[0140] In certain embodiments, the anti-ceramide antibodies and antigen-binding fragments thereof may be prepared using standard molecular biology techniques with regard to selecting antibodies that have a desired specificity. In some embodiments, the anti-ceramide antibodies and antigen-binding fragments thereof are produced using recombinant DNA technologies. Procedures for the expression and purification of recombinant proteins are well established in the art.

Polynucleotides and Methods of Protein Expression

[0141] The disclosure also includes polynucleotides (*e.g.*, DNA or RNA) encoding the anti-ceramide antibodies and antigen-binding fragments thereof of the present disclosure. In some embodiments, the polynucleotides encode a polypeptide that is substantially identical to a

polypeptide listed in Tables 1, 2, 3 and 4. In some embodiments, the polynucleotides encode a polypeptide that is at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at about least 99%, or at about least 100% identical to a polypeptide listed in Tables 1, 2, 3 and 4. Polynucleotides of the disclosure also include complementary nucleic acids. In some instances, the sequences will be fully complementary (no mismatches) when aligned. In other instances, there can be up to about a 20% mismatch in the sequences. The polynucleotide sequences provided herein can be exploited using codon optimization, degenerate sequence, silent mutations, and other DNA techniques to optimize expression in a particular host, and the present disclosure encompasses such sequence modifications.

[0142] In some embodiments, the disclosure provides an isolated polynucleotide encoding an anti-ceramide antibody or antigen-binding fragment thereof, wherein said polynucleotide is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or is 100% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 65-73. In some embodiments, the disclosure provides an isolated polynucleotide encoding an anti-ceramide antibody or antigen-binding fragment thereof, wherein said polynucleotide comprises or consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 65-73.

[0143] In some embodiments, the polynucleotides of the present disclosure are inserted into a nucleic acid vector. The nucleic acid vector may be a viral vector or a non-viral vector, e.g. a plasmid. Vectors include, without limitation, plasmids, phagemids, cosmids, transposons, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. In some embodiments, the vector is a plasmid selected from pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, and pSVLSV40 (Pharmacia). In some embodiments, the vector is a viral vector selected from viral vectors based on vaccinia virus; poliovirus; adenovirus (see, *e.g.*, Li *et al.*, Invest Ophthalmol Vis Sci 35:2543 2549, 1994; Borras *et al.*, Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto *et al.*, H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191 ; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, *e.g.*, U.S. Patent No. 7,078,387; Ali *et al.*, Hum Gene Ther 9:81 86, 1998, Flannery *et al.*, PNAS 94:6916 6921 , 1997; Bennett *et al.*, Invest Ophthalmol Vis Sci 38:2857 2863, 1997; Jomary *et al.*, Gene Ther 4:683 690, 1997, Rolling *et al.*, Hum Gene Ther 10:641 648, 1999; Ali *et al.*, Hum Mol

Genet 5:591-594, 1996; Srivastava in WO 93/09239, Samulski *et al.*, J. Vir. (1989) 63:3822-3828; Mendelson *et al.*, Virol. (1988) 166:154-165; and Flotte *et al.*, PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, *e.g.*, Miyoshi *et al.*, PNAS 94:10319-23, 1997; Takahashi *et al.*, J Virol 73:7812-7816, 1999); a retroviral vector (*e.g.*, Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like. Examples of vectors are pCIneo vectors (Promega) for expression in mammalian cells; pLenti4/V5-DEST™, pLenti6/V5-DEST™, and pLenti6.2/V5-GW/lacZ (Invitrogen) for lentivirus-mediated gene transfer and expression in mammalian cells.

[0144] In some embodiments, the polynucleotide is inserted into a nucleic acid vector and is operably linked to one or more regulatory sequences that control transcription, such as promoters, enhancers, terminators, inducers, or repressors. Exemplary promoters include Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include those from cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, a viral simian virus 40 (SV40) (*e.g.*, early and late SV40), a spleen focus forming virus (SFFV) promoter, long terminal repeats (LTRs) from retrovirus (*e.g.*, a Moloney murine leukemia virus (MoMLV) LTR promoter or a Rous sarcoma virus (RSV) LTR), a herpes simplex virus (HSV) (thymidine kinase) promoter, H5, P7.5, and P11 promoters from vaccinia virus, an elongation factor 1- α (EF1 α) promoter, early growth response 1 (EGR1) promoter, a ferritin H (FerH) promoter, a ferritin L (FerL) promoter, a Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promoter, a eukaryotic translation initiation factor 4A1 (EIF4A1) promoter, a heat shock 70kDa protein 5 (HSPA5) promoter, a heat shock protein 90kDa beta, member 1 (HSP90B1) promoter, a heat shock protein 70kDa (HSP70) promoter, a β -kinesin (β -KIN) promoter, the human ROSA 26 locus (Irions *et al.*, *Nature Biotechnology* 25, 1477-1482 (2007)), a Ubiquitin C (UBC) promoter, a phosphoglycerate kinase-1 (PGK) promoter, a cytomegalovirus enhancer/chicken β -actin (CAG) promoter, a β -actin promoter and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) promoter, and mouse metallothionein-I.

[0145] In some embodiments, the vector is introduced into a host cell for expression of the anti-ceramide antibody or antigen-binding fragment thereof. Accordingly, proteins for use within the present disclosure can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or

transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells (including cultured cells of multicellular organisms), particularly cultured mammalian cells. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001), and Ausubel *et al.*, *Short Protocols in Molecular Biology* (4th ed., John Wiley & Sons, 1999).

[0146] The gene product encoded by a polynucleotide of the disclosure is expressed in any convenient expression system, including, for example, bacterial, yeast, insect, amphibian, and mammalian systems. Examples of suitable mammalian host cells include African green monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44; CHO DXB11 (Hyclone, Logan, UT); *see also, e.g.*, Chasin *et al.*, *Som. Cell. Molec. Genet.* 12:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658). Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Manassas, Virginia. Introduction of the DNA construct can use any convenient method, including, e.g. conjugation, bacterial transformation, calcium-precipitated DNA, electroporation, fusion, transfection, infection with viral vectors, biolistics, and the like.

[0147] For example, for recombinant expression of an anti-ceramide antibody or antigen-binding fragment thereof as described herein, an expression vector will generally include a nucleic acid segment encoding one or more of the amino acid sequences provided in Tables 1, 2, 3 and 4, operably linked to a promoter. The expression vector is introduced to a host cell by conventional techniques, and the host cells are then cultured by conventional techniques to produce the encoded polypeptide(s) to produce the corresponding anti-ceramide antibodies or antigen-binding fragments thereof.

[0148] To direct a recombinant protein into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence) is provided in the expression vector. The secretory signal sequence can be that of the native form of the recombinant protein or can be derived from another secreted protein or synthesized *de novo*. The secretory signal sequence is

operably linked to the polypeptide-encoding DNA sequence, *i.e.*, the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences can be positioned elsewhere in the DNA sequence of interest (*see, e.g.*, U.S. Patent Nos. 5,037,743 and 5,143,830). In some embodiments, the secretory signal sequence is SEQ ID NO: 75.

[0149] Cultured mammalian cells are suitable hosts for production of recombinant polypeptides and proteins of the present disclosure (*e.g.*, anti-ceramide antibodies and antigen-binding fragments thereof). Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler *et al.*, Cell 14:725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603, 1981; Graham and Van der Eb, Virology 52:456, 1973), electroporation (Neumann *et al.*, EMBO J. 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel *et al.*, *supra*), and liposome-mediated transfection (Hawley-Nelson *et al.*, Focus 15:73, 1993; Ciccarone *et al.*, Focus 15:80, 1993). The production of recombinant polypeptides in cultured mammalian cells is disclosed by, for example, U.S. Patent Nos. 4,713,339; 4,784,950; 4,579,821; and 4,656,134.

[0150] Transformed or transfected host cells to produce the polypeptides and proteins of the present disclosure (*e.g.*, ceramide-binding polypeptides) are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins, and minerals. Media can also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

[0151] The anti-ceramide antibodies and antigen-binding fragments thereof of the present disclosure may be purified by conventional protein purification methods, typically by a combination of chromatographic techniques. *See generally Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988); Scopes, *Protein Purification: Principles and Practice* (Springer-Verlag, New York 1994). Proteins comprising an immunoglobulin Fc region can be purified by affinity chromatography on immobilized protein A or protein G. Additional purification steps, such as gel filtration, can be

used to obtain the desired level of purity or to provide for desalting, buffer exchange, and the like.

Methods of Humanized Antibody Generation

[0152] The present disclosure discloses humanized anti-ceramide antibodies and antigen-binding fragments thereof. Humanized antibodies have the same or similar binding specificity and affinity as a mouse or other nonhuman antibody that provides the starting material for construction of a humanized antibody. A preferred antibody for humanization is the murine 6B5 antibody described in US 2017-03334133, incorporated herein by reference in its entirety. The VH and VL sequences of the murine 6B5 antibody are provided below in Table 5. The CDRs are indicated by underlined text. Linker sequences are indicated by italicized text. Signal sequences are indicated by bold text. C-terminal tag sequences are indicated by bold and italicized text.

Table 5. Murine 6B5 VH and VL sequences from anti-ceramide antibodies

6B5 Fragment	Amino Acid Seq	SEQ ID:
6B5 VH	QVQLQQSDAELVKPGASVKISCKVSGYTFDHTIHWMKQRPEQGLEWIGYNYPRD GSTKYNEKFKGKATLTADKSSSTAYMQLNSLTSEDSAVYFCAKGFITTVVPSAYW GQGTLVTVSA	63
6B5 VL	DVQITQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQ SGIPSRFSGSGSGTDFTLTISSELPEDFAMYQCQHNEYPWTFGGGTKLEIK	64
6B5 scFv	MKKTAIAIAVALAGFATVAQA QVQLQQSDAELVKPGASVKISCKVSGYTFDHTI HWMKQRPEQGLEWIGYNYPRDGSTKYNEKFKGKATLTADKSSSTAYMQLNSLTSE DSAVYFCAKGFITTVVPSAYWGQGLVTVSAGGGGSGGGGSGGGGSDVQITQSPS YLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSG SGSGTDFTLTISSELPEDFAMYQCQHNEYPWTFGGGTKLEIK GSHHHHHH	62

[0153] The substitution of mouse CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework adopts the same or similar conformation to the murine variable framework from which the CDRs originated. This is achieved by obtaining the human variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine variable framework domains from which the CDRs were derived. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. The human variable framework region sequences can be the sequences of naturally occurring human antibodies or can be consensus sequences of several human antibodies. See Kettleborough et al., *Protein Engineering* 4:773 (1991); Kolbinger et al., *Protein Engineering* 6:971 (1993) and Carter et al., WO 92/22653.

[0154] Having identified the CDRs of the murine donor immunoglobulin and appropriate human acceptor framework region, the next step is to determine which, if any, residues from these components should be modified to optimize the properties of the resulting humanized antibody. In general, substitution of human amino acid residues with murine residues should be minimized, because introduction of murine residues increases the risk of the antibody eliciting a human-anti-mouse-antibody (HAMA) response in humans. Art-recognized methods of determining immune response can be performed to monitor a HAMA response in a particular patient or during clinical trials.

[0155] In some embodiments, the selection of amino acid residues for modification is determined, in part, by computer modeling. Computer hardware and software are described herein for producing three-dimensional images of immunoglobulin molecules. In general, molecular models are produced starting from solved structures for immunoglobulin chains or domains thereof. The chains to be modeled are compared for amino acid sequence similarity with chains or domains of solved three-dimensional structures, and the chains or domains showing the greatest sequence similarity is/are selected as starting points for construction of the molecular model. Chains or domains sharing at least 50% sequence identity are selected for modeling, and preferably those sharing at least 60%, 70%, 80%, 90%, or more sequence identity are selected for modeling. The solved starting structures are modified to allow for differences between the actual amino acids in the immunoglobulin chains or domains being modeled, and those in the starting structure. The modified structures are then assembled into a composite immunoglobulin. Finally, the model is refined by energy minimization and by verifying that all atoms are within appropriate distances from one another and that bond lengths and angles are within chemically acceptable limits.

[0156] The selection of amino acid residues for modification can also be determined, in part, by examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutagenesis of particular amino acids.

[0157] Additional exemplary humanization techniques that can be used for humanizing the immunoglobulins of the disclosure are described in, for example, Presta et al., *J. Immunol.*, 151: 2623-2632 (1993); Carter et al., *Proc. Natl. Acad. Sci. USA.*, 89: 4285-4289 (1992); Couto et al., *Cancer Res.*, 55: 5973s-77s (1995); O'Conner et al., *Protein Eng.*, 11: 321-328 (1998); and *Antibody Engineering-Methods and Protocols* by Lo, Vol. 248 (2004).

[0158] Usually the CDR regions in humanized antibodies are identical to the corresponding CDR regions of the murine donor antibody. For example, in some embodiments, the CDR amino acid sequences of the humanized antibodies and fragments thereof are identical to the CDR amino acid sequences of the murine 6B5 antibody (*e.g.*, SEQ ID NOs: 11-16).

[0159] In some embodiments, it may be desirable to modify one or more CDR regions to modify the antigen binding specificity of the antibody and/or reduce the immunogenicity of the antibody. In some embodiments, one or more residues of a CDR are altered to modify binding to achieve a more favored on-rate of binding, a more favored off-rate of binding, or both, such that an idealized binding constant is achieved. Using this strategy, an antibody having high binding affinity of, for example, 10^{10} M^{-1} or more, can be achieved. Affinity maturation techniques, as described herein, can be used to alter the parent CDR region(s) followed by screening of the resultant binding molecules for the desired change in binding. The method may also be used to alter the parent CDR to be less immunogenic such that a potential human anti-mouse antibody (HAMA) response is minimized or avoided. Accordingly, as CDR(s) are altered, changes in binding affinity as well as immunogenicity can be monitored and scored such that an antibody optimized for the best combined binding and low immunogenicity are achieved (see, *e.g.*, U.S. Pat. No. 6,656,467 and U.S. Pat. Pub. US20020164326A1). Moreover, it is sometimes possible to make one or more conservative amino acid substitutions of CDR residues without appreciably affecting the binding affinity of the resulting humanized immunoglobulin. Conservative substitutions are intended combinations such as gly, ala; val, ile, leu; asp, glu; asn, gin; ser, thr; lys, arg; and phe, tyr.

[0160] In some embodiments, the humanized anti-ceramide antibodies and antigen-binding fragments thereof comprise one or more mutations relative to the corresponding CDR sequence of the murine 6B5 antibody (*e.g.*, relative to one or more of SEQ ID NOs: 11-16). Exemplary mutations to the CDR amino acid sequences of the murine 6B5 CDRs are shown in bold text in FIG. 4A.

[0161] The framework regions of humanized immunoglobulins are usually substantially identical, and more usually, identical to the framework regions of the human antibodies from which they were derived. In some embodiments, humanized antibodies of the disclosure include a variable region framework sequence selected from human antibody genes (*e.g.*, germline antibody gene segments) which include one or more canonical CDR structure types that are identical or similar to the canonical CDR structure types for the corresponding non-human antibody (*e.g.*, murine) which is humanized. See, U.S. Pat. No. 6,881,557 and Tan et

al., *Journal of Immunol* 169:1119-1125 (2002) (incorporated by reference in their entirety for all purposes).

[0162] Of course, many of the amino acids in the framework region make little or no direct contribution to the specificity or affinity of an antibody. Thus, many individual conservative substitutions of framework residues can be tolerated without appreciable change of the specificity or affinity of the resulting humanized immunoglobulin. Framework residues can be analyzed to determine which, if any, residues should be substituted to optimize the properties of the resulting humanized antibody. For example, computer modeling can be used to identify residues which have a good probability of directly or indirectly influencing antigen binding.

[0163] Thus, in one embodiment the variable framework region of the humanized anti-ceramide antibody or antigen-binding fragment thereof shares at least 85% sequence identity to a human variable framework region sequence or consensus of such sequences. In some embodiments, the variable framework region of the humanized anti-ceramide antibody or antigen-binding fragment thereof shares at least 90%, preferably 95%, more preferably 96%, 97%, 98%, or 99% sequence identity to a human variable framework region sequence or consensus of such sequences.

[0164] In some embodiments, the humanized anti-ceramide antibody or antigen-binding fragment thereof exhibits a specific binding affinity for antigen of at least 10^7 M^{-1} , 10^8 M^{-1} , 10^9 M^{-1} , or 10^{10} M^{-1} . Usually the upper limit of binding affinity of the humanized antibodies for antigen is within a factor of three, four, or five of that of the parental immunoglobulin. Often the lower limit of binding affinity is also within a factor of three, four, or five of that of the parent immunoglobulin. Alternatively, the binding affinity can be compared to that of a humanized antibody having no substitutions (e.g., an antibody having donor CDRs and acceptor FRs, but no FR substitutions). In such instances, the binding of the optimized antibody (with substitutions) is preferably at least two- to three-fold greater, or three- to four-fold greater, than that of the unsubstituted antibody. For making comparisons, activity of the various antibodies can be determined, for example, by BIACORE (i.e., surface plasmon resonance using unlabelled reagents) or competitive binding assays.

Methods of treatment

[0165] The present application discloses a method of inhibiting cell death in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-ceramide antibody or antigen-binding fragment thereof of the present disclosure. In some

embodiments, the antibody or antigen-binding fragment thereof is a humanized scFv that specifically binds to ceramide.

[0166] In some embodiments, the cell death is associated with a disease selected from the group consisting of graft versus host disease, radiation disease, GI syndrome, and autoimmune disease. In some further embodiments, the disease is radiation disease or GI syndrome and the anti-ceramide antibody or antigen-binding fragment thereof is administered before the subject is exposed to radiation.

[0167] Radiation targets both the gastrointestinal microvasculature and intestinal stem cell compartments. Dysfunction of the microvascular endothelium, detected as apoptosis at four hours following radiation, represents a principle lesion leading to the GI syndrome. Endothelial dysfunction converts lesions to cycling crypt base columnar (CBC) cells from sublethal to lethal, resulting in loss of regenerative crypts and promoting GI toxicity. Immunohistochemical and labeling studies with [3H]TdR and BrdUrd revealed that crypt stem cell death does not occur acutely after radiation exposure. Rather, the earliest detectable response is a temporary dose-dependent delay in progression through a late S-phase checkpoint and mitotic arrest, apparently signaled by radiation-induced DNA double strand breaks (DSBs). A rapid apoptotic death occurs in growth arrested cells during the first 24 hours post irradiation that, at 12 Gy, equals 33% of the total death. In mammalian cells, DNA dsbs activate pathways of DNA damage recognition and repair, and a coordinated regulation of cell cycle checkpoint activity. The intestinal stem cell mitotic arrest appears to represent a regulated event in this pathway. A mitotic form of death occurs during this second 24 hours, representing 66% of overall death. No significant change in crypt number per intestinal circumference is apparent at this stage although crypt size progressively decreases due to continued normal migration of crypt transit and differentiated cells from the crypt into the epithelial lining of the villus and loss from the villus tip. Resumption of mitotic activity at 12-18 hours is associated with a rapid depletion of crypt stem cell clonogens and reduction in crypt number per circumference. Stem cell clonogen loss leads to a near total collapse of the crypt-villus system, mucosal denudation, and death from the GI syndrome.

[0168] Ceramide mediated raft clustering is involved in radiation-induced apoptosis and clonogenic cell death. It has long been accepted that the clonogenic compartment of the gastrointestinal (GI) mucosa is the specific and direct target for radiation in inducing GI damage.

[0169] The present disclosure is directed to a method for the mitigation of cell death in GI syndrome in a subject in need thereof. The method comprises the administration of an effective amount of an anti-ceramide antibody or antigen-binding fragment thereof. In some embodiments, the method comprises administering said anti-ceramide antibody or antigen-binding fragment thereof to said subject immediately after exposure of said subject to penetrating radiation. In other embodiments, the method comprises administering said anti-ceramide antibody or antigen-binding fragment thereof to said subject within one hour after exposure of said subject to penetrating radiation. In some embodiments, the method comprises administering said anti-ceramide antibody or antigen-binding fragment thereof to said subject within 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 18 hours after exposure of said subject to penetrating radiation. In some embodiments, the method comprises administering said anti-ceramide antibody or antigen-binding fragment thereof to said subject within 24 hours after exposure of said subject to penetrating radiation. In other embodiments, the method comprises administering said anti-ceramide antibody or antigen-binding fragment thereof to said subject within 30, 36, 42, 48, 54, 60, 66 or 72 hours after exposure of said subject to penetrating radiation. In other embodiments, the method comprises administering said anti-ceramide antibody or antigen-binding fragment thereof to said subject within 48, 36, 24, 18, 12, 10, 8, 6, 4, 2 or 1 hour(s), or within 45, 30 or 15 minutes before exposure of said subject to penetrating radiation.

[0170] In some embodiments, the disease is graft versus host disease and the anti-ceramide antibody or antigen-binding fragment thereof is administered before the subject receives a transplant. In some embodiments, the transplant is a bone marrow transplant. In other embodiments, the anti-ceramide antibody or antigen-binding fragment thereof is administered after the subject receives a transplant, but before the onset of graft versus host disease. In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof is administered to a subject in need thereof after the onset of graft versus host disease in an amount effective for the mitigation of apoptosis in graft versus host disease.

[0171] The disclosure also encompasses anti-ceramide antibodies and antigen-binding fragments thereof for the manufacture of a medicament for treatment of a disorder (*e.g.*, GI syndrome or GvHD) characterized by expression of ceramide. In some embodiments, the protein or polypeptide comprises an anti-ceramide binding domain and inhibits formation of ceramide rich platforms. In some embodiments, the disclosure relates to a method for treating a disorder in a subject, wherein said disorder is characterized by expression of ceramide rich

platforms, the method comprising administering to the subject a therapeutically effective amount of a protein or polypeptide of the present disclosure comprising a ceramide binding domain that specifically binds an epitope of human ceramide (*e.g.*, ceramide-binding polypeptides).

[0172] In some embodiments, the disclosure provides a method of treating a patient with GI syndrome or GvHD, comprising administering to the patient an anti-ceramide antibody or antigen-binding fragment thereof comprising the amino acid sequence set forth herein (*e.g.*, an amino acid sequence selected from the group consisting of SEQ ID NOs: 48-61). In some embodiments, the disclosure provides a method of treating a patient with GI syndrome or GvHD comprising administering the anti-ceramide antibody or antigen-binding fragment thereof of SEQ ID NO: 48 or 51.

[0173] In some embodiments, the anti-ceramide antibodies and antigen-binding fragments thereof prevent radiation-induced cell death or cell death associated with GvHD or autoimmune disease. Cell death can be measured by a variety of means known in the art including flow cytometry, immunofluorescence, and immunohistochemistry to assess changes in marker expression in dying cells, cell counts to assess viability, qPCR to assess changes in gene expression, and the like.

[0174] In some embodiments, the anti-ceramide antibodies and antigen-binding fragments thereof prevent cell death by inhibiting ceramide-rich platform formation. For example, in some embodiments, the anti-ceramide scFvs inhibit ceramide-rich platform formation in irradiated Jurkat T cells (*See*, Example 3 and FIG. 1). In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof reduces ceramide-rich platform formation by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% or about 100%.

[0175] In some embodiments, the anti-ceramide antibodies and antigen-binding fragments thereof inhibit cell death. For example, in some embodiments, the anti-ceramide scFvs inhibit cell death in irradiated Jurkat T cells (*See*, Example 4 and FIGs. 2A-2D). In some embodiments, the anti-ceramide antibody or antigen-binding fragment thereof reduces cell death or apoptosis by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% or about 100%.

[0176] In some embodiments, for treatment methods and uses described herein, the anti-ceramide antibody or antigen-binding fragment thereof is delivered in a manner consistent with

conventional methodologies associated with management of the disease or disorder for which treatment is sought. In accordance with the disclosure herein, a therapeutically effective amount of the protein or polypeptide is administered to a subject in need of such treatment for a time and under conditions sufficient to prevent or treat the disease or disorder.

[0177] In prophylactic applications, pharmaceutical compositions or medicants comprising the anti-ceramide antibody or antigen-binding fragment thereof are administered to a patient susceptible to, or otherwise at risk of, a particular disorder in an amount sufficient to eliminate or reduce the risk or delay the onset of the disorder. In therapeutic applications, compositions or medicants comprising a protein of the present disclosure are administered to a patient suspected of, or already suffering from such a disorder in an amount sufficient to cure, or at least partially arrest, the symptoms of the disorder and its complications. An amount adequate to accomplish this is referred to as a therapeutically effective dose or amount. In both prophylactic and therapeutic regimes, agents are usually administered in several dosages until a sufficient response (*e.g.*, inhibition of inappropriate angiogenesis activity) has been achieved. Typically, the response is monitored and repeated dosages are given if the desired response starts to fade.

[0178] For administration, the anti-ceramide antibody or antigen-binding fragment thereof may be formulated as a pharmaceutical composition. A pharmaceutical composition may comprise: (i) a ceramide-binding polypeptide; and (ii) a pharmaceutically acceptable carrier, diluent or excipient. A pharmaceutical composition comprising a ceramide-binding polypeptide can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic molecule is combined in a mixture with a pharmaceutically acceptable carrier, diluent, or excipient. A carrier is said to be a “pharmaceutically acceptable carrier” if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers, diluents, or excipients are well-known to those in the art. (*See, e.g.*, Gennaro (ed.), *Remington's Pharmaceutical Sciences* (Mack Publishing Company, 19th ed. 1995).) Formulations can further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, *etc.*

[0179] A pharmaceutical composition comprising a polypeptide or protein described herein may be formulated in a dosage form selected from the group consisting of: an oral unit dosage form, an intravenous unit dosage form, an intranasal unit dosage form, a suppository unit dosage form, an intradermal unit dosage form, an intramuscular unit dosage form, an

intraperitoneal unit dosage form, a subcutaneous unit dosage form, an epidural unit dosage form, a sublingual unit dosage form, and an intracerebral unit dosage form. The oral unit dosage form may be selected from the group consisting of: tablets, pills, pellets, capsules, powders, lozenges, granules, solutions, suspensions, emulsions, syrups, elixirs, sustained-release formulations, aerosols, and sprays.

[0180] A pharmaceutical composition comprising the anti-ceramide antibody or antigen-binding fragment thereof may be administered to a subject in a therapeutically effective amount. According to the methods of the present disclosure, the anti-ceramide antibody or antigen-binding fragment thereof can be administered to subjects by a variety of administration modes, including, for example, by intramuscular, subcutaneous, intravenous, intra-atrial, intra-articular, parenteral, intranasal, intrapulmonary, transdermal, intrapleural, intrathecal, and oral routes of administration. For prevention and treatment purposes, an agonist (*e.g.*, ceramide-binding polypeptides) can be administered to a subject in a single bolus delivery, via continuous delivery (*e.g.*, continuous transdermal delivery) over an extended time period, or in a repeated administration protocol (*e.g.*, on an hourly, daily, weekly, or monthly basis).

[0181] Effective doses of the compositions of the present disclosure vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, whether treatment is prophylactic or therapeutic, as well as the specific activity of the composition itself and its ability to elicit the desired response in the individual. Usually, the patient is a human, but in some diseases, the patient can be a nonhuman mammal. Typically, dosage regimens are adjusted to provide an optimum therapeutic response, *i.e.*, to optimize safety and efficacy.

[0182] Determination of effective dosages in this context is typically based on animal model studies followed up by human clinical trials and is guided by determining effective dosages and administration protocols that significantly reduce the occurrence or severity of the subject disorder in model subjects. Accordingly, a “therapeutically effective amount,” as used herein, refers to an amount of a compound is an amount that achieves the desired biologic or therapeutic effect, namely an amount that prevents, reduces or ameliorates one or more symptoms of the enumerated diseases being treated or prevented. For example, the therapeutically effective amount of the antibody, or antigen-binding fragment thereof, will depend on the condition to be treated, the severity and course of the condition, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient’s clinical history and response to the antibody, the type of antibody, or antigen-binding fragment

thereof, used, and the discretion of the attending physician. The anti-ceramide antibody or antigen-binding fragment thereof is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards. The anti-ceramide antibody or antigen-binding fragment thereof may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question.

[0183] In some embodiments, the therapeutically effective amount of the anti-ceramide antibody or antigen-binding fragment thereof is between about 1 ng/kg body weight/day to about 100 mg/kg body weight/day. In some embodiments, the range of antibody administered is from about 1 ng/kg body weight/day to about 1 µg/kg body weight/day, 1 ng/kg body weight/day to about 100 ng/kg body weight/day, 1 ng/kg body weight/day to about 10 ng/kg body weight/day, 10 ng/kg body weight/day to about 1 µg/kg body weight/day, 10 ng/kg body weight/day to about 100 ng/kg body weight/day, 100 ng/kg body weight/day to about 1 µg/kg body weight/day, 100 ng/kg body weight/day to about 10 pg/kg body weight/day, 1 µg/kg body weight/day to about 10 pg/kg body weight/day, 1 µg/kg body weight/day to about 100 pg/kg body weight/day, 10 pg/kg body weight/day to about 100 pg/kg body weight/day, 10 pg/kg body weight/day to about 1 mg/kg body weight/day, 100 µg/kg body weight/day to about 10 mg/kg body weight/day, 1 mg/kg body weight/day to about 100 mg/kg body weight/day and 10 mg/kg body weight/day to about 100 mg/kg body weight/day. Dosages within this range can be achieved by single or multiple administrations, including, *e.g.*, multiple administrations per day or daily, weekly, bi-weekly, or monthly administrations. The anti-ceramide antibody or antigen-binding fragment thereof may be administered, as appropriate or indicated, as a single dose by bolus or by continuous infusion, or as multiple doses by bolus or by continuous infusion. Multiple doses may be administered, for example, multiple times per day, once daily, every 2, 3, 4, 5, 6 or 7 days, weekly, every 2, 3, 4, 5 or 6 weeks or monthly. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques.

[0184] For administration to a human adult patient, the therapeutically effective amount may be administered in doses in the range of 0.0006 mg to 1000 mg per dose, including but not limited to 0.0006 mg per dose, 0.001 mg per dose, 0.003 mg per dose, 0.006 mg per dose, 0.01 mg per dose, 0.03 mg per dose, 0.06 mg per dose, 0.1 mg per dose, 0.3 mg per dose, 0.6 mg per dose, 1 mg per dose, 3 mg per dose, 6 mg per dose, 10 mg per dose, 30 mg per dose, 60 mg per dose, 100 mg per dose, 300 mg per dose, 600 mg per dose and 1000 mg per dose, and

multiple, usually consecutive daily doses may be administered in a course of treatment. The anti-ceramide antibody or antigen-fragment thereof can be administered at different times of the day. In one embodiment the optimal therapeutic dose can be administered in the evening. In another embodiment the optimal therapeutic dose can be administered in the morning. As expected, the dosage will be dependent on the condition, size, age, and condition of the patient.

[0185] Dosage of the pharmaceutical composition comprising the anti-ceramide antibody or antigen-binding fragment thereof can be varied by the attending clinician to maintain a desired concentration at a target site. For example, if an intravenous mode of delivery is selected, local concentration of the agent in the bloodstream at the target tissue can be between about 0.01-50 nM, sometimes between about 1.0 nM and 10, 15, or 25 nM depending on the subject's status and projected measured response. Higher or lower concentrations can be selected based on the mode of delivery, *e.g.*, trans-epidermal delivery versus delivery to a mucosal surface. Dosage should also be adjusted based on the release rate of the administered formulation, *e.g.*, nasal spray versus powder, sustained release oral or injected particles, transdermal formulations, *etc.* To achieve the same serum concentration level, for example, slow-release particles with a release rate of 5 nM (under standard conditions) would be administered at about twice the dosage of particles with a release rate of 10 nM.

[0186] Pharmaceutical compositions comprising the anti-ceramide antibody or antigen-binding fragment thereof can be supplied as a kit comprising a container that comprises the pharmaceutical composition as described herein. A pharmaceutical composition can be provided, for example, in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a pharmaceutical composition. Such a kit can further comprise written information on indications and usage of the pharmaceutical composition.

EXAMPLES

[0187] The present disclosure is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and Tables, are incorporated herein by reference.

Example 1: 6B5 antibody humanization and production

[0188] The goal of the current study was to maximize the amount of human sequence in the humanized ceramide antibodies while retaining the original specificity of the 6B5 mouse antibody.

[0189] Antibody humanization design: Humanization design of the parental 6B5 mouse antibody) was performed using *in silico* analysis. Briefly, a 3D structure of the parental 6B5 antibody was generated using homology modeling and a profile of the parental antibody was created based on the 3D model. Two human heavy chain (HC) and two human light chain (LC) acceptor frameworks were selected based on overall sequence identity across the framework, matching interface position, similarly classed CDR canonical positions, and the presence of N-glycosylation sites. Humanized antibodies were then designed by creating multiple hybrid sequences that fused select parts of the parental antibody sequence with the human framework sequences. Using the 3D structure of the parental 6B5 antibody, these humanized sequences were methodically analyzed by eye and computer modeling to isolate the sequences that would most likely retain antigen binding. Three humanized HCs were designed based on the two different human HC acceptor frameworks and three humanized LCs were designed based on the two different human LC acceptor frameworks. The framework combinations are shown below in Table 6.

Table 6. Acceptor frameworks for humanized 6B5 antibodies

Chain Name	Human Acceptor Framework
VH1 + VL1	HC framework 1/LC framework 1
VH1 + VL2	HC framework 1/LC framework 1
VH1 + VL3	HC framework 1/LC framework 2
VH2 + VL1	HC framework 1/LC framework 1
VH2 + VL2	HC framework 1/LC framework 1
VH2 + VL3	HC framework 1/LC framework 2
VH3 + VL1	HC framework 2/LC framework 1
VH3 + VL2	HC framework 2/LC framework 1
VH3 + VL3	HC framework 2/LC framework 2

[0190] Humanized VH1 and VL1 utilize the first respective framework and contain the most human sequence with minimal parental antibody framework sequence. VH2 and VL2 use the same framework as VH1 and VL1 but contain additional parental sequences. VH3 and VL3 utilize the second respective framework and similar to humanized VH2 and VL2, also contain additional parental sequences fused with the human framework.

[0191] The humanized heavy and light chains described above were combined to create fully humanized antibodies. The combinations of humanized heavy and light chains were tested for their expression level and antigen binding affinity to identify antibodies that perform similarly to the parental 6B5 mouse antibody.

[0192] Calculation of humanness scores of engineered antibodies: A tool was developed to calculate the humanness scores of engineered humanized antibodies (Gao *et. al.*, BMC Biotechnology 2013, 13:55). The T20 score represents the degree of antibody humanness by analyzing the primary sequences of the variable regions. The T20 scores for the murine 6B5 and humanized 6B5 heavy and light chains are shown in Table 7 and 8 below.

Table 7. Humanness scores of Humanized 6B5 VH Chains

VH	Framework only Humanness	Framework + CDR Humanness
Murine 6B5 VH	69	62
Humanized VH1	90	82
Humanized VH2	87	78
Humanized VH3	85	75

Table 8. Humanness scores of Humanized 6B5 VL Chains

VL	Framework only Humanness	Framework + CDR Humanness
Murine 6B5 VL	79	76
Humanized VL1	96	89
Humanized VL2	93	86
Humanized VL3	95	87

[0193] For full-length HCs, a score of 79 or above is indicative of a human-like heavy chain and for full-length kappa LCs, a score of 86 or above is indicative of a human-like light chain. As shown in Tables 7 and 8, the humanness scores of full-length VH1, VL1, VL2, and VL3 signified that these chains were human-like.

[0194] The T20 scores for the full-length antibodies are significantly influenced by the low humanness of the CDR regions from the mouse 6B5 antibody, which are generally kept untouched during humanization. Thus, the T20 scores for the framework only region, i.e., without the murine CDR sequences, was also calculated for the humanized antibodies. For HC frameworks, a score of 84 or above is indicative of human-like heavy chain and for kappa LC frameworks, a score of 90 or above is indicative of human-like light chain. As shown in Tables 7 and 8, the humanness scores of the 6B5 heavy and light chains (framework only) signified that the VH and VL regions were human-like.

[0195] Construction and small-scale production of humanized scFvs: The humanized VH and VL chains described above were reformatted into scFvs with octa-His tags at the C-terminus (SEQ ID NO: 76). The murine parental 6B5 scFv was expressed in parallel for comparison. For antibody production, plasmids for the indicated scFvs were transformed into BL21(DE3) *E. coli*, and scFvs were then purified from lysed cells using His-tag affinity purification columns.

Example 2: Production and analysis of a humanized anti-ceramide scFv

[0196] Expression of antibody constructs: The nucleotide sequences of the anti-ceramide VH2 and VL1 were cloned into a high expression mammalian vector (Lake Pharma) and nucleic acid sequences of the cloned constructs were verified. Each construct was then scaled up to the appropriate amount for transfection and quality of the plasmid DNA was assessed by gel electrophoresis.

[0197] CHO cells were seeded in a shake flask and expanded using serum-free chemically defined medium. On the day of transfection, the expanded cells were seeded into a new flask with fresh medium, and each antibody-expression construct was transiently transfected into CHO cells using standard procedures known in the art. The cells were maintained as a batch-fed culture (C4611 and C4612, Medna) until the end of production. The conditioned media from the transient transfection was harvested and clarified by centrifugation and filtration. Antibodies were then purified by Protein L or IMAC purification as described below.

[0198] Protein L Affinity Purification: The supernatant was loaded over a Protein L column pre-equilibrated with binding buffer. Washing buffer was passed through the column until the OD280 value (NanoDrop, ThermoScientific) was zero. The target protein was eluted with a low pH buffer, fractions were collected, and the OD280 value of each fraction was recorded. Fractions containing the target protein were pooled and filtered through a 0.2 µm membrane. The protein concentration was calculated from the OD280 value and the calculated extinction coefficient.

[0199] IMAC Purification of His-tagged Protein: The supernatant was loaded over an Immobilized Metal (Nickel) Affinity Chromatography (IMAC) column pre-equilibrated with binding buffer. Washing buffer containing 40 mM imidazole was passed through the column until the OD280 value (NanoDrop, ThermoScientific) was close to zero. The target protein was eluted with a linear gradient of increasing imidazole concentration up to 0.5 M. The eluate was collected in fractions and the OD280 value of each fraction was recorded. CE-SDS (LabChip

GXII, Perkin Elmer) of each fraction was performed and analyzed. Fractions containing the target protein were pooled and dialyzed into the client specified buffer. The protein was filtered through a 0.2 μ m membrane and protein concentration was calculated from the OD280 value (NanoDrop, Thermo Scientific) and the calculated extinction coefficient.

[0200] Antibody analysis: Size exclusion chromatography (SEC) was performed using a HiLoad 26/600 Superdex 200 column (GE Healthcare Life Sciences). Collected fractions were analyzed by CE-SDS (LabChip GXII, Perkin Elmer). Fractions containing the target protein were pooled and analyzed by SE-UPLC. A summary of the analysis report for antibody construct 2766 using each purification method is shown in Table 9.

Table 9. Purification of the 2766 6B5 antibody

Antibody	Purification method	Concentration (mg/mL)	Total yield (mg)
2766	IMAC	0.77	32.34
2766	Protein L	0.60	35.52

Example 3: Humanized 6B5 scFvs inhibit formation of ceramide-rich platforms *in vitro*

[0201] The humanized anti-ceramide scFvs produced in Example 1 and 2 were then tested for their ability to inhibit formation of ceramide-rich platforms.

[0202] For cell culture and stimulation, Jurkat T lymphocyte (clone E6-1, TIB-152, ATCC) were maintained in RPMI 1640 media supplemented with 10% heat inactivated FBS in a humidified incubator at 37°C with 5% CO₂. Cells were washed and resuspended in RPMI 1640 media supplemented with 1% FBS or delipidated FBS. Briefly, cells were counted on a hemocytometer and 1x10⁶ cells per 0.5ml were plated in a 24 well plate at 37°C. After 2h, cells were stimulated with 0.6 μ g/ml human anti-CD28 antibody (AF-342-PB, R&D systems). In addition, Jurkat T lymphocytes were pre-treated with 50 ng/mL to 800 ng/mL of humanized 6B5 scFvs for 1 hour prior to stimulation with anti-CD28 antibody (0.6 μ g/mL, Cat. No. AF-342-PB, R&D Systems) for 45 seconds. Cells were then immediately fixed with 4% paraformaldehyde for 10 min at 4°C.

[0203] Ceramide-rich platforms (CRPs) were detected as described previously (J. A. Rotolo et al., 2005; Stancevic et al., 2013). Non-specific sites were blocked by incubating cells with 2% normal donkey serum (D9663, Sigma) for 2h on ice. Following a PBS wash, cells were stained for surface ceramide using mouse anti-ceramide 1° Ab MID 15B4 IgM (1:50, Alexis Biochemicals) overnight at 4°C. Irrelevant mouse IgM was used as isotype control. Cells were

then washed 3X with PBST and stained with Cy3-conjugated anti-mouse IgM 2° Ab (1:400, Jackson ImmunoResearch) for 1h at 4°C. Cells were washed 3X with PBST and mounted on glass slides using Vectashield fluorescent mounting media containing DAPI (Vector Laboratories). Platforms were imaged on Leica SP5 Inverted microscope with HyD hybrid detector photon counter and HCX PL APO CS 63.0x 1.40 oil objective lens with digital zoom at 7 for volumetric analysis and HCX PL APO CS 40.0x 1.25 oil objective lens with digital zoom 1 for incidence analysis at room temperature. Z-stacks were acquired using Leica LAS AF software.

[0204] 3-dimensional CRP volumetric analysis was conducted created using Imaris software (Bitplane AG, Zurich, Switzerland). Briefly, 10-12 individual Z-stacks for each sample were acquired under optimized conditions, and data were transported to Imaris software. Intensity normalization was performed for control and treated samples. Briefly, intensity mean threshold for z-column was obtained from untreated control samples. Based on intensity mean threshold, surfaces were created in for untreated control, CD28-treated and scFv-treated cells using the “Surfaces” feature in Imaris. Intensity mean threshold allows only a certain range of intensity values to be considered as positive value for surface volume quantitation, while anything outside this range is considered negative. Volume for each surface is generated by surface volume function of Imaris. Individual volume for each sample set is summed up and mean calculated for final analysis. To calculate IC₅₀ values, inhibitory effects of each antibody were normalized to a scale of 0-100% CRP inhibition, where 0% represents untreated control (CD28 antibody alone) and 100% represents antibody treatment that achieved the best inhibitory effect. Data were plotted using Graph Pad Prism 8 and curves fitted using nonlinear regression analysis.

[0205] As shown in FIG. 1, pre-incubation with different concentrations of 6B5 scFvs inhibited CRP formation in CD28-activated T cells. VH2-VL1 was the most effective at preventing CRP formation with an IC₅₀ value of 6.1 ng/mL. The IC₅₀ values for the various humanized 6B5 scFvs tested are shown in Table 10 below.

Table 10. Inhibition of CRP formation with humanized 6B5 scFvs

Humanized 6B5 scFv	SEQ ID NO	IC₅₀ (ng/mL)
VH1-VL1	54	180.8
VH1-VL3	56	85.7
VH2-VL1	51	6.1
VH2-VL2	57	12.1
VH3-VL1	59	12.7

Humanized 6B5 scFv	SEQ ID NO	IC ₅₀ (ng/mL)
VH3-VL2	60	8.4

Example 4: Humanized 6B5 scFvs inhibit radiation-induced apoptosis *in vitro*

[0206] The humanized anti-ceramide scFvs produced in Example 1 and 2 were then tested for their ability to inhibit radiation-induced cell death.

[0207] Apoptosis assay was performed as described (Haimovitz-Friedman et al., 1994; J. A. Rotolo et al., 2005). Jurkat T lymphocytes were resuspended in RPMI 1640 supplemented with 1% FBS and pre-treated with 100 ng/mL, 200 ng/mL, 400 ng/mL, 600 ng/mL, or 800 ng/mL of humanized 6B5 scFvs for 1 hour at 37°C before exposure to 10 Gy (~1.5 Gy/minute) of radiation. Irradiated Jurkat T cells were incubated at 37°C for 16 hours and then fixed in 4% paraformaldehyde. Cells were then washed with PBS, stained with 24 µg/ml of the fluorophore bisbenzimidazole trihydrochloride (Hoechst 33258; Sigma-Aldrich), and placed on glass slides for counting on an Olympus IMT-2 microscope using a 40× DPlanApo40UV 0.850160/0.11-0.23 objective lens at room temperature. Using a fluorescence microscope, apoptosis was quantified based on morphologic characteristics of apoptotic nuclei including chromatin condensation, segmentation, and compaction along with nuclear periphery and the appearance of apoptotic bodies. A minimum of 100 cells was counted per sample. Percent inhibition of apoptosis was determined by normalizing values to the radiation only control (FIG. 2A). IC₅₀ values were calculated using non-linear regression on GraphPad Prism v7 software.

[0208] As shown in FIG. 2A, pre-incubation with different concentrations of 6B5 scFvs inhibited apoptosis in irradiated Jurkat T cells. VH2-VL1 was the most effective at inhibiting radiation-induced apoptosis with an IC₅₀ value of 130.3 ng/mL. The IC₅₀ values for the various humanized 6B5 scFvs tested are shown in Table 11 below.

Table 11. Inhibition of T cell apoptosis with humanized 6B5 scFvs

6B5 scFv	SEQ ID NO	IC ₅₀ (ng/mL)
VH1-VL1	54	3707
VH1-VL3	56	509.9
VH2-VL1	51	130.3
VH2-VL2	57	332.7
VH3-VL1	59	339.8
VH3-VL2	60	197.6
Murine 6B5 antibody	62	274.8
Genscript		299.2

[0209] Two additional humanized 6B5 scFvs, VH4-VL1 (SEQ ID NO: 52) and VH5-VL1 (SEQ ID NO: 53) were tested for their ability to inhibit radiation-induced T cell apoptosis using a similar experimental setup (FIGs. 2B-2E). In FIG. 2B, each of VH4-VL1, VH5-VL1, and parental murine 6B5 scFv was incubated with Jurkat T cells at 25 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL, 400 ng/mL, 600 ng/mL or 800 ng/mL for 1 hour, and the ability of each scFv to inhibit radiation-induced apoptosis was normalized to the protective effect of parental murine 6B5 scFv at 800 ng/mL. In FIG. 2C, each of VH4-VL1, VH5-VL1, and parental murine 6B5 scFv was incubated with Jurkat T cells at 25 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL, 400 ng/mL, or 800 ng/mL for 1 hour, and the ability of each scFv to inhibit radiation-induced apoptosis was normalized to the protective effect of VH5-VL1 at 800 ng/mL. In FIG. 2D, VH4-VL1 and VH5-VL1 was incubated with Jurkat T cells at 25 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL, 400 ng/mL, 600 ng/mL or 800 ng/mL for 1 hour, and the ability of each construct to inhibit radiation-induced apoptosis was normalized to the protective effector to the protective effect of VH4-VL1 at 800 ng/mL. IC₅₀ values based on these sets of experiments are provided below in Table 12.

Table 12. Inhibition of T cell apoptosis with humanized 6B5 scFvs

6B5 scFv	SEQ ID NO	IC ₅₀ (ng/mL)	Corresponding Figure
Parental murine 6B5 scFv	62	19.4	FIG. 2C
VH4-VL1	52	15.1	FIG. 2C
VH5-VL1	53	30.0	FIG. 2C
VH4-VL1	52	18.9	FIG. 2D
VH5-VL1	53	80.7	FIG. 2D

[0210] FIG. 2E shows the protective effect of VH4-VL1 and VH5-VL1 at given concentrations (400 ng/mL and 800 ng/mL) as evaluated by the percentage of radiation-induced apoptotic T cells under these conditions. Overall, these results demonstrate that humanized 6B5 scFvs can effectively protect T cells against radiation induced apoptosis.

Example 5: Treatment with humanized 6B5 scFv prevents radiation-induced cell death *in vivo*

[0211] Anti-ceramide VH2-VL1 (SEQ ID NO: 51) was tested *in vivo* for treatment in mice exposed to lethal doses of radiation. A LD₉₀ dose of 15 gray units (Gy) was delivered to C57BL/6 mice using a cesium irradiator and mice were left either untreated or intravenously treated with 6B5 VH2-VL1 (150 µg/25 g) 24 hours following irradiation. Mice were euthanized at different time points following irradiation and sequential segments of proximal jejunum from

the ligament of Treitz were isolated, fixed in 4% paraformaldehyde, and embedded in paraffin. Tissue was sectioned at 5 μ m and apoptotic endothelial cells were identified using lethal the Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) assay with MECA-32 antibody (pan-endothelial cell marker). Double-stained apoptotic endothelial cells were quantified.

[0212] Mice irradiated with an LD₉₀ dose of 15 Gy resulted in cell death of the small intestinal endothelium, which peaked 24 hours following irradiation (FIG. 3A). Treatment with 6B5 VH2-VL1 24 hours after irradiation significantly reduced endothelial cell apoptosis compared to untreated mice (FIG. 3B). Overall, these data indicate that inhibiting ceramide prevents endothelial cell death in the gastrointestinal tract in a mouse model of GI syndrome. Humanized anti-ceramide scFvs may be beneficial for the treatment of diseases associated with increased cell death, including GI syndrome, GvHD, and autoimmunity.

FURTHER NUMBERED EMBODIMENTS

[0213] Further embodiments of the instant disclosure are provided in the numbered embodiments below:

[0214] Embodiment 1. An anti-ceramide antibody or antigen-binding fragment thereof comprising an immunoglobulin heavy chain variable region (VH) comprising a heavy chain complementarity determining region (CDR) 1 (HCDR1), a heavy chain CDR2 (HCDR2), and a heavy chain CDR3 (HCDR3), and an immunoglobulin light chain variable region (VL) comprising a light chain complementarity determining region (CDR) 1 (LCDR1), a light chain CDR2 (LCDR2), and a light chain CDR3 (LCDR3), wherein: (a) the HCDR1 comprises an amino acid sequence selected from SEQ ID NO: 1 and 2; (b) the HCDR2 comprises an amino acid sequence selected from SEQ ID NO: 3, 4, 5, and 6; (c) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 7; (d) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 8; (e) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 9; and (f) the LCDR3 comprises the amino acid sequence of SEQ ID NO: 10.

[0215] Embodiment 2. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the HCDR1 comprises SEQ ID NO: 1 and the HCDR2 comprises SEQ ID NO: 3.

[0216] Embodiment 3. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the HCDR1 comprises SEQ ID NO: 1 and the HCDR2 comprises SEQ ID NO: 4.

[0217] Embodiment 4. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the HCDR1 comprises SEQ ID NO: 1 and the HCDR2 comprises SEQ ID NO: 5.

[0218] Embodiment 5. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the HCDR1 comprises SEQ ID NO: 1 and the HCDR2 comprises SEQ ID NO: 6.

[0219] Embodiment 6. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the HCDR1 comprises SEQ ID NO: 2 and the HCDR2 comprises SEQ ID NO: 3.

[0220] Embodiment 7. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the HCDR1 comprises SEQ ID NO: 2 and the HCDR2 comprises SEQ ID NO: 4.

[0221] Embodiment 8. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the HCDR1 comprises SEQ ID NO: 2 and the HCDR2 comprises SEQ ID NO: 5.

[0222] Embodiment 9. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the HCDR1 comprises SEQ ID NO: 2 and the HCDR2 comprises SEQ ID NO: 6.

[0223] Embodiment 10. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 22.

[0224] Embodiment 11. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 10, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22.

[0225] Embodiment 12. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 10, wherein the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0226] Embodiment 13. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at

least 95%, or at least 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 23.

[0227] Embodiment 14. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 13, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23.

[0228] Embodiment 15. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 13, wherein the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0229] Embodiment 16. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 24.

[0230] Embodiment 17. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 16, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24.

[0231] Embodiment 18. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 16, wherein the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0232] Embodiment 19. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 22.

[0233] Embodiment 20. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 19, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22.

[0234] Embodiment 21. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 19, wherein the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0235] Embodiment 22. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 23.

[0236] Embodiment 23. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 22, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23.

[0237] Embodiment 24. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 22, wherein the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0238] Embodiment 25. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 24.

[0239] Embodiment 26. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 25, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24.

[0240] Embodiment 27. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 25, wherein the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0241] Embodiment 28. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 22.

[0242] Embodiment 29. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 28, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22.

[0243] Embodiment 30. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 28, wherein the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0244] Embodiment 31. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 23.

[0245] Embodiment 32. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 31, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23.

[0246] Embodiment 33. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 31, wherein the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0247] Embodiment 34. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 24.

[0248] Embodiment 35. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 34, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24.

[0249] Embodiment 36. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 34, wherein the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0250] Embodiment 37. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 20 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 22.

[0251] Embodiment 38. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 37, wherein the VH comprises an amino acid sequence that is 100% identical

to SEQ ID NO: 20 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22.

[0252] Embodiment 39. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 37, wherein the VH amino acid sequence consists of SEQ ID NO: 20 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0253] Embodiment 40. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 1, wherein the VH comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 21 and the VL comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 22.

[0254] Embodiment 41. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 40, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 21 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22.

[0255] Embodiment 42. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 40, wherein the VH amino acid sequence consists of SEQ ID NO: 21 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0256] Embodiment 43. An anti-ceramide antibody or antigen-binding fragment thereof comprising an immunoglobulin heavy chain variable region (VH) and an immunoglobulin light chain variable region (VL), wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to a sequence selected from SEQ ID NO: 17, 18, 19, 20, and 21; and wherein the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to a sequence selected from SEQ ID NO: 22, 23 and 24.

[0257] Embodiment 44. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 22.

[0258] Embodiment 45. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 44, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22.

[0259] Embodiment 46. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43-45, wherein the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0260] Embodiment 47. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 23.

[0261] Embodiment 48. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 47, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23.

[0262] Embodiment 49. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43, 47 or 48, wherein the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0263] Embodiment 50. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 24.

[0264] Embodiment 51. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 50, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 17 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24.

[0265] Embodiment 52. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43, 50 or 51, wherein the VH amino acid sequence consists of SEQ ID NO: 17 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0266] Embodiment 53. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 22.

[0267] Embodiment 54. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 53, wherein the VH comprises an amino acid sequence that is 100%

identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22.

[0268] Embodiment 55. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43, 53 or 54, wherein the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0269] Embodiment 56. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 23.

[0270] Embodiment 57. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 56, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23.

[0271] Embodiment 58. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43, 56 or 57, wherein the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0272] Embodiment 59. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 24.

[0273] Embodiment 60. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 59, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 18 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24.

[0274] Embodiment 61. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43, 59 or 60, wherein the VH amino acid sequence consists of SEQ ID NO: 18 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0275] Embodiment 62. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 22.

[0276] Embodiment 63. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 62, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22.

[0277] Embodiment 64. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43, 62 or 63, wherein the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0278] Embodiment 65. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 23.

[0279] Embodiment 66. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 65, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 23.

[0280] Embodiment 67. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43, 65 or 66, wherein the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 23.

[0281] Embodiment 68. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 24.

[0282] Embodiment 69. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 68, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 19 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 24.

[0283] Embodiment 70. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43, 68 or 69, wherein the VH amino acid sequence consists of SEQ ID NO: 19 and the VL amino acid sequence consists of SEQ ID NO: 24.

[0284] Embodiment 71. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%,

95%, or 97% identical to SEQ ID NO: 20 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 22.

[0285] Embodiment 72. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 71, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 20 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22.

[0286] Embodiment 73. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43, 71 or 72, wherein the VH amino acid sequence consists of SEQ ID NO: 20 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0287] Embodiment 74. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43, wherein the VH comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 21 and the VL comprises an amino acid sequence that is at least 90%, 95%, or 97% identical to SEQ ID NO: 22.

[0288] Embodiment 75. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 43 or 74, wherein the VH comprises an amino acid sequence that is 100% identical to SEQ ID NO: 21 and the VL comprises an amino acid sequence that is 100% identical to SEQ ID NO: 22.

[0289] Embodiment 76. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 43, 74 or 75, wherein the VH amino acid sequence consists of SEQ ID NO: 21 and the VL amino acid sequence consists of SEQ ID NO: 22.

[0290] Embodiment 77. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 1-76, wherein the antibody or antigen-binding fragment is humanized.

[0291] Embodiment 78. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 1-76, wherein the antibody or antigen-binding fragment is fully human.

[0292] Embodiment 79. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 1-78, wherein the anti-ceramide antibody comprises one or more point mutations in the Fc domain of the antibody.

[0293] Embodiment 80. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 1-79, wherein the antigen-binding fragment is a single chain variable fragment (scFv).

[0294] Embodiment 81. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 80, wherein the scFv comprises an amino acid sequence that is at least 90%, at least 95%, or at least 97% identical to a sequence selected from the group consisting of SEQ ID NOs: 48-61.

[0295] Embodiment 82. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 80, wherein the scFv comprises an amino acid sequence that is 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 48-61.

[0296] Embodiment 83. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 80, wherein the scFv consists of a sequence selected from the group consisting of SEQ ID NOs: 48-61.

[0297] Embodiment 84. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 80, wherein the light chain variable region of said scFv is carboxy-terminal to the heavy chain variable region of said scFv.

[0298] Embodiment 85. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 80, wherein the light chain variable region of said scFv is amino-terminal to the heavy chain variable region of said scFv.

[0299] Embodiment 86. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 80-85, wherein the scFv comprises a linker polypeptide.

[0300] Embodiment 87. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 86, wherein the linker polypeptide is between the light chain variable region and the heavy chain variable region of the said scFv.

[0301] Embodiment 88. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 86 or 87, wherein the linker polypeptide comprises a Gly4Ser linker.

[0302] Embodiment 89. The anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 86-88, wherein the linker polypeptide comprises the formula (Gly4Ser)_n, wherein $n = 1-5$.

[0303] Embodiment 90. An anti-ceramide single chain variable fragment (scFv) comprising an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least

95%, or at least 97% identical to a sequence selected from the group consisting of SEQ ID NOs: 48-61.

[0304] Embodiment 91. The anti-ceramide scFv of Embodiment 90, wherein the scFv comprises an amino acid sequence that is 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 48-61.

[0305] Embodiment 92. The anti-ceramide scFv of Embodiment 90, consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 48-61.

[0306] Embodiment 93. A polynucleotide encoding the anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 1-92.

[0307] Embodiment 94. An anti-ceramide single chain variable fragment (scFv) comprising an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 97% identical to SEQ ID NO: 48 or 51.

[0308] Embodiment 95. The anti-ceramide scFv of Embodiment 94, wherein the scFv comprises an amino acid sequence that is 100% identical to SEQ ID NO: 48 or 51.

[0309] Embodiment 96. The anti-ceramide scFv of Embodiment 94, consisting of the amino acid sequence of SEQ ID NO: 48 or 51.

[0310] Embodiment 97. A polynucleotide encoding the anti-ceramide single chain variable fragment (scFv) of any one of Embodiments 94-96.

[0311] Embodiment 98. An expression vector comprising the polynucleotide of any one of Embodiments 93 or 97.

[0312] Embodiment 99. A host cell comprising the polynucleotide of any one of Embodiments 93 or 97 or the expression vector of Embodiment 97.

[0313] Embodiment 100. A method of manufacturing the anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 1-89 or the anti-ceramide single chain variable fragment (scFv) of any one of Embodiments 94-96, comprising introducing the expression vector of Embodiment 98 into a host cell.

[0314] Embodiment 101. A method of inhibiting apoptosis in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 1-88 or the anti-ceramide scFv of any one of Embodiments 94-96.

[0315] Embodiment 102. The method of Embodiment 101, wherein the apoptosis is associated with a disease selected from the group consisting of graft versus host disease, radiation disease, GI syndrome, and autoimmune disease.

[0316] Embodiment 103. The method of Embodiment 102, wherein the disease is radiation disease or GI syndrome and the anti-ceramide antibody or antigen-binding fragment thereof is administered before the subject is exposed to radiation.

[0317] Embodiment 104. The method of Embodiment 102, wherein the disease is graft versus host disease and the anti-ceramide antibody or antigen-binding fragment thereof is administered before the subject receives a transplant.

[0318] Embodiment 105. The method of Embodiment 104, wherein the transplant is a bone marrow transplant.

[0319] Embodiment 106. The method of Embodiment 101, wherein the anti-ceramide antibody or antigen-binding fragment thereof is administered intravenously, intramuscularly, intraperitoneally, intracerebrospinally, subcutaneously, intrasynovially, intrathecally, orally, topically, or via inhalation.

[0320] Embodiment 107. A method for mitigating apoptosis in a subject with GI syndrome comprising administering to the subject an effective amount of the anti-ceramide antibody or antigen-binding fragment thereof of any one of Embodiments 1-89 or the anti-ceramide scFv of any one of Embodiments 94-96, after the subject is exposed to penetrating radiation.

[0321] Embodiment 108. The method of Embodiment 107, wherein the anti-ceramide antibody or antigen-binding fragment thereof is administered immediately after the subject is exposed to penetrating radiation.

[0322] Embodiment 109. The method of Embodiment 107, wherein the anti-ceramide antibody or antigen-binding fragment thereof is administered within 24 hours after the subject is exposed to penetrating radiation.

[0323] Embodiment 110. A method for inhibiting apoptosis in a subject with GvHD comprising administering to the subject an effective amount of anti-ceramide antibody or antigen binding fragment thereof of any one of Embodiments 1-89 or the anti-ceramide scFv of any one of Embodiments 94-96, either before the subject receives a transplant or after the subject receives a transplant prior to the onset of GvHD.

[0324] Embodiment 111. The method of Embodiment 110, wherein the transplant is a bone marrow transplant.

[0325] Embodiment 112. An anti-ceramide antibody or antigen-binding fragment thereof comprising an immunoglobulin heavy chain variable region (VH) comprising a heavy chain complementarity determining region (CDR) 1 (HCDR1), a heavy chain CDR2 (HCDR2), and a heavy chain CDR3 (HCDR3), and an immunoglobulin light chain variable region (VL) comprising a light chain complementarity determining region (CDR) 1 (LCDR1), a light chain CDR2 (LCDR2), and a light chain CDR3 (LCDR3), wherein: (a) the HCDR1 comprises an amino acid sequence selected from GYTFTDHTIH (SEQ ID NO: 1) and GYTFTDHTMH (SEQ ID NO: 2); (b) the HCDR2 comprises an amino acid sequence selected from YNYPRDGSTKYNEKFQG (SEQ ID NO: 3), YNYPREGSTKYNEKFQG (SEQ ID NO: 4), YNYPRDVSTKYNEKFQG (SEQ ID NO: 5), and YNYPRDGSTKYAEKFQG (SEQ ID NO: 6); (c) the HCDR3 comprises the amino acid sequence of GFITTVVPSAY (SEQ ID NO: 7); (d) the LCDR1 comprises the amino acid sequence of RASKSISKYLA (SEQ ID NO: 8); (e) the LCDR2 comprises the amino acid sequence of SGSTLQS (SEQ ID NO: 9); and (f) the LCDR3 comprises the amino acid sequence of QQHNEYPWT (SEQ ID NO: 10).

[0326] Embodiment 113. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 112, wherein the HCDR1 comprises GYTFTDHTIH (SEQ ID NO: 1) and the HCDR2 comprises YNYPRDGSTKYNEKFQG (SEQ ID NO: 3).

[0327] Embodiment 114. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 112, wherein the HCDR1 comprises GYTFTDHTIH (SEQ ID NO: 1) and the HCDR2 comprises YNYPREGSTKYNEKFQG (SEQ ID NO: 4).

[0328] Embodiment 115. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 112, wherein the HCDR1 comprises GYTFTDHTIH (SEQ ID NO: 1) and the HCDR2 comprises YNYPRDVSTKYNEKFQG (SEQ ID NO: 5).

[0329] Embodiment 116. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 112, wherein the HCDR1 comprises GYTFTDHTIH (SEQ ID NO: 1) and the HCDR2 comprises YNYPRDGSTKYAEKFQG (SEQ ID NO: 6).

[0330] Embodiment 117. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 112, wherein the HCDR1 comprises GYTFTDHTMH (SEQ ID NO: 2) and the HCDR2 comprises YNYPRDGSTKYNEKFQG (SEQ ID NO: 3).

[0331] Embodiment 118. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 112, wherein the HCDR1 comprises GYTFTDHTMH (SEQ ID NO: 2) and the HCDR2 comprises YNYPREGSTKYNEKFQG (SEQ ID NO: 4).

[0332] Embodiment 119. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 112, wherein the HCDR1 comprises GYTFTDHTMH (SEQ ID NO: 2) and the HCDR2 comprises YNYPRDVSTKYNEKFQG (SEQ ID NO: 5).

[0333] Embodiment 120. The anti-ceramide antibody or antigen-binding fragment thereof of Embodiment 112, wherein the HCDR1 comprises GYTFTDHTMH (SEQ ID NO: 2) and the HCDR2 comprises YNYPRDGSTKYAEKFQG (SEQ ID NO: 6).

INCORPORATION BY REFERENCE

[0334] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

SEQUENCE LISTING OF THE PRESENT DISCLOSURE:

Seq Desc.	Chain	Sequence	SEQ ID
HCDR1-IH	Heavy	GYTFTDHTIH	1
HCDR1-IH-MH	Heavy	GYTFTDHTMH	2
HCDR2-DG	Heavy	YNYPRDGSTKYNEKFQG	3
HCDR2-DG-EG	Heavy	YNYPREGSTKYNEKFQG	4
HCDR2-DG-DV	Heavy	YNYPRDVSTKYNEKFQG	5
HCDR2-NE-AE	Heavy	YNYPRDGSTKYAEKFQG	6
HCDR3	Heavy	GFITTVVPSAY	7
LCDR1	Light	RASKSISKYLA	8
LCDR2	Light	SGSTLQS	9
LCDR3	Light	QQHNEYPWT	10
Parental 6B5 HCDR1	Heavy	GYTFTDHTIH	11
Parental 6B5 HCDR2	Heavy	YNYPRDGSTKYNEKFQG	12
Parental 6B5 HCDR3	Heavy	GFITTVVPSAY	13
Parental 6B5 LCDR1	Light	RASKSISKYLA	14
Parental 6B5 LCDR2	Light	SGSTLQS	15
Parental 6B5 LCDR3	Light	QQHNEYPWT	16
Humanized VH1	Heavy	<u>QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHTMHWRQAPGQGLEWMGYNYP</u> <u>PRDGSTKYAEKFQGRVTMTADKSTSTVYMESSLRSED</u> <u>TAVYYCAKGFITTV</u> <u>VPSAYWGQGT</u> <u>TLTVSS</u>	17
Humanized VH2	Heavy	<u>QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHTIHWMRQAPGQGLEWMGYNYP</u> <u>PRDGSTKYNEKFQGRVTMTADKSTSTVYMESSLRSED</u> <u>TAVYYCAKGFITTV</u> <u>VPSAYWGQGT</u> <u>TLTVSS</u>	18
Humanized VH3	Heavy	<u>QVQLVQSGAEVKKPGATVKISCKVSGYTFTDHTIHWMRQAPGQGLEWMGYNYP</u> <u>PRDGSTKYNEKFQGRVTITADKSTSTAYMESSLRSED</u> <u>TAVYYCAKGFITTV</u> <u>VPSAYWGQGT</u> <u>TLTVSS</u>	19
Humanized VH4	Heavy	<u>QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHTIHWMRQAPGQGLEWMGYNYP</u> <u>PREGSTKYNEKFQGRVTMTADKSTSTVYMESSLRSED</u> <u>TAVYYCAKGFITTV</u> <u>VPSAYWGQGT</u> <u>TLTVSS</u>	20
Humanized VH5	Heavy	<u>QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHTIHWMRQAPGQGLEWMGYNYP</u> <u>PRDVSTKYNEKFQGRVTMTADKSTSTVYMESSLRSED</u> <u>TAVYYCAKGFITTV</u> <u>VPSAYWGQGT</u> <u>TLTVSS</u>	21
Humanized VL1	Light	<u>DIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPKAPKLLIYSGS</u> <u>TLQSGVPSRFRSGSGSGTEFTLTISLQPEDFATYYC</u> <u>QQHNEYPWTFGGGTKV</u> <u>EIK</u>	22
Humanized VL2	Light	<u>DVQITQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPKAPKLLIYSGS</u> <u>TLQSGVPSRFRSGSGSGTDFTLTISLQPEDFATYYC</u> <u>QQHNEYPWTFGGGTKV</u> <u>EIK</u>	23
Humanized VL3	Light	<u>DVQLTQSPSSVSASVGDRVTITCRASKSISKYLAWYQQKPKAPKLLIYSGS</u> <u>TLQSGVPSRFRSGSGSGTDFTLTISLQPEDFATYYC</u> <u>QQHNEYPWTFGGGTKV</u> <u>EIK</u>	24
STD1 linker		NYGGGGSGGGSGGGSGNS	25
STD2 linker		NYGGGGSGGGSGGGSGNYGGGGSGGGSGGGSGNS	26

Seq Desc.	Chain	Sequence	SEQ ID
H1 linker		NS	27
H2 linker		GGGGSGNS	28
H3 linker		NYGGGGSGNS	29
H4 linker		GGGGSGGGSGNS	30
H5 linker		NYGGGGSGGGSGNS	31
H6 linker		GGGGSGGGSGGGSGNS	32
H7 linker		GCPPCPNS	33
Gly ₄ Ser linker		GGGGS	34
(G ₄ S) ₃ linker		GGGGSGGGSGGGGS	35
H105 linker		SGGGSGGGSGGGGS	36
(G ₄ S) ₄ linker		GGGGSGGGSGGGSGGGGS	37
H75 linker (NKG2A quadruple mutant)		QRHNNSSLNTGTQMAGHSPNS	38
H83 linker (NKG2A derived)		SSLNTGTQMAGHSPNS	39
H106 linker (NKG2A derived)		QRHNNSSLNTGTQMAGHS	40
H81 linker (NKG2D derived)		EVQIPLTESYSPNS	41
H91 linker (NKG2D derived)		NSLANQEVQIPLTESYSPNS	42
H94 linker		SGGGSGGGSGGGSPNS	43
H111 linker		SGGGSGGGSGGGSPGS	44
H113 linker		SGGGSGGGSGGGSPAS	45
H114 linker		SGGGSGGGSGGGSPS	46
H115 linker		SGGGSGGGSGGGSPSS	47
VH2-VL1 (Lead) scFv		MEWSWVFLFFLSVTTGVHVS QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHT IHWMRQAPGQGLEWMGYNYPDGSTKYNEKFQGRVTMTADKSTSTVYMELSS LRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDI QLTQSPSFLSASVGDRTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTL QSGVPSRFRSGSGTEFTLTISLQPEDFATYYCQQHNEYPTFGGGTKVEI KRAAAGGGSGGGSHHHHHHHH	48
VH4-VL1 scFv		MEWSWVFLFFLSVTTGVHVS QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHT IHWMRQAPGQGLEWMGYNYPREGSTKYNEKFQGRVTMTADKSTSTVYMELSS LRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDI QLTQSPSFLSASVGDRTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTL QSGVPSRFRSGSGTEFTLTISLQPEDFATYYCQQHNEYPTFGGGTKVEI KRAAAGGGSGGGSHHHHHHHH	49
VH5-VL1 scFv		MEWSWVFLFFLSVTTGVHVS QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHT IHWMRQAPGQGLEWMGYNYPDVSTKYNEKFQGRVTMTADKSTSTVYMELSS LRSEDTAVYYCAKGFITTVVPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDI QLTQSPSFLSASVGDRTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTL QSGVPSRFRSGSGTEFTLTISLQPEDFATYYCQQHNEYPTFGGGTKVEI KRAAAGGGSGGGSHHHHHHHH	50
VH2-VL1 scFv – no signal sequence		QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHTIHWMRQAPGQGLEWMGYNYP PDGSTKYNEKFQGRVTMTADKSTSTVYMELSSLRSEDTAVYYCAKGFITTV VPSAYWGQGLTVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRTIT TCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFRSGSGTEFTL TISLQPEDFATYYCQQHNEYPTFGGGTKVEIK RRAAAGGGSGGGSHHHH HHHH	51

Seq Desc.	Chain	Sequence	SEQ ID
VH4-VL1 scFv – no signal sequence		QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHTIHWMRQAPGQGLEWMGYNYPREGSTKYNEKFQGRVTMTADKSTSTVYMESSLRSEDTAVYYCAKGFITTVVPSAYWGQGTTLVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGKVEIK RAAAGGGGSGGGGSHHHH	52
VH5-VL1 scFv – no signal sequence		QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHTIHWMRQAPGQGLEWMGYNYPADVSTKYNEKFQGRVTMTADKSTSTVYMESSLRSEDTAVYYCAKGFITTVVPSAYWGQGTTLVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGKVEIK RAAAGGGGSGGGGSHHHH	53
VH1-VL1 scFv		QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHTMHWRQAPGQGLEWMGYNYPDGGSTKYAEKFQGRVTMTADKSTSTVYMESSLRSEDTAVYYCAKGFITTVVPSAYWGQGTTLVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGKVEIK RAAAGGGGSGGGGSHHHH	54
VH1-VL2 scFv		QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHTMHWRQAPGQGLEWMGYNYPDGGSTKYAEKFQGRVTMTADKSTSTVYMESSLRSEDTAVYYCAKGFITTVVPSAYWGQGTTLVTVSSGGGGSGGGSGGGGSDVQITQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGGGKVEIK RAAAGGGGSGGGGSHHHH	55
VH1-VL3 scFv		QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHTMHWRQAPGQGLEWMGYNYPDGGSTKYAEKFQGRVTMTADKSTSTVYMESSLRSEDTAVYYCAKGFITTVVPSAYWGQGTTLVTVSSGGGGSGGGSGGGGSDVQITQSPSSVSASVGDRVTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGPGTKVEIK RAAAGGGGSGGGGSHHHH	56
VH2-VL2 scFv		QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHTIHWMRQAPGQGLEWMGYNYPDGGSTKYNEKFQGRVTMTADKSTSTVYMESSLRSEDTAVYYCAKGFITTVVPSAYWGQGTTLVTVSSGGGGSGGGSGGGGSDVQITQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGGGKVEIK RAAAGGGGSGGGGSHHHH	57
VH2-VL3 scFv		QVQLVQSGAEVKKPGASVKVSCKVSGYTFTDHTIHWMRQAPGQGLEWMGYNYPDGGSTKYNEKFQGRVTMTADKSTSTVYMESSLRSEDTAVYYCAKGFITTVVPSAYWGQGTTLVTVSSGGGGSGGGSGGGGSDVQITQSPSSVSASVGDRVTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGPGTKVEIK RAAAGGGGSGGGGSHHHH	58
VH3-VL1 scFv		QVQLVQSGAEVKKPGATVKISCKVSGYTFTDHTIHWMQAPGKGLEWMGYNYPDGGSTKYNEKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGTTLVTVSSGGGGSGGGSGGGGSDIQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQQHNEYPWTFGGGKVEIK RAAAGGGGSGGGGSHHHH	59
VH3-VL2 scFv		QVQLVQSGAEVKKPGATVKISCKVSGYTFTDHTIHWMQAPGKGLEWMGYNYPDGGSTKYNEKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGTTLVTVSSGGGGSGGGSGGGGSDVQITQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGGGKVEIK RAAAGGGGSGGGGSHHHH	60
VH3-VL3 scFv		QVQLVQSGAEVKKPGATVKISCKVSGYTFTDHTIHWMQAPGKGLEWMGYNYPDGGSTKYNEKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAKGFITTVVPSAYWGQGTTLVTVSSGGGGSGGGSGGGGSDVQITQSPSSVSASVGDRVTITCRASKSISKYLAWYQQKPGKAPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHNEYPWTFGGGKVEIK RAAAGGGGSGGGGSHHHH	61

Seq Desc.	Chain	Sequence	SEQ ID
		TISSIQPEDFATYYCQQHNEYPWTFGPGTKVEIK RAAAGGGGSGGGGSHHHH HHHH	
Mouse 6B5 scFv		MKKTAIAIAVALAGFATVAQAQVQLQQSDAELVKPGASVKISCKVSGYTFTDHTIHWKQRPEQGLEWIGYNYPRDGSTKYNEKFKGKATLTADKSSSTAYMQLNSLTSEDSAVYFCAKGFITTVVPSAYWGQGLTVTVSAGGGGSGGGGSGGGGSDVQITQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISSELEPEDFAMYYCQQHNEYPWTFGGGTKLEIKGSHHHHHH	62
Murine 6B5 VH parental		QVQLQQSDAELVKPGASVKISCKVSGYTFTDHTIHWKQRPEQGLEWIGYNYPRDGSTKYNEKFKGKATLTADKSSSTAYMQLNSLTSEDSAVYFCAKGFITTVVPSAYWGQGLTVTVSA	63
Murine 6B5 VL parental		DVQITQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQSGIPSRFSGSGSGTDFTLTISSELEPEDFAMYYCQQHNEYPWTFGGGTKLEIK	64
VH3-VL3 scFv nucleic acid sequence		ATGAAGTACCTTCTTCCACCGCCGCGGCTGGGTTGTTGTTACTTGCAGCAC AACCGGCGATGGCTCAAGTCCAGTTAGTTCAAGTCGGGGGCCGAAGTAAAGAA GCCAGGTGCTACTGTGAAAATTTCTGTCAAAGTGAGCGGATACACATTCACG GACCACACGATTCACTGGATGCAGCAAGCTCCGGGCAAGGCTCTTGAGTGGA TGGGCTACAACATATCCGCGTGACGGATCAACAAAATACAATGAAAAATTTCA AGGGCGTGTTACAATTACCGCGGATAAATCCACGAGTACTGCCTACATGGAG TTGTCTCACTTCTGTTCAAGAGCACAGCAGTGTACTATTGTGCTAAAGGGT TCATCAGCACCCTGGTCCCTCTGCTTATTGGGGCCAAGGAACACTTGTGAC TGTGTCTTCTGGGGGCGGGGGGAGCGGAGGCGGAGGTAGCGGTGGCGGGGGT AGTGACGTTCAACTGACTCAATCGCTTCATCCGTTAGCGCCAGCGTAGGCG ACCGTGTGACCATCACGTGCCGCGCGTCCAAAAGTATCAGCAAGTACCTGGC CTGGTACCAACAAAACCCGGAAGGCACCGAAGTTGTTGATTTATTCCGGT TCAACATTACAAAGCGCGTTCCGAGTCGTTTCAGTGGTAGCGGTTCCGGCA CAGATTTTACCTTGACCATTTCTCCCTGCAGCCAGAAGACTTTGCTACTTA TTACTGTCAACAGCACAATGAGTACCCGTGGACGTTTGGGCTGGAACATAAG GTTGAGATTAAGCGTGTCTGCTGCCGGGGCGGTGGTTCCGAGGAGGAGGAA GTCACCACCACCATCACCATCATCATGGT	65
VH3-VL2 scFv nucleic acid sequence		ATGAAATATCTGCTGCCAACTGCCGCGAGCAGGTCTGTTGCTTCTGGCCGCTC AACCGGCCATGGCTCAAGTCCAGTTAGTTCAAGTGAGTGGGGCAGAGGTTAAAA ACCGGGAGCTACAGTAAAGATCAGCTGTAAAGTATCAGGTTATACATTTACT GACCATACTATCCACTGGATGCAACAGGCGCCAGGGAAAGGTTTGGAGTGGA TGGGATATAATTATCCACGCGACGGGTCAACTAAGTACAACGAGAAGTTTCA AGGCCGTGTAACGATTACCGCGGACAAAAGCACGAGTACAGCGTACATGGAA TTATCATCTTTGCGCTCGGAGGACACAGCGGTCTATTACTGTGCCAAGGGTT TCATCACTACTGTGGTACCAAGTGCCTATTGGGGTCAAGGAACCCCTTGTAC GGTGTATCAGGGGTGGCGGAAGTGGCGGTGGTGGATCGGGCGGAGGTGGA AGCGATGTTCAAGTACACAGTCCGCTCGTTCTGAGTGCAAGTGTCCGTG ATCGTGTGACTATTACGTGTCGTGCATCGAAATCAATCTCAAAGTATCTGGC TTGGTACCAGCAAAAACCTGGAAGGCTAACAAATTGTTGATCTATTCCGGG AGCACCCCTTCAAAGCGGTGTCCCTCTCGTTTCTCCGATCCGGGTACAGGA CCGACTTTACTCTGACTATTTCCAGTCTTCAGCCGGAAGATTTTGCAGCTTA CTATTGCCAGCAGCATAATGAGTACCCATGGACTTTTGGCGGTGGCACAAAG GTCGAGATTAAGCGTGCAGTGCCGGTGGTGGAGGTTCCGGTGGGGGCGGTT CTCATCATCACCATCATCACCATCATGGT	66
VH3-VL1 scFv nucleic acid sequence		ATGAAATATTTGCTTCCGACTGCCGCGCAGGTCTGTTACTTTTAGCAGCGC AGCCCGCTATGGCACAGGTCCAGTTAGTTCAATCAGGAGCGGAGGTGAAGAA GCCGGGAGCCACGGTTAAATCTCATGCAAGGTGTCCGGCTATACATTTACC GACCACACGATTCACTGGATGCAGCAAGCTCCCGGAAGGGCTTGAATGGA TGGGGTACAATTATCCACGCGATGGTTCACCAAGTATAACGAAAAATTTCA AGGTCTGTCAACATTACCGCTGACAAAAGCACGTCTACACGCTACATGGAG CTGAGTAGCTTGCCTCAGAGGACACAGCCGTATACTATTGCGCAAAAGGTT TCATCAGCACCCTAGTCCCTCTGCGTATTGGGGACAGGGGACCCTGGTCAC GGTCTCTCCGGGGCGGAGGATCGGGAGGCGGGGGTCTGGAGGTGGTGA	67

Seq Desc.	Chain	Sequence	SEQ ID
		TCTGACATCCAGCTTACCCAATCGCCTAGCTTCTTGAGTGCTAGTGTAGGCG ACCGTGTTACTATCACTTGTGCGCTTCTAAGAGCATTAGCAAATACTTAGC CTGGTACCAACAAAAGCCGGGAAAGGCACCCAAGCTGTTAATTTATAGTGGG TCTACCCTTCAGTCCGGCGTACCCTCACGCTTTTCCGGCAGTGGCTCGGGTA CCGAATTTACCCTTACGATTTCTTCGCTTCAACCCGAAGATTTTGCAACGTA CTACTGTCAGCAGCACAACGAGTATCCCTGGACATTCCGGCGAGGGACAAAA GTAGAAATCAAGCGTGCAGCCGAGGTGGCGGCGGGTCTGGCGGCGGAGGGA GTCACCATCACCATCATCATCATCATGGA	
VH2-VL3 scFv nucleic acid sequence		ATGAAATATTTGTTACCTACCGCTGCGGCAGGACTTCTGCTTTTGGCCGCTC AGCCCGCAATGGCTCAAGTGCAATTGGTTCAGTCAGGGGCCGAAGTAAAAA GCCAGGCGCGAGCGTAAAAGTGAGTTGCAAGGTCTCCGGTTACACGTTTACC GATCACACGATTCACTGGATGCGTCAAGCTCCAGGCCAGGGTCTTGAATGGA TGGGTTACAACATCTCTCGCGATGGTTCGACCAAGTATAACGAGAAGTTTCA GGGCCGTGTCAATGACCGCCGACAAATCAACGAGCACTGTCTACATGGAG CTGTCTATCCCTGCGCAGCGAGGATACAGCTGTGTACTACTGTGCCAAGGGCT TCATTACTACCGTCTGTCAGCGCTACTGGGGTCAAGGTACACTGGTAAC AGTTTCATCCGGAGGAGGGGGAAGCGGCGGGTGGATCTGGCGGCGGTGGG TCAGACGTTCAACTTACCCAGTCGCCTTCTTCCGTGAGTGCCTCTGTCCGGG ATCGTGTACCATCACTTGGCGCGCATCTAAGTCAATTTCTAAATATTTGGC CTGGTATCAGCAGAAACCCGGTAAAGCGCCCAAATTTGTTGATCTATTCCGGC AGTACCTTGCAATCCGGGGTGCCCTTCGCGCTTCTCGGGGAGTGGAAGCGGCA CGGATTTCACTTTAACGATCTCGAGTTTGCAGCCGGAAGATTTTGCAGCTTA CTATTGTCAGCAGCATAACGAATATCCATGGACCTTTGGCCCTGGTACGAAA GTAGAAATCAAACGTGCAGCAGCCGGTGGGGTGGCTCCGGTGGCGGTGGGT CGCATCACCACCACCATCATCATCACGGT	68
VH2-VL2 scFv nucleic acid sequence		ATGAAGTACCTGTTGCCCACAGCCGCTGCTGGGTTACTGCTTCTTGCTGCCC AGCCCGCAATGGCGCAAGTGCAATTAGTCCAGTCTGGAGCAGAGGTTAAAAA GCCGGGAGCTTCAGTGAAGGTCTCCTGTAAGGTAAGTGGTTATACATTCACC GATCACACAATCCATTGGATGCGCCAGGCCCCAGGTGAGGGTCTTGAATGGA TGGGCTATAACTACCCGCGTGATGGCTCAACTAAGTATAACGAGAAGTTTCA GGGGCGCGTTACAATGACTGCGGATAAAAGCACGTCGACGGTGTACATGGAA TTGTCAAGCCTTCGTTCCGAAGATACCGCAGTTTACTATTGTGCTAAGGGCT TTATCACTACCGTGGTACCCAGTGCCTACTGGGGGAGGGAACCTTGGTAAC CGTGTATCGGGCGAGGCGGAAGCGGGGAGGTGGCAGTGGAGGTGGCGGG TCTGACGTTCAAATCACCCAGTCACCGTCATTTCTTTCCGCGTCTGTGGGTG ACCGCGTAACAATTACTTGGCGTGCTTCGAAAAGCATTTGAAATACCTTGC CTGGTACCAACAGAAACCGGGGAAAGCAAACAACTTTTAACTACAGCGGG AGCACGCTTCAATCCGGCGTCCCATCCCGTTTTTCTGGCTCCGGCAGCGGGA CCGACTTCACCTTAACATTTCTTCCCTTCAACCGGAGGACTTTGCGACGTA TTACTGTCAACAACACAATGAGTATCCGTGGACTTTTCGGAGCGGGAATAG GTTGAAATCAAGCGTGCTGCTGCTGGCGGGGAGGCTCAGCGGAGGGGGAT CACATCATCATCACCATCATCATCACGGA	69
VH2-VL1 scFv nucleic acid sequence		ATGAAATACTTGCTGCCGACTGCCGAGCGGGATTATTATTACTTGCGGCTC AGCCGGCTATGGCGCAGGTCCAGTTAGTTCAGAGTGGGGCAGAAGTGAAGAA ACCCGGTGCATCTGTAAAGGTCTCATGTAAAGTATCTGGCTACACCTTCACT GACCACACTATCCACTGGATGCGTCAAGCACAGGACAAGTTTAGAGTGGA TGGGATATAACTATCCGCGTGACGGGAGTACGAAGTATAACGAAAAATTTCA GGGCCGTGTAACCATGACCGCGGATAAATCCACTTCCACTGTCTATATGGAA TTATCGTCGTTACGTAGCGAGGACACGGCGGTATATTATTGCGCCAAAGGT TTATCACGACTGTTGTGCCGTCCGCTTACTGGGGGCAAGGGACTTTAGTGAC CGTGTCAGCGGAGGGGGGGTAGCGGGGTGGCGGAGTGGTGGTGGGGGT TCGGACATTCACTTACCCAATCCCTTAGCTTTTGTGACGCTCCGTTGGCG ATCGTGTAATACTATTACGTGCCGCGCCTCCAAGTCCATTTCAAATATTTAGC CTGGTACCAACAGAAGCCAGGAAAAGCTCCTAAATTAAGTACTGATTTACTCAGGT TCCACATTGCAGTCGGGCGTACCAAGCCGTTTTTCGGGAAGTGGTAGCGGGA CCGAGTTTACATTGACGATTAGTAGTCTTCAACCGGAGGATTTTGCAACCTA CTATTGTCAGCAGCACAACGAGTACCATGGACCTTTGGAGGCGGTACCAAA GTGGAATTAACGCGCAGCAGCAGCGGCGGTGGTTCAGGGGGAGGAGGCT CGCATCACCACCACCATCATCATCACCGT	70

Seq Desc.	Chain	Sequence	SEQ ID
VH1-VL3 scFv nucleic acid sequence		ATGAAATACCTTTTGGCGACTGCCGCCGAGGATTATTGTTACTGGCGGCTC AGCCGGCTATGGCCCAAGTCCAATTAGTTCAATCAGGGGCAGAAGTTAAGAA GCCCGGAGCAAGTGTCAAGGTCTCATGCAAAGCCTCCGGTTACACCTTTACC GACCACACGATGCACTGGGTTTCGCCAAGCTCCCGGACAGGGCCTGGAATGGA TGGGTTATAATTATCCGCGTGATGGTAGCACGAAATACGCGGAAAAATTCCA AGGTCGCGTTACGATGACCGCGGACAAGTCTACTAGCACCGTGTACATGGAA CTTAGCTCGCTGCGTTCGGAAGATACGGCGGTGTACTACTGTGCGAAAGGTT TCATCACTACAGTAGTGCCAAGTGCGTATTGGGGTCAAGGCACGCTGGTAAC GGTTCAGTGGTGGCGGCGGTAGCGGCGGCGGCGGCGAGCGGAGGAGGGGGA TCGGATGTCCAATTACGCAATCTCCGTCTCTGTTTCCGCAAGTGTGGGCG ACCGCGTAACCATCACTTGCCGTGCTAGTAAAGCATTCTAAGTATTTAGC GTGGTATCAACAGAAGCCCGGAAAGGCGCCAAAGCTGTTGATTTATCCGGG AGTACATTACAATCTGGCGTGCCGTCCCGCTTCTCCGGCAGCGGTAGTGTA CTGACTTCACGCTGACAATTAGTTCACTTCAGCCAGAGGATTTTGCACATA TTATTGCCAGCAGCATAACGAATACCCATGGACCTTCGGTCTCGGAACCAAG GTTGAAATCAAACGTGCTGCCGCCGCGCGGCGGTGGGAGCGGAGGGGCGGGT CACATCATCATCATCATCACCATCATGGT	71
VH1-VL2 scFv nucleic acid sequence		ATGAAATACCTTCTTCCACCGCAGCAGCGGGATTGTTATTATTGGCTGCAC AACCTGCGATGGCTCAAGTACAGTTGGTGCAGTCAGGGGCGGAAGTCAAGAA ACCAGGCGCTTCAGTGAAAGTTTCGTGTAAAGCATCCGGCTATACTTTTACC GACCACACCATGCACTGGGTTTCGCCAAGCTCCTGGACAGGGTCTGGAATGGA TGGGGTACAACATATCCCGCGATGGATCCACAAAGTACGCGGAGAAGTTCCA AGGACGTGTAACAATGACGCGCGATAAAAGCACTTCACAGTCTATATGGAA CTTTCGTCTCTTCGTTCTGAGGACACAGCAGTTTACTACTGTGCTAAAGGAT TTATCACTACCGTCGTTCCGTCTGCATATTGGGGGCAGGGCAGCTTAGTAAC AGTCAGCTCCGGGGGCGGGGGCTCAGGGGGCGGTGGATCAGGGGGAGGCGGC TCTGACGTGCAGATCACTCAGTCGCCTAGCTTTCTTTCCGGTAGTGTCCGGG ATCGCGTCACTATCACATGCCGCGCCAGCAAAAGCATCTTAAATACCTGGC TTGGTACCAACAAAAACCCGGAAGGCTAATAAGTTGCTGATCTACAGTGGG AGTACTTTGCAGTCGGGGGTGCCGAGTCGTTTTTCAGGTAGCGGGTCGGGCA CGGACTTTACCCTTACAATTTCTCCCTGCAGCCCGAGGACTTTGCCACATA TTATTGTCAACAGCACAACGAGTACCCGTGGACGTTTGGGGGAGGTACCAAA GTGGAATCAAGCGTGACGCGCCGGGGAGGTGGAAGCGGCGGCGGTGGAA GTCACCATCATCACCACCACCATCACGGA	72
VH1-VL1 scFv nucleic acid sequence		ATGAAATACCTGTTACCTACTGCTGCCCGAGGTTTGTGCTCTGGCGGCTC AACCGGCCATGGCAGAGTTTCACTGGTGCAATCAGGCGCCGAAGTCAAAAA ACCGGGTGCAAGCGTCAAGGTGAGCTGTAAGGCAAGCGGATATACTTTTACA GATCATACAATGCACTGGGTACGCCAGGCACAGGCCAGGGCCTTGAATGGA TGGGATATAACTACCCACGTGATGGATCTACTAAATATGCCGAGAAGTTCCA GGGTCGCGTTACCATGACTGCTGATAAAAGTACATCAACTGTATACATGGAA CTTAGCTCATTGCGTTCCGAAGATACTGCCGTGTACTATTGTGCAAAGGGGT TTATCAGCAGTCGTTCCATCGGCATATTGGGGCCAGGGAACGTTAGTAAC AGTCAGCAGTGGGGGTGGGGGTCAGGCGGAGGAGGGTCTGGTGGGGGGGT TCTGACATCCAGTTGACACAGTCCCATCCTTTTTGTCTGCATCGGTTGGGG ACCGCGTGACGATTACTTGTGCGCGCTCCAAGTCTATCAGCAAATATTTAGC ATGGTATCAACAAAAACCTGGCAAGGCTCCCAAGTTACTTATTTACTCCGT AGTACTCTTCAGTCGGGAGTGCCTTCCCGTTTTAGCGGGTCTGGTTCAGGCA CGGAGTTTACCCTTACAATTAGTTCTCTGCAGCCCGAGGATTTTGCACTTA CTACTGCCAGCAACACAATGAGTACCCCTGGACATTTCGGCGGTGGGACAAAA GTCGAGATTAAGCGTGCCGCTGCCGGTGGAGGAGGGTCAGGGGGTGGCGGCA GTCATCACCACCATCACCATCATGGA	73
6B5 scFv nucleic acid sequence		ATGAAGTACTTATTGCCCACTGCTGCTGCTGGATTATTATTGTTGGCAGCGC AACCGGCCATGGCGCAGGTCCAATTTCAGCAGTCAGATGCCGAAGTTGTAAA GCCGGGTGCAAGCGTGAAATCTCATGTAAAGTATCTGGTTATACGTTTACA GATCACACCATCCACTGGATGAAGCAACGCCCTGAGCAGGGTTTAGAGTGA TTGGCTATAATTATCCGCGCGACGGTTCGACAAAAATACAACGAAAAGTTCAA GGGCAAGGCAACGCTTACCGCCGATAAGAGTTCAAGTACAGCATATATGCAA CTGAACAGCTTAAGTAGCGAAGATAGCGCAGTTTACTTTTTCGCGCAAGGGAT TTATCACCACCGTGGTTCCGAGTGCCTACTGGGGACAGGGCACATTGGTTAC	74

Seq Desc.	Chain	Sequence	SEQ ID
		AGTCAGTGCAGGAGGAGGAGGGTCCGGTGGCGGAGGATCGGGTGGGGGTGGA TCAGATGTCCAAATCACACAATCGCCATCCTACTTGGCCGCCTCGCCCGGAG AAACCATCACTATTAATTGCCGCGCGAGCAAGTCTATTTCCAAGTATTTGGC ATGGTACCAAGAAAAGCCCGGTAAACTAACAACCTTTTGATCTACTCTGGG TCCACGCTGCAGTCAGGAATCCCATCCCGTTTCTCGGGCAGCGGATCTGGGA CAGACTTCACGTTGACAATTTGAGCCTTGAGCCGGAAGATTTGCAATGTA CTACTGTCAGCAACATAACGAGTACCCTTGGACATTTGGCGGGGGCACAAG CTTGAAATTAAACGCGCTGCTGCCGGAGGAGGTGGTTCTGGGGGGAGGGGGTT CTCATCATCACCACCATCACCACCACGGA	
Signal sequence		MEWSWVFLFFLSVTGVHS	75
C-terminal tag		RAAAGGGGSGGGGSHHHHHHH	76