

BTLA AGONIST ANTIBODIES AND USES THEREOF

The present invention is in the field of medicine. More particularly, the present invention relates to antibodies directed against B and T Lymphocyte Attenuator (BTLA) and pharmaceutical compositions thereof. The antibodies of the present invention are
5 expected to be useful in the treatment of autoimmune diseases such as lupus.

Lupus is an autoimmune disease with heterogeneous features, including skin, oral, muscle & joint, cardiac, peripheral blood, lung, kidney, reproductive, and CNS manifestations. Lupus patients are at risk for serious and life-threatening cardiovascular, renal and neuropsychiatric disease. The standard of care includes numerous steroids,
10 which have many unfavorable and/or dangerous side effects. There is a need for therapies to manage disease and allowing for reduction or elimination of steroid use.

B and T Lymphocyte Attenuator (BTLA; CD272) is an Ig superfamily member and part of a family of checkpoint receptors that negatively regulate immune cell activation. BTLA is primarily expressed on B cells, T cells, and dendritic cells. The
15 natural ligand for BTLA is the TNF receptor superfamily member, herpes virus entry mediator (HVEM; TNFRSF14).

Human HVEM-Fc has been reported to bind to human BTLA expressed in 293T cells with a K_D of 112 nM as detected by flow cytometry. (Cheung et al., PNAS, Sept. 13, 2005, 102:37;13218-13223). Binding of HVEM to BTLA leads to tyrosine-
20 phosphorylation of two conserved immunoreceptor tyrosine-based inhibitory motif domains on the cytoplasmic domain of BTLA. This phosphorylation leads to recruitment of, via two Src homology 2 domains, protein tyrosine phosphatases that impart the inhibitory activity of BTLA by dephosphorylating and down-regulating positive cell receptor signaling (eg. T cell receptor or B cell receptor signal transduction cascades),
25 thus leading to suppression of immune cell activation. In a mouse model prone to spontaneously develop lupus-like diseases (MRL-lpr mice), BTLA-deficient mice have more severe lymphocytic infiltration in salivary glands, lungs, pancreas, kidneys and joints compared to BTLA-expressing mice. Therefore, BTLA agonist antibodies may provide a benefit for patients having autoimmune diseases such as lupus.

30 Agonist antibodies to BTLA are known in the art. For example, U.S. Patent Number 8,563,694 (the '694 patent) discloses BTLA agonist antibodies that either block (Mab21H6 and Mab19A7) or do not block (Mab8D5 and Mab8A3) HVEM binding to

BTLA. The '694 patent describes an ongoing need to develop treatments that exploit the inhibitory role of BTLA in lymphocyte responses, while allowing for BTLA-HVEM binding. However, there is a lack of BTLA agonist antibodies that mimic the binding of HVEM to BTLA for the treatment of autoimmune diseases. An antibody "mimics" HVEM binding to BTLA if the antibody has an epitope that significantly overlaps the binding site of HVEM, and there is structural similarity between the antibody and HVEM. There is also a lack of BTLA agonist antibodies that bind human BTLA and are useful to study in *in vivo* pre-clinical models of autoimmune diseases such as murine and cynomolgus monkey models. Thus, there remains a need for alternative BTLA agonist antibodies.

The antibodies of the present invention seek to provide alternative BTLA agonist antibodies. Such BTLA agonist antibodies may be useful in the treatment of autoimmune diseases such as lupus. Such BTLA agonist antibodies are able to bind BTLA from multiple species such as human, cynomolgus monkey, and/or murine BTLA. In addition, such BTLA agonist antibodies demonstrate increased *in vitro* activity compared to an antibody having the same heavy chain variable region and light chain variable region as Mab8D5. The antibodies of the present inventions possess at least one of these desirable characteristics.

One such BTLA agonist antibody is able to bind human, cynomolgus monkey, and murine BTLA. Surprisingly, this antibody has this desired cross-reactivity because it mimics HVEM binding to BTLA. This antibody also has a higher binding affinity to BTLA as compared to HVEM binding BTLA. This may provide a benefit for patients having disease states with transient levels of HVEM, wherein it may be desirable to have a BTLA-mimicking agonist antibody on-board during times when the patient has a reduction in HVEM.

The present inventions provide antibodies that bind to BTLA and activate and/or enhance BTLA-mediated signaling (BTLA agonist antibodies). The present inventions provide an antibody that comprises a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein the LCVR comprises complementarity determining regions (CDRs) LCDR1, LCDR2, and LCDR3 and the HCVR comprises CDRs HCDR1, HCDR2, and HCDR3, and wherein the amino acid sequence of LCDR1 is SEQ ID NO: 22, the amino acid sequence of LCDR2 is SEQ ID NO: 25, the amino acid

sequence of LCDR3 is SEQ ID NO: 28, the amino acid sequence of HCDR1 is SEQ ID NO: 13, the amino acid sequence of HCDR2 is SEQ ID NO: 16, and the amino acid sequence of HCDR3 is SEQ ID NO: 19. In an embodiment, the antibody comprises a LCVR and a HCVR, and wherein the amino acid sequence of the LCVR is SEQ ID NO: 4, and the amino acid sequence of the HCVR is SEQ ID NO: 3. In another embodiment, the antibody comprises a light chain (LC) and a heavy chain (HC), and wherein the amino acid sequence of the LC is SEQ ID NO: 2, and the amino acid sequence of the HC is SEQ ID NO: 1. In yet another embodiment, the antibody comprises 2 LCs and 2 HCs, wherein the amino acid sequence of each LC is SEQ ID NO: 2, and the amino acid sequence of each HC is SEQ ID NO: 1.

The present inventions also provide a BTLA agonist antibody wherein the amino acid sequence of LCDR1 is SEQ ID NO: 23, the amino acid sequence of LCDR2 is SEQ ID NO: 26, the amino acid sequence of LCDR3 is SEQ ID NO: 29, the amino acid sequence of HCDR1 is SEQ ID NO: 14, the amino acid sequence of HCDR2 is SEQ ID NO: 17, and the amino acid sequence of HCDR3 is SEQ ID NO: 20. In an embodiment, the amino acid sequence of the LCVR is SEQ ID NO: 8, and the amino acid sequence of the HCVR is SEQ ID NO: 7. In another embodiment, the amino acid sequence of the LC is SEQ ID NO: 6, and the amino acid sequence of the HC is SEQ ID NO: 5. In yet another embodiment, the antibody comprises 2 LCs and 2 HCs, wherein the amino acid sequence of each LC is SEQ ID NO: 6, and the amino acid sequence of each HC is SEQ ID NO: 5.

The present inventions also provide a BTLA agonist antibody wherein the amino acid sequence of LCDR1 is SEQ ID NO: 24, the amino acid sequence of LCDR2 is SEQ ID NO: 27, the amino acid sequence of LCDR3 is SEQ ID NO: 30, the amino acid sequence of HCDR1 is SEQ ID NO: 15, the amino acid sequence of HCDR2 is SEQ ID NO: 18, and the amino acid sequence of HCDR3 is SEQ ID NO: 21. In an embodiment, the amino acid sequence of the LCVR is SEQ ID NO: 12, and the amino acid sequence of the HCVR is SEQ ID NO: 11. In another embodiment, the amino acid sequence of the LC is SEQ ID NO: 10, and the amino acid sequence of the HC is SEQ ID NO: 9. In yet another embodiment, the antibody comprises 2 LCs and 2 HCs, wherein the amino acid sequence of each LC is SEQ ID NO: 10, and the amino acid sequence of each HC is SEQ ID NO: 9.

The present invention also provides an antibody that binds BTLA, wherein the antibody is generated by steps comprising immunizing rabbits with Fc-tagged extracellular domain (ECD) domain of human BTLA and boosting with human and mouse BTLA-Fc tagged proteins. The amino acid sequence of the human BTLA ECD is
5 amino acids 31-150 of SEQ ID NO: 31.

The present invention provides a BTLA agonist antibody that mimics HVEM binding to BTLA. The present invention also provides a BTLA agonist antibody that is able to bind human, cynomolgus monkey, and murine BTLA.

The present invention also provides a pharmaceutical composition comprising an
10 antibody of the present invention, and one or more pharmaceutically acceptable carriers, diluents, or excipients. In some embodiments, pharmaceutical compositions of the present invention can be used in the treatment of one or more of rheumatic, neural, and dermatology disease, whereby such treatment comprises administering to a patient in need thereof an effective amount of a pharmaceutical composition of the present
15 invention. In some particular embodiments, the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis. In other particular embodiments, the dermatology disease is at least one of atopic dermatitis and psoriasis. In other particular embodiments, the neural disease is multiple sclerosis.

The present invention also provides a method of treating a patient having one or
20 more of rheumatic, neural, and dermatology disease, comprising administering to a patient in need thereof an effective amount of an antibody of the present invention. In some such embodiments, the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis. In other particular embodiments, the dermatology disease is at least one of atopic dermatitis and psoriasis. In other particular
25 embodiments, the neural disease is multiple sclerosis.

The present invention also provides an antibody of the present invention or pharmaceutical composition thereof for use in therapy. In some embodiments, the present invention provides an antibody of the present invention or pharmaceutical composition thereof for use in the treatment of one or more of rheumatic, neural, and dermatology
30 disease. In some such embodiments, the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis. In other particular

embodiments, the dermatology disease is at least one of atopic dermatitis and psoriasis. In other particular embodiments, the neural disease is multiple sclerosis.

The present invention also provides the use of an antibody of the present invention or a pharmaceutical composition thereof in the manufacture of a medicament for the treatment of one or more of rheumatic, neural, and dermatology disease. In some such
5 embodiments, the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis. In other particular embodiments, the dermatology disease is at least one of atopic dermatitis and psoriasis. In other particular embodiments, the neural disease is multiple sclerosis.

10 The present invention provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:1, SEQ ID NO:5, or SEQ ID NO:9. The present invention also provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:6, or SEQ ID NO:10.

15 The present invention provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 1, and comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2. The present invention also provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid
20 sequence of SEQ ID NO: 1, and a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2. In a particular embodiment the polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 1 is SEQ ID NO: 35 and the polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2 is SEQ ID NO: 36.

25 The present invention also provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 5, and comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6. The present invention also provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid
30 sequence of SEQ ID NO: 5, and a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6. In a particular embodiment the polynucleotide sequence encoding a polypeptide having the

amino acid sequence of SEQ ID NO: 5 is SEQ ID NO: 37, the polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6 is SEQ ID NO: 38.

5 The present invention also provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 9, and comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 10. The present invention also provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 9, and a DNA molecule comprising a polynucleotide sequence
10 encoding a polypeptide having the amino acid sequence of SEQ ID NO: 10. In a particular embodiment the polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 9 is SEQ ID NO: 39, the polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 10 is SEQ ID NO: 40.

15 Further, the present invention provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 1 and a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2. The present invention also provides a mammalian cell comprising a DNA molecule
20 comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 5 and a polypeptide having the amino acid sequence of SEQ ID NO: 6. The present invention also provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 9 and a polypeptide having the amino acid sequence of
25 SEQ ID NO: 10. In an embodiment the mammalian cell line is a Chinese Hamster Ovary (CHO) or Hamster embryonic kidney (HEK) cell line.

The present invention also provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 1 and/or a DNA molecule comprising a polynucleotide
30 sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 2, wherein the cell is capable of expressing an antibody comprising a HC having the amino acid sequence of SEQ ID NO: 1 and a LC having the amino acid sequence of SEQ ID NO:

2. Preferably the mammalian cell comprises a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:1 and a polypeptide having the amino acid sequence SEQ ID NO: 2. In an embodiment the mammalian cell line is a CHO or HEK cell line.

5 The present invention also provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:5 and/or a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 6, wherein the cell is capable of expressing an antibody comprising a HC having the amino
10 acid sequence of SEQ ID NO:5 and a LC having the amino acid sequence of SEQ ID NO: 6. Preferably the mammalian cell comprises a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:5 and a polypeptide having the amino acid sequence SEQ ID NO: 6. In an embodiment the mammalian cell line is a CHO or HEK cell line.

15 The present invention also provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:9 and/or a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 10, wherein the cell is capable of expressing an antibody comprising a HC having the amino
20 acid sequence of SEQ ID NO:9 and a LC having the amino acid sequence of SEQ ID NO: 10. Preferably the mammalian cell comprises a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:9 and a polypeptide having the amino acid sequence SEQ ID NO: 10. In an embodiment the mammalian cell line is a CHO or HEK cell line.

25 In another embodiment, the present invention provides a process for producing an antibody comprising a LC having an amino acid sequence of SEQ ID NO: 2 and a HC having an amino acid sequence of SEQ ID NO: 1, wherein the process comprises cultivating a mammalian cell comprising a DNA encoding a LC having an amino acid sequence of SEQ ID NO: 2 and/or a HC having an amino acid sequence of SEQ ID NO: 1
30 under conditions such that the antibody is expressed, and recovering the expressed antibody. The invention includes an antibody obtainable by the process of the invention as described immediately above.

The present invention also provides a process for producing an antibody comprising a LC having an amino acid sequence of SEQ ID NO: 6 and a HC having an amino acid sequence of SEQ ID NO: 5, wherein the process comprises cultivating a mammalian cell comprising a DNA encoding a LC having an amino acid sequence of
5 SEQ ID NO: 6 and/or a HC having an amino acid sequence of SEQ ID NO: 5 under conditions such that the antibody is expressed, and recovering the expressed antibody. The invention includes an antibody obtainable by the process of the invention as described immediately above.

The present invention also provides a process for producing an antibody
10 comprising a LC having an amino acid sequence of SEQ ID NO: 10 and a HC having an amino acid sequence of SEQ ID NO: 9, wherein the process comprises cultivating a mammalian cell comprising a DNA encoding a LC having an amino acid sequence of SEQ ID NO: 10 and/or a HC having an amino acid sequence of SEQ ID NO: 9 under conditions such that the antibody is expressed, and recovering the expressed antibody.
15 The invention includes an antibody obtainable by the process of the invention as described immediately above.

The present invention includes a process for producing an antibody, which antibody comprises two HCs and two LCs, in which the amino sequence of each of the two HCs is SEQ ID NO: 1, and the amino acid sequence of each of the two LCs is SEQ
20 ID NO: 2, and which process comprises: a) cultivating a mammalian cell of the invention, as described above, under conditions such that the antibody is expressed, and b) recovering the expressed antibody. The invention includes an antibody obtainable by the process of the invention as described immediately above.

The present invention also includes a process for producing an antibody, which
25 antibody comprises two HCs and two LCs, in which the amino sequence of each of the two HCs is SEQ ID NO: 5 and the amino acid sequence of each of the two LCs is SEQ ID NO: 6, and which process comprises: a) cultivating a mammalian cell of the invention, as described above, under conditions such that the antibody is expressed, and b) recovering the expressed antibody. The invention includes an antibody obtainable by the process of
30 the invention as described immediately above.

The present invention also includes a process for producing an antibody, which antibody comprises two HCs and two LCs, in which the amino sequence of each of the

two HCs is SEQ ID NO: 9 and the amino acid sequence of each of the two LCs is SEQ ID NO: 10, and which process comprises: a) cultivating a mammalian cell of the invention, as described above, under conditions such that the antibody is expressed, and b) recovering the expressed antibody. The invention includes an antibody obtainable by the process of the invention as described immediately above.

The present invention provides an antibody that contacts human BTLA at a structural and functional epitope having the following residues of SEQ ID NO: 31: Arg at position 42 and His at position 127. The present invention also provides an antibody that contacts human BTLA at a structural and functional epitope comprising Arg at position 42 of the amino acid sequence given by SEQ ID NO: 31.

The present invention provides an antibody that contacts human BTLA at a novel structural epitope having the following residues of SEQ ID NO: 31: Asp at position 35, Gln at position 37, Arg at position 42, Leu at position 74, Gly at position 76, Cys at position 79, Arg at position 114, Phe at position 119, Gln at position 120, Asn at position 122, Ser at position 128. In a preferred embodiment, the antibody 22B3 is said to mimic HVEM binding to BTLA because the HCDR3 of antibody 22B3 is structurally similar to HVEM. Preferably, when the BTLA:antibody crystal structure is aligned with the BTLA:HVEM crystal structure in a program such as PyMOL™, an antibody CDR loop adopts a conformation similar to the HVEM loop comprising amino acid residues 69 to 72 (amino acids ELTG of SEQ ID NO:41).

The present invention provides an antibody that contacts human BTLA at a functional epitope having Asp at position 52 of SEQ ID NO:31. The antibody contacts a novel structural epitope having the following residues of SEQ ID NO: 31: His at position 46, Glu at position 55, Glu at position 103, Pro at position 104, Leu at position 106, Pro at position 107, Thr at position 134, Ala at position 139.

The present invention provides an antibody that contacts human BTLA at a functional epitope having His at position 68 and Lys at position 81 of SEQ ID NO:31. In an embodiment, the antibody contacts a novel structural epitope having the following residues of SEQ ID NO: 31: Tyr at position 62, Ala at position 64, His at position 68, Arg at position 85, Glu at position 91, Phe at position 98, Asn at position 118.

The present invention provides an antibody that contacts human BTLA at a novel structural epitope having the following residues of SEQ ID NO: 31: Asp at position 35,

Gln at position 37, Arg at position 42, Leu at position 74, Gly at position 76, Cys at position 79, Arg at position 114, Phe at position 119, Gln at position 120, Asn at position 122, and Ile at position 124, Ser at position 128.

As used herein, an “antibody” is an immunoglobulin molecule comprising 2 HCs and 2 LCs interconnected by disulfide bonds. The amino terminal portion of each LC and HC includes a variable region of about 100-120 amino acids primarily responsible for antigen recognition via the CDRs contained therein. The CDRs are interspersed with regions that are more conserved, termed framework regions (“FR”). Each LCVR and HCVR is composed of 3 CDRs and 4 FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The 3 CDRs of the LC are referred to as “LCDR1, LCDR2, and LCDR3,” and the 3 CDRs of the HC are referred to as “HCDR1, HCDR2, and HCDR3.” The CDRs contain most of the residues which form specific interactions with the antigen. That is, the CDRs contain most of the residues that are in contact with (within 4.5 Å) the antigen’s residues. The functional ability of an antibody to bind a particular antigen is, thus, largely influenced by the amino acid residues within the six CDRs. Assignment of amino acids to CDR domains within the LCVR and HCVR regions of the antibodies of the present invention is based on the well-known Kabat numbering convention (Kabat, et al., Ann. NY Acad. Sci. 190:382-93 (1971); Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)), and Chothia (Chothia C, Lesk AM. Canonical structures for the hypervariable regions of immunoglobulins. J. Mol. Biol. 1987;196:901–17. Chothia C, Lesk AM, Tramontano A, Levitt M, Smith-Gill SJ, Air G, Sheriff S, Padlan EA, Davies D, Tulip WR, et al. Conformations of immunoglobulin hypervariable regions. Nature. 1989;342:877–83). The starting amino acid residue of HCDR1 is defined by Chothia and the ending amino acid residue for HCDR1 is defined by Kabat. The starting and ending amino acid residues for HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are defined by Kabat.

The term “epitope” as used herein may refer to a structural epitope (sites of an antigen that are in contact with the variable region of an antibody) and/or a functional epitope (sites of an antigen that may or may not be in contact with the variable region of an antibody and are necessary for antibody binding). The structural epitope is

determined by X-ray crystallography wherein any residue on human BTLA within 4.5 Å of another residue on the bound Fab is considered to be a contact site.

The antibodies of the present invention may be prepared and purified using known methods. For example, cDNA sequences encoding a HC (for example the amino acid
5 sequence given by SEQ ID NO: 1) and a LC (for example, the amino acid sequence given by SEQ ID NO: 2) may be cloned and engineered into a GS (glutamine synthetase) expression vector. The engineered immunoglobulin expression vector may then be stably transfected into CHO cells. As one skilled in the art will appreciate, mammalian
10 expression of antibodies will result in glycosylation, typically at highly conserved N-glycosylation sites in the Fc region. Stable clones may be verified for expression of an antibody specifically binding to BTLA. Positive clones may be expanded into serum-free culture medium for antibody production in bioreactors. Medium, into which an antibody has been secreted, may be purified by conventional techniques. For example, the medium
15 may be conveniently applied to a Protein A or G Sepharose FF column that has been equilibrated with a compatible buffer, such as phosphate buffered saline. The column is washed to remove nonspecific binding components. The bound antibody is eluted, for example, by pH gradient and antibody fractions are detected, such as by SDS-PAGE, and then pooled. The antibody may be concentrated and/or sterile filtered using common
20 techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The product may be immediately frozen, for example at -70°C, or may be lyophilized.

An antibody of the present invention can be incorporated into a pharmaceutical composition which can be prepared by methods well known in the art and comprise an
25 antibody of the present invention and one or more pharmaceutically acceptable carriers, diluents, or excipients.

A pharmaceutical composition comprising an effective amount of an antibody of the present invention can be administered to a patient at risk for, or exhibiting, diseases or disorders as described herein by parental routes (e.g., subcutaneous, intravenous,
30 intraperitoneal, intramuscular, or transdermal). An “effective amount” refers to an amount necessary (at dosages and for periods of time and for the means of administration) to achieve the desired therapeutic result. An effective amount of the antibody may vary

according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the antibody of the present invention are outweighed by the therapeutically beneficial effects.

5 The antibodies of the present invention can be used in the treatment of patients. More particularly the antibodies of the present invention are expected to treat one or more of rheumatic, neural, and dermatology disease. Rheumatic diseases are characterized by inflammation that can affect a person's joints, muscles, and/or organs. One such rheumatic disease is systemic lupus erythematosus (SLE).

10 As used interchangeably herein, "treatment" and/or "treating" and/or "treat" are intended to refer to all processes wherein there may be a slowing, interrupting, arresting, controlling, stopping, or reversing of the progression of the disorders described herein, but does not necessarily indicate a total elimination of all disorder symptoms. Treatment includes administration of an antibody of the present invention for treatment of a disease
15 or condition in a human that would benefit from an increase in BTLA activity, and includes: (a) inhibiting further progression of the disease; and (b) relieving the disease, i.e., causing regression of the disease or disorder or alleviating symptoms or complications thereof.

Antibody Engineering

20 The antibodies of the present invention were generated by immunizing rabbits with Fc-tagged extracellular domain (ECD) domain of human BTLA and boosting with mouse BTLA-Fc tagged protein (25F7) or alternately with human and mouse BTLA-Fc tagged proteins (22B3 and 23C8). Screening was done with histidine-tagged human, mouse, and cynomolgus monkey BTLA to identify cross reactivity. The amino acid
25 sequence of human BTLA is given by SEQ ID NO: 31, the amino acid sequence of Balbc mouse BTLA is given by SEQ ID NO: 32, the amino acid sequence of C57BL6 is given by SEQ ID NO:33, and the amino acid sequence of cynomolgus monkey BTLA is given by SEQ ID NO: 34. The antibodies were then humanized and affinity matured.

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Examples

Expression and purification of engineered BTLA agonist antibodies

BTLA agonist antibodies of the present invention can be expressed and purified essentially as follows. An appropriate host cell, such as HEK 293 or CHO, can be either transiently or stably transfected with an expression system for secreting antibodies using an optimal predetermined HC:LC vector ratio (such as 1:3 or 1:2) or a single vector system encoding both the HC and the LC. Clarified media, into which the antibody has been secreted, may be purified using any of many commonly-used techniques. For example, the medium may be conveniently applied to a MabSelect column (GE Healthcare), or KappaSelect column (GE Healthcare) for Fab fragment, that has been equilibrated with a compatible buffer, such as phosphate buffered saline (pH 7.4). The column may be washed to remove nonspecific binding components. The bound antibody may be eluted, for example, by pH gradient (such as 20 mM Tris buffer, pH 7.0 to 10 mM sodium citrate buffer, pH 3.0, or phosphate buffered saline pH 7.4 to 100 mM glycine buffer, pH 3.0). Antibody fractions may be detected, such as by SDS-PAGE, and then may be pooled. Further purification is optional, depending on intended use. The antibody may be concentrated and or sterile filtered using common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, multimodal, or hydroxyapatite chromatography. The purity of the antibody after these chromatography steps is between about 95% to about 99%. The product may be held refrigerated, immediately frozen at -70°C, or may be lyophilized. Amino acid SEQ ID NOs for exemplified antibodies of the present invention are shown below.

Table 1. Amino acid sequences of exemplified BTLA agonist antibodies.

Antibody SEQ ID NOs						
Antibody	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
22B3	13	16	19	22	25	28
23C8	14	17	20	23	26	29
25F7	15	18	21	24	27	30

Binding affinity and kinetics

The binding affinity and kinetics of the BTLA agonist antibodies of the present invention (22B3, 23C8, and 25F7) to BTLA are measured by surface plasmon resonance

- using Biacore® 3000 (GE Healthcare). The binding affinity is measured by immobilizing about 120 RU BTLA protein (human, rat, murine (Balbc or C57BL6), or cynomolgus monkey BTLA) via amine coupling on a Biacore® CM5 chip, and flowing BTLA agonist antibody, starting from 500 nM in 2-fold serial dilution down to 15.6 nM. The
- 5 experiments are carried out at 25°C in HBS-EP buffer (GE Healthcare BR100669; 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% surfactant P20, pH 7.4). For each cycle, 250 µL antibody sample is flowed through flow cell 1 and 2 at 50 µl/min, and then dissociated for 10 minutes. The chip surface is regenerated with 5 µL injection of glycine buffer at pH 1.5 at 10 µL/mL flow rate. The data are fit to a 1:1 Langmuir binding model
- 10 to derive k_{on} , k_{off} , and to calculate K_D . Following procedures essentially as described above, the following parameters (shown in Table 2) were observed. Data shown below are the average of three experiments for human, cyno, rat and murine for 22B3.

Table 2. Binding affinity and kinetics.

Antibody	Antigen (BTLA)	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
22B3	Human	5.87E+06	2.19E-03	0.365
	Cyno	2.45E+06	6.47E-04	0.27
	Murine (balbc)	2.60E+06	8.58E-02	32.5
	Murine (C57BL6)	1.89E+06	2.65E-01	147
	Rat	2.10E+06	4.62E-02	24.1
23C8	Human	1.59E+05	2.93E-04	1.93 (n=3)
	Cyno	8.71E+04	3.09E-03	35.35 (n=2)
	Murine (balbc)	No Binding	No Binding	No Binding
	Murine (C57BL6)	No Binding	No Binding	No Binding
	Rat	Not Tested	Not Tested	Not Tested
25F7	Human	6.8622E+04	1.42E-02	206.2 (n=2)
	Cyno	Not Tested	Not Tested	Not Tested
	Murine (balbc)	Not Tested	Not Tested	Not Tested
	Murine (C57BL6)	7.70E+04	3.50E-04	4.63 (n=1)

	Rat	Not Tested	Not Tested	Not Tested
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As shown above in Table 2, the BTLA agonist antibodies of the present invention bind BTLA. Specifically, antibody 22B3 is able to bind human, murine, and cynomolgus monkey BTLA.

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Binding to primary cells

The ability of BTLA antibodies of the present invention (22B3, 23C8, and 25F7) to bind primary cells from different species is determined by FACS. Human peripheral blood mononuclear cells (PBMCs) are isolated from a donor blood sample (San Diego Blood Bank, #LRS-WBC) using Ficoll (GE #17-1440-02) and SepMate tubes (STEMCELL #15450), per manufacturer's protocol. Cyno PBMCs (WorldWide Primates #CA-10) are thawed from liquid nitrogen and washed once with FACS buffer (same as above).

Spleens from male C57BL6 mice (JAX) or female Sprague Dawley rats (Harlan) are harvested, pooled, and dissociated into single cell suspensions using a cell strainer and syringe plunger over a 50 mL conical tube rinsed with RPMI 1640 complete with 10% heat inactivated FBS and 2 mM EDTA. Cells are pelleted, media removed, and red blood cells lysed by resuspending pellet in 2 ml ACK Lysing Buffer (gibco #A10492-01) for approximately 2 minutes before quenching with complete RPMI. Lysed cells may be pelleted and washed once in FACS buffer (DPBS 1X containing 3% FBS, 20 mM HEPES, and 2 mM EDTA).

Isolated primary cells are quantified using a Countess cell counter, and resuspended at 2×10^6 cells per ml in FACS buffer. Flow cytometry experiments is performed the same day as cell isolation by plating 50 μ l ($\sim 0.1 \times 10^6$) cells into a 96 well plate (Greiner #650101). Non-specific antibody binding is prevented by adding 1 μ l Fc block (for example, from BD #553142) for 15 minutes at 4 °C without washing.

BTLA antibody binding is tested at various concentrations, by serial dilution in FACS buffer. For example, a purified antibody and controls starting at different concentrations are first diluted to 30 μ g/mL and serial 1:3 dilutions of the starting material is performed for a total of 10 titrations (plus untreated control). Antibody titrations are incubated with cells for 20 minutes at 4°C, and washed with FACS buffer

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prior to stain. Cells are stained using fluorochrome-conjugated antibodies to identify specific cell types (eg. CD19 B cells, CD4 T cells or CD8 T cells) or using a secondary antibody to identify the presence or absence of antibody binding to that subset. Staining is performed for 20 minutes at 4°C and washed 3 times with FACS buffer prior to
5 analysis on a flow cytometer. Results are analyzed using standard FACS analysis software (eg. FACSDiva) and reported as mean fluorescent intensity of the secondary antibody for each titration. A positive result, which indicates binding, is determined by mean fluorescent intensity staining above background.

Following procedures essentially as described above, antibody 22B3 binds to
10 human, cynomolgus monkey, rat, and mouse BTLA-expressing cells, antibody 23C8 binds human and cynomolgus monkey BTLA-expressing cells, and antibody 25F7 binds human, cynomolgus monkey, and mouse BTLA-expressing cells.

BTLA agonist antibody-induced phosphorylation

15 To determine the ability of BTLA agonist antibodies of the present invention (22B3 and 25F7) to induce tyrosine phosphorylation in a human B cell line, a BTLA antibody is bound to a 24-well culture plate at 10 µg/mL for one hour at 37°C. The plate is washed with PBS to remove any unbound antibody. A human BTLA-expressing B cell line, such as Ramos.2G6.4C10 human B Lymphocyte cell line (ATCC), may be
20 added to the wells at 10×10^6 cells/mL and incubated for 37°C for 30 min. The cells are removed and lysed in Complete Lysis Buffer (MSD), and frozen at -80°C for at least 30 min.

Phosphorylated-BTLA is detected by Meso Sector S 600. Streptavidin detection
plates are prepared by incubating in blocking solution (MSD) for one hour at room
25 temperature. A biotinylated-BTLA capture antibody (5A5) is coated onto the plate for one hour at room temperature followed by three or more Tris-wash steps. The cell lysates are incubated for two hours at room temperature. Total BTLA is detected with a SULFO-TAG anti-BTLA antibody (ANC6E9) and phosphorylated BTLA is measured with a
SULFO-TAG anti-phosphotyrosine antibody (PY20; MSD) followed by three or more
30 Tris-wash steps. Addition of 2x Read Buffer T (MSD) is then added to the wells immediately prior to analysis using a Meso Sector S 600.

Following procedures essentially as described above, antibody 22B3 resulted in a 2.41-fold increase in tyrosine phosphorylation of BTLA over background compared to negative control, and antibody 25F7 resulted in a 1.47-fold increase in tyrosine phosphorylation of BTLA over background compared to negative control. These data demonstrate that the BTLA agonist antibodies 22B3 and 25F7 are able to induce BTLA phosphorylation in a human B cell line.

Inhibition of human primary B cell proliferation

The *in vitro* potency of BTLA agonist antibodies of the present invention are evaluated by the ability to inhibit human primary B cell proliferation. Human primary B cells are isolated from healthy human peripheral blood mononuclear cells using human B cell isolation kit (EasySep) and are resuspended in appropriate human primary cell media. Anti-IgM is coated to plates along with titrations of isotype control or BTLA antibody and incubated for one hour at 37 °C followed by PBS wash step. Isolated human B cells are added to each well and incubated for 72 hours at 37 °C with 5% CO₂ followed by [³H]-thymidine pulse for the last 18 hours. Post incubation plates are removed and placed on dry ice for 30 minutes and then stored at -20°C until ready to harvest. Cells are lysed by thawing and harvested with Harvester9600 (Tomtec). Proliferation is assessed by measuring [³H]-thymidine incorporation with a MicroBeta² 2450 Microplate Counter (Perkin Elmer).

Counts are used to assess relative proliferative response in this assay, and percent inhibition is calculated using the equation [% Inhibition= (AVGmaxsignal-signalsample)/AVGmaxsignal x 100], which can be used to determine IC₅₀ values using graphing software (GraphPad Prism).

Following procedures essentially as described above, the BTLA agonist antibody 22B3 was able to inhibit primary B cell proliferation *in vitro* with a calculated IC₅₀ of 0.32 +/- 0.1 nM, antibody 23C8 was able to inhibit primary B cell proliferation *in vitro* with a calculated IC₅₀ of 0.14 nM, and antibody 25F7 was able to inhibit primary B cell proliferation *in vitro* with a calculated IC₅₀ of 0.17 nM. In a similar experiment, antibody 22B3 was able to inhibit primary B cell proliferation with a calculated IC₅₀ of 0.32 nM, and an antibody having the same HCVRs and LCVRs as Mab8D5 (SEQ ID NO:

11 and 18 of the '694 patent, respectively) inhibited primary B cell proliferation with a calculated IC₅₀ of 6.38 nM. These data demonstrate that the BTLA agonist antibodies 22B3, 23C8, and 25F7 are able to inhibit B cell proliferation *in vitro*, and that antibody 22B3 has greater *in vitro* activity as compared to Mab8D5.

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Humanized NSG mouse model of GvHD

Prevention of human PBMC-driven graft vs. host disease (GvHD) is determined *in vivo*.

10 Briefly, female NSG mice (JAX Labs, Stock # 05557), approximately 8-10 weeks old, are normalized and divided into treatment groups (n=8 mice per treatment group) based on baseline body weight measurements. Peripheral blood mononuclear cells (PBMCs) are isolated from a blood donor program (San Diego Blood Bank, # LRS-WBC) using Ficoll (GE # 17-1440-02) and SepMate tubes (STEMCELL # 15450), per manufacturer's protocol. PBMCs are resuspended at approximately 150 x10⁶ cells per
15 ml of PBS. Treatment groups are blinded prior to dosing.

On day 1, 100 μ l (15 x10⁶ cells) of PBMCs suspended in PBS (as described above) (or 100 μ l PBS for non-engrafted controls) are injected intravenously (IV) into the tail of each mouse. Mice are dosed weekly (QW) with antibody of the present invention (22B3 or 23C8) or controls at varying concentrations in PBS vehicle, by subcutaneous
20 (SQ) injections. Three independent studies are performed essentially as described herein. Dosing concentrations for each study is [Study 1 (antibody 22B3): 0.1, 1.0, 5.0, 10.0, and 20.0 mg/kg; Study 2 (antibody 22B3 or 23C8): 0.001, 0.01, 10.0, and 100 mpk; and Study 3: 0.001, 0.005, 0.01, 0.1, 0.5, and 1.0mpk].

The study is terminated and mice are euthanized prior to isotype control animals
25 losing 20% loss of baseline body weight (Studies 1 and 2) or day 28 (Study 3). Weights are recorded (Study 1 and Study 2), serum is collected for cytokine analysis (Study 1; analysis is performed by MSD ELISA; cytokines analyzed are TNF α , IL-10, IL-6, IL-4, IL12p70, IL-13, IL-2, and IL-8), and spleens are harvested for phenotyping/pharmacodynamic analyses (measured by a reduction in CD 8 T cell
30 population; Study 1 and Study 3).

Following procedures essentially as described above, the following data were obtained.

Antibody 22B3-treated animals in Study 1 demonstrated the following (at doses 0.1, 1.0, 5.0, 10.0, or 20.0 mg/kg antibody): (i) similar body weights at the end of the study compared to the body weights of non-engrafted control animals; (ii) a reduction in the cytokines TNF α , IL-10, IL-6, IL-4, and IL-12p70 compared to isotype control animals; and (iii) a reduction in CD 8 T cell population compared to isotype control animals (phenotyping/pharmacodynamic analyses).

Data from Study 2 demonstrate that mice treated with 0.01mg/kg antibody 22B3, or 1.0, 5.0, or 10.0 mg/kg antibody 23C8 had similar body weights at the end of the study compared to the body weights of non-engrafted control animals. Study 2 did not demonstrate activity of 22B3 on body weight at 10.0 mg/kg, which may reflect natural donor variability of this model. In Study 3, antibody 22B3 demonstrated pharmacodynamic activity in vivo at the following doses of antibody: 0.01, 0.1, 0.5, and 1.0 mg/kg. Taken together, these data demonstrate that antibody 22B3 and antibody 23C8 were efficacious in preventing GvHD in vivo.

mIFN α -induced lupus nephritis

The interferon- alpha (IFN α)-induced lupus nephritis model is a mouse model of systemic lupus erythematosus (SLE) in which IFN α is used to synchronize onset and accelerate progression of disease in a cross with New Zealand Black and New Zealand White (NZB/W) mice. The NZB/W mouse model is a classical model of spontaneous lupus nephritis. The disease progression in these mice could be accelerated with exogenous administration of IFN α using adenovirus vectors. This lupus nephritis model is used to demonstrate the activity of the BTLA agonist antibodies of the present invention.

One day before the study start, eleven week old female NZB/W mice are randomly sorted based on body weight. Mice are distributed into the following treatment groups: (1) LacZ adeno-associated virus (AAV + 10 mg/kg human IgG4 PAA isotype control (PAA is S228P, F234A, and L235A mutations), (2) IFN α AAV + 10 mg/kg human IgG4 PAA isotype control, (3) IFN α AAV + 3 mg/kg 22B3 antibody, (4) IFN α AAV + 10 mg/kg 22B3 antibody, or (5) IFN α AAV + 50 mg/kg cyclophosphamide. On study start date (Day 0), mice are either administered once with 10¹¹ genome copies (GC) of AAV expressing LacZ gene (non-diseased) or mouse IFN α (diseased) in PBS intravenously. In groups 1-4, the mice are treated with isotype control or 22B3 antibody

antibodies in PBS subcutaneously once every week starting on Day 0. In group 5, mice are treated with cyclophosphamide in PBS intraperitoneally every 10 days. Urine samples are collected from the mice every 2 weeks until study termination 6 weeks after treatment initiation. The Kamiya Biomedical™ mouse microalbumin ELISA is used to quantitate urine albumin levels. Urine creatinine is measured by using an enzymatic creatinine assay (Roche Diagnostics). Albuminuria, a biomarker of renal function, is defined as greater than 300 µg albumin per mg creatinine detected in the urine.

Following procedures essentially as described above, by week 4, the incidence of albuminuria in the isotype treated diseased group (IFN α AAV + hIgG4 PAA) reached 100% and stayed elevated until end of study, while the LacZ AAV treated (non-diseased) mice did not show any incidence of albuminuria. Cyclophosphamide, which can be acutely nephrotoxic, caused a transient increase in albuminuria in diseased mice, but the incidence of albuminuria in the cyclophosphamide group was reduced to zero by study end. Antibody 22B3 at 3 mg/kg and 10 mg/kg was able to reduce incidence of albuminuria to 50% and 20%, respectively, at day 28, and 60% and 70%, respectively, at day 42. These results indicated that antibody 22B3 was able to preserve renal function in the model.

A Kaplan-Meier plot (data not shown) of percent survival during the study showed that renal insufficiency in the isotype treated diseased group led to deaths starting at day 28. By the end of the study, survival rate in the isotype treated diseased group was 60%. The non-diseased and cyclophosphamide treated groups had survival rates of 100%. The mice treated with 10 mg/kg antibody 22B3 also showed 100% survival at the end of the study, while the mice treated with 3 mg/kg showed 80% survival. These results indicated that antibody 22B3 was able to prevent disease related deaths in this model.

25

Imiquimod-induced model of psoriasis

The ability of an antibody of the present invention to limit the severity of psoriasis-like dermatitis induced by application of the TLR7/8 agonist imiquimod (IMQ) is tested. Seven-week-old, female B6.SJL-*Ptprca*^a *Pepc*^b/BoyJ mice (JAX stock number: 002014), or HVEM^{-/-} mice (described in Wang et al, J. Clin. Invest., 115:3, 711-717, March 2005) are injected intraperitoneally with 3 mg/kg or 1 mg/kg of antibody 22B3 or antibody 25F7, respectively, on day 0, and the backs of the mice are shaved. Animals

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injected with hIgG4 isotype control served as controls. On days 1-3, mice are anesthetized with inhaled isoflurane (VetOne), and 5% IMQ cream (50mg, Fougera) is then applied to a defined area of the shaved skin. On day 4, the treated area of skin is excised and analyzed for disease severity and inflammation-related gene expression.

- 5 Following procedures essentially as described above, histological analysis demonstrated thickening of the epidermal layer with parakeratosis and hyperkeratosis in the groups treated with hIgG4 isotype control or 1mg/kg antibody 22B3. Mice treated with 3mg/kg antibody 22B3 or 3mg/kg antibody 25F7 showed a significant reduction in epidermal thickness, with some areas appearing histologically normal. Gene expression in the skin was analyzed by qPCR using the iTaq Universal SYBR Green Supermix (Bio-
10 Rad). Mice treated with 3mg/kg antibody 22B3 exhibited a significant decrease in expression of Type I IFNs (IFN α , IFN β) and IFN γ , as well as IFN-response genes (Isg15, Mx1, Mx2, Oas2). Analysis of cytokines involved in establishing IMQ-induced dermatitis also demonstrated a significant reduction in IL-22 and IL-23 expression in the 3mg/kg
15 antibody 22B3 treatment group. These data demonstrate that the BTLA agonist antibodies 22B3 and 25F7 are able to reduce epidermal thickness in a mouse model of psoriasis-like dermatitis.

Epitope determination

- 20 The functional epitopes of the BTLA agonist antibodies of the present invention are determined by ELISA, and the structural epitopes are determined by x-ray crystallography.

Methods

ELISA: Functional epitope

- 25 The following set of surface mutations of BTLA were introduced individually into a human BTLA protein fused to (human) Fc: D35R, Q37R, Y39E, R42D, Q43A, E45R, S47H, L49R, D52R, E55R, E57R, D84R, N65R, H68A, V80R, K81E, E83R, S88H, K90H, E91H, I95R, E103H, L106R, N108R, R114V, S121Y, N122R, E125H, H127E, T130R, Y132R, and T134H.
- 30 Binding of 22B3 and 23C8 was determined using an ELISA wherein the antibody to be epitope mapped was captured by an immobilized anti-rabbit antibody and after washing each BTLA mutant was incubated as a 4 point 4-fold dilution series with the

captured antibody and detected with an enzyme linked anti-human Fc reagent. The resulting signal was compared among antibodies and to control antibodies. The functional epitope normally indicated itself by a dramatic reduction in signal for one or two mutants. For the 25F7 antibody, a sandwich ELISA was performed, wherein humanized 22B3 was
5 immobilized, BTLA mutants were captured, and bound by rabbit 25F7. This gave a much stronger signal and the 25F7 epitope could be identified after eliminating the 22B3 epitope.

X-ray Crystallography: Structural epitope

10 In order to determine interacting interfaces and therefore the physical epitope on BTLA of the various antibodies, human BTLA was co-crystallized with the Fab portion of an antibody of the present invention and a crystal structure was determined. From the resulting crystal structure, the BTLA residues within 4.5Å of any antibody atom were counted as part of the epitope (using the Pymol visualization software). 4.5 angstroms is
15 measured from atom center to atom center. Any residue with at least one atom that is 4.5 angstroms close to any atom in the antibody is part of the epitope.

Two 22B3 structures were determined in complex with human BTLA. The first utilized the parent rabbit 22B3 antibody Fab, Histidine tagged and purified with a S47H mutant (stabilizing mutation) of human BTLA expressed as an Fc fusion and then cleaved
20 and purified. These two proteins were mixed at an approximately equimolar ratio and screened in commercially available screens for crystallization. Crystals were obtained and diffraction data collected at the Advanced Photon Source. This data was reduced and solved by molecular replacement and refined to yield a high resolution structure of the complex between 22B3 and BTLA. The second complex was between an affinity matured
25 version (with HC mutations I56Q/T57H/G98A and LC S95H) of the humanized 22B3 (Fab portion) and human BTLA. These were co-expressed, purified as a complex and similarly screened. The resulting structure and epitope were similar to the first structure.

The structure of 23C8 in complex was obtained in the same way as the first 22B3 complex, namely by purifying the His tagged rabbit parent Fab, mixing with monomeric
30 S47H human BTLA and crystallizing.

The structure of 25F7 in complex with human BTLA was obtained as per the second 22B3 complex, namely by co-expression, co-purification and crystallization. A

double mutant of the humanized 25F7 with improved binding to human BTLA was utilized (humanized 25F7 used for epitope determination has mutations at HC S30W/LC E27R).

Results

5 22B3 antibody: Among a set of BTLA surface mutants, R42D and H127E had a significant negative impact on binding to rabbit 22B3 antibody (comprising the same CDRs as 22B3 but with a rabbit framework). The functional epitope comprises Arg at position 42 and His at position 127 of human BTLA (SEQ ID NO:31). BTLA residues that are within 4.5 angstroms of 22B3 in the crystal structure complex between human
10 BTLA and rabbit 22B3 Fab, and are the structural epitope, are the following residues of SEQ ID NO: 31: Asp at position 35, Gln at position 37, Arg at position 42, Leu at position 74, Gly at position 76, Cys at position 79, Arg at position 114, Phe at position 119, Gln at position 120, and Asn at position 122, Ser at position 128. BTLA residues that are within 4.5 angstroms of 22B3 in the crystal structure complex between human
15 BTLA and a human 22B3 variant (HC I56Q/T57H/G98A LC S95H) Fab are Asp at position 35, Gln at position 37, Arg at position 42, Leu at position 74, Gly at position 76, Cys at position 79, Arg at position 114, Phe at position 119, Gln at position 120, Asn at position 122, and Ile at position 124, Ser at position 128 of SEQ ID NO:31.

In a similar study, the structural epitope for HVEM binding BTLA was the
20 following amino acids of BTLA: Gln at position 37, Arg at position 42, Leu at position 74, Gly at position 76, Thr at position 77, Ser at position 112, Arg at position 114, Asn at position 118, Ser at position 121, Ser at position 128, and Thr at position 130. Structural similarity between antibody 22B3 and HVEM was assessed by superimposing the antibody:BTLA crystal structure onto the HVEM:BTLA crystal structure aligning the
25 BTLA molecules. The backbone root-mean-square deviation in the HVEM region containing amino acid residues 69-72 and the corresponding antibody region was determined to be 1.4 angstroms.

23C8 antibody: D52R blocks binding of rabbit 23C8 (comprising the same HCDR1, HCDR2, HCDR3, LCDR1 and LCDR2 as 23C8, having the LCDR3 of
30 QCTYGGVVGSTSDNP, and having a rabbit framework) to human BTLA in an ELISA. The functional epitope comprises Asp at position 52 of human BTLA (SEQ ID NO:31). BTLA residues that are within 4.5 angstroms of 23C8 in the crystal structure

complex between human BTLA (S47H) and rabbit 23C8 Fab, and are the structural epitope, are His at position 46, Glu at position 55, Glu at position 103, Pro at position 104, Leu at position 106, Pro at position 107, Thr at position 134, and Ala at position 139 of SEQ ID NO:31. Antibody 23C8 does not mimic HVEM binding.

- 5 25F7 antibody: Among a set of BTLA surface mutants, H68A and K61E had a significant negative impact on binding to rabbit 25F7 antibody (comprising the same CDRs as 25F7 but with a rabbit framework). The functional epitope comprises His at position 68, and Lys at position 81, of human BTLA (SEQ ID NO:31). BTLA residues that are within 4.5 angstroms of 25F7 in the crystal structure complex between human
- 10 BTLA and humanized 25F7 Fab variant (HC S30W, LC E27R), and are the structural epitope, are Tyr at position 62, Ala at position 64, His at position 68, Arg at position 85, Glu at position 91, Phe at position 98, and Asn at position 118 of SEQ ID NO:31. Antibody 25F7 does not mimic HVEM binding.

Sequences

HC of Antibody 22B3 (SEQ ID NO: 1)

QVQLVQSGAEVKKPGASVKVSCKASGFSLSYGVSWVRQAPGQGLEWMGAISY
5 DGITYYASWAKSRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGDYYDDYVY
VYALDIWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT
VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK
VDKRVESKYGPCCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVQSQ
DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC
10 KVSNGKLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMH
LHNHYTQKSLSLGLG

LC of Antibody 22B3 (SEQ ID NO: 2)

EIVLTQSPGTLSPGERATLSCQASQSISTALAWYQQKPGQAPRLLIYAASLAS
15 GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGYSSSNLDNVFGGGTKVEIKRT
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
EQDSKDYSTLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

HCVR of Antibody 22B3 (SEQ ID NO: 3)

QVQLVQSGAEVKKPGASVKVSCKASGFSLSYGVSWVRQAPGQGLEWMGAISY
20 DGITYYASWAKSRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARGDYYDDYVY
VYALDIWGQGTLVTVSS

LCVR of Antibody 22B3 (SEQ ID NO: 4)

EIVLTQSPGTLSPGERATLSCQASQSISTALAWYQQKPGQAPRLLIYAASLAS
GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGYSSSNLDNVFGGGTKVEIK

25 HC of Antibody 23C8 (SEQ ID NO: 5)

EVQLVESGGGLVQPGGSLRLSCAASGFDISKYNIQWVRQAPGKGLEWVGFINYG
GSAYYASRAKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCARGLSNSDLWGQ
GTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG
30 PPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVQSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP

ENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLG

LC of Antibody 23C8 (SEQ ID NO: 6)

DIQMTQSPSSLSASVGDRVITTCQASQSISSWLSWYQQKPGKAPKLLIYRASTLAS
5 GVPSRFSGSGSGTDFTFTISSLQPEDATYYCQSTYGGVVGSTSDNPFGGGTKVEI
KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

HCVR of Antibody 23C8 (SEQ ID NO: 7)

EVQLVESGGGLVQPGGSLRLSCAASGFDISKYNIQWVRQAPGKGLEWVGFYNYG
10 GSAYYASRAKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCARGLSNSDLWGQ
GTLVTVSS

LCVR of Antibody 23C8 (SEQ ID NO: 8)

DIQMTQSPSSLSASVGDRVITTCQASQSISSWLSWYQQKPGKAPKLLIYRASTLAS
GVPSRFSGSGSGTDFTFTISSLQPEDATYYCQSTYGGVVGSTSDNPFGGGTKVEI
15 K

HC of Antibody 25F7 (SEQ ID NO: 9)

QVQLVQSGAEVKKPGASVKVSCKASGFSLSSTYAMNWRQAPGQGLEWMGIISD
DGTYYATWAKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARDAGAGGVQ
DYLTLWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPTV
20 SWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNTKV
DKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED
PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
25 HNHYTQKSLSLSLG

LC of Antibody 25F7 (SEQ ID NO: 10)

DIVMTQSPDSLAVSLGERATINCQASENIYNFLAWYQQKPGQPPLLIYSASTLAS
GVPPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQGSSNSNIDNPFGGGTKVEIKRT
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
30 EQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

HCVR of Antibody 25F7 (SEQ ID NO: 11)

QVQLVQSGAEVKKPGASVKVSCKASGFSLSITYAMNWVRQAPGQGLEWMGIISD
DGTITYATWAKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARDAGAGGVQ
DYLT LWGQGT LVT VSS

LCVR of Antibody 25F7 (SEQ ID NO: 12)

5 DIVMTQSPDSLAVSLGERATINCQASENIYNFLAWYQQKPGQPPLLIYSASTLAS
GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQGSSNSNIDNPFGGGKVEIK

HCDR1 of Antibody 22B3 (SEQ ID NO: 13)

GFSLSSYGVS

HCDR1 of Antibody 23C8 (SEQ ID NO: 14)

10 GFDISKYNIQ

HCDR1 of Antibody 25F7 (SEQ ID NO: 15)

GFSLSTYAMN

HCDR2 of Antibody 22B3 (SEQ ID NO: 16)

AISYDGITYYASWAKS

15 **HCDR2 of Antibody 23C8 (SEQ ID NO: 17)**

FINYGGSAYYASRAKG

HCDR2 of Antibody 25F7 (SEQ ID NO: 18)

IISDDGTTYYATWAKG

HCDR3 of Antibody 22B3 (SEQ ID NO: 19)

20 GDYYDDYVYVYALDI

HCDR3 of Antibody 23C8 (SEQ ID NO: 20)

GLSNSDL

HCDR3 of Antibody 25F7 (SEQ ID NO: 21)

DAGAGGVQDYLT

25 **LCDR1 of Antibody 22B3 (SEQ ID NO: 22)**

QASQSISTALA

LCDR1 of Antibody 23C8 (SEQ ID NO: 23)

QASQSISSWLS

LCDR1 of Antibody 25F7 (SEQ ID NO: 24)

30 QASENIYNFLA

LCDR2 of Antibody 22B3 (SEQ ID NO: 25)

AASSTLAS

LCDR2 of Antibody 23C8 (SEQ ID NO: 26)

RASTLAS

LCDR2 of Antibody 25F7 (SEQ ID NO: 27)

SASTLAS

5 **LCDR3 of Antibody 22B3 (SEQ ID NO: 28)**

QQGYSSSNLDNV

LCDR3 of Antibody 23C8 (SEQ ID NO: 29)

QSTYGGVVGSTSDNP

LCDR3 of Antibody 25F7 (SEQ ID NO: 30)

10 QQGSSNSNIDNP

Human BTLA (SEQ ID NO: 31)

MKTLPAMLGTGKLFVWFFLIPYLDIWNHKGESCDVQLYIKRQSEHSILAGDPFEL
ECPVKYCANRPHVTWCKLNGTTCVKLEDRTSWKEEKNISFFILHFEPVLPNDNG
SYRCSANFQSNLIESHSTLYVTDVKSASERPSKDEMASRPWLLYRLLPLGGLPLL
15 ITTCFCLFCCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSET
GIYDNDPDLCFRMQEGSEVYSNPCLEENKPGIVYASLNHSGVIGPNSRLARNVKEA
PTEYASICVRS

Mouse Balbc BTLA (SEQ ID NO: 32)

MKTVPAMLGTPRLFREFFILHLGLWSILCEKATKRNDDEECPVQLNIKRNSKHS
20 TGELFKIECPVKYCVHRPNVTWCKHNGTIWVPLEVGPQLYTSWEENRSVPVFL
HFKPIHLSDNQSYSCSTNFNSQVINSHSVTIHVRERTQNSSEHPLITVSDIPDATNAS
GPSTMEERPGRTWLLYTLLPLGALLLLACVCLLCFLKRIQGKEKKPSDLGRDT
NLVDIPASSRTNHQALPSGTGIYDNDPWSSMQDESELTISLQSERNNQGIVYASLN
HCVIGRNPRQENNMQEAPTEYASICVRS

25 **Mouse C57BL6 BTLA (SEQ ID NO: 33)**

MKTVPAMLGTPRLFREFFILHLGLWSILCEKATKRNDDEECPVQLTITRNSKQSART
GELFKIQCPVKYCVHRPNVTWCKHNGTICVPLEVSPQLYTSWEENQSVPVFLHF
KPIHLSDNQSYSCSTNFNSQVINSHSVTIHVTERTQNSSEHPLITVSDIPDATNASGP
STMEERPGRTWLLYTLLPLGALLLLACVCLLCFLKRIQKG
30 EKKPSDLGRDTNLVDIPASSRTNHQALPSGTGIYDNDPWSSMQDESELTISLQSE
RNNQGIVYASLNHCVIGRNPRQENNMQEAPTEYASICVRS

Cynomolgus Monkey BTLA (SEQ ID NO: 34)

MKTLPAMLGSGRLFWVFLIPYLDIWNHKGKESCDVQLYIKRQSYHSIFAGDPFK
LECPVKYCAHRPQVTWCKLNGTTCVKLEGRHTSWKQEKNLFFILHFEPVLPD
NGSYRCSANFLSAIIESHSTTLVYTDVKASERPSKDEMASRPWLLYSLLPLGGLP
LLITTCFCLFCFLRRHQKQNELSDTTGREITLVDVPFKSEQTEASTRQNSQVLLSE
5 TGIYDNEPDCFRMQEGSEVYSNPCLEENKPGIYASLNHSIIGLNSRQARNVKEA
PTEYASICVRS

**Exemplified DNA for Expressing Antibody 22B3 Heavy Chain of SEQ ID NO:1
(SEQ ID NO: 35)**

caggtgcagctggcgagctctggggctgaggtgaagaagcctggggcctcagtgaaaggttcctgcaaggcatctggattctcc
10 ctcagtagctatggagtgagctgggtgcgacaggcccctggacaaggccttgagtgatgggagccattagttatgatgtatta
catactacgcgagctggcgaaaagcagagtcacatgaccaggacacgtccacgagcacagctctacatggagctgagcag
cctgagatctgaggacacggccgtgtattactgtcgcgagaggggactactacgatgattatgtttatgtttatgcttagacatctgg
ggccagggcaccctgggtcaccgtctcctcagcttctaccaaggggccatcggtcttcccgtagcgcctgtccaggagcacc
tccgagagcacagccgcccctggggctgcctgggtcaaggactacttccccgaaccgggtgacgggtgctcgtggaactcaggcgccct
15 gaccagcggcggtgcacaccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtgggtgaccgtgccctccag
cagcttgggcacgaagacctacacctgcaacgtagatcacaagcccagcaacaccaagggtggacaagagagttgagtccaaa
tatgttccccatgccaccctgccagcacctgaggccggcggggaccatcagtcttctgttcccccaaaacccaagga
cactctcatgatctcccgacccctgaggtcacgtgcgtgggtgggtgacgtgagccaggaagaccccagggtccagtccaactg
gtacgtggatggcggtggaggtgcataatgccaaagaaagccgaggaggagcagttcaacagcacgtaccgtgtgtgcagc
20 gtctctaccgtctgcaccaggactgggtgaacggcaaggagtacaagtgaagggtctccaacaaaggcctcccgtctccatc
gagaaaacctctccaagccaaaggcgacccccgagagccacaggtgtacacctgcccccatcccaggaggagatgacc
aagaaccagggtcagcctgacctgcctgggtcaaaggcttctacccagcgacatcgccgtggagtgggaaagcaatgggcagc
cggagaacaactacaagaccacgcctcccgtgctggactccgacgggtccttcttctctacagcaggctaaccgtggacaaga
gcaggtggcaggagggaatgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacagaagagcctctccc
25 tgtctctgggt

**Exemplified DNA for Expressing Antibody 22B3 Light Chain of SEQ ID NO:2 (SEQ
ID NO: 36)**

Gaaattgtgtgacgcagctctccaggcacctgtcttctccaggggaaagagccaccctctcctgccaggccagtcagagc
30 attagtactgcattagcctgggtaccagcagaacctggccaggctcccagggtcctcatctatgtctgcatccactctggcatctgg
catcccagacaggttcagtgagcagtggtgtgggacagacttactctcaccatcagcagactggagcctgaagattttgagtg
tattactgtcaacagggttatagtagtaatcttgataatgttttcggcggaggggaccaagggtggagatcaaaccggaccgtgggt

gcaccatctgtcttcatcttcccgcctctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatccc
agagaggccaaagtacagtggaggtggataacgccctccaatcgggtaactcccaggagagtgacacagagcaggacagc
aaggacagcacctacagcctcagcagcaccctgacgctgagcaaaagcagactacgagaaacacaaagtctacgcctgcgaa
gtcacccatcagggcctgagctcgccgtcacaaagagcttcaacaggggagagtg

5

**Exemplified DNA for Expressing Antibody 23C8 Heavy Chain of SEQ ID NO:5
(SEQ ID NO: 37)**

gaggtgcagctggtggagctctgggggaggttggtccagcctggagggtccctgagactctcctgtgcagcctctggattcgac
atcagtaagtacaacatccaatgggtccgaggtccagggaaggggctggagtggttggttcattaattatggtgtagcg
10 catactacgcgagccggcgaaaggcagattcaccatctcaagagatgattcaaagaactcactgtatctgcaaatgaacagcct
gaaaaccgaggacacggccgtgtattactgtgctagaggactaagtaatagcgacctctggggccagggcaccctggtcaccg
tctctcagcttctaccaaggggccatcggtcttcccgtagcgccttgcctcaggagcacctccgagagcacagccgcctgg
gctgcctggtcaaggactacttccccgaaccggtgacgggtgctgtggaactcaggcgcctgaccagcggcgtgcacacctc
ccgggtgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacgaagacctac
15 acctgcaacgtagatcacaaagcccagcaacaccaagggtggacaagagagttgagtcacaatatggtccccatgccaccctg
cccagcacctgaggcccgccggggaccatcagttcttctgttcccccaaaaccaaaggacactctcatgatctccggagcccc
tgaggtcagctgcgtggtggtggacgtgagccagggaagaccccagggtccagtccaactggtacgtggatggcgtggaggtg
cataatgccaaagacaaagccgcccggaggagcagttcaacagcacgtaccgtgtgtgacgctcctaccgtcctgcaccagg
actggtgaacggcaaggagtacaagtgaaggtctccaacaaaggcctcccgtcctccatcgagaaaaccatctccaaagcc
20 aaagggcagccccgagagccacaggtgtacacctgtccccatcccaggaggagatgaccaagaaccaggtcagcctgacc
tgcctggtcaaaagcttctacccagcgacatcgccgtggagtggaagcaatgggcagccggagaaactacaagacca
cgcttcccgtgctggactccgacggctccttcttctctacagcaggctaaccgtggacaagagcaggtggcaggagggaat
gtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacagaaagagccttccctgtctctggt

**25 Exemplified DNA for Expressing Antibody 23C8 Light Chain of SEQ ID NO:6 (SEQ
ID NO: 38)**

gacatccagatgaccagctctccatctccctgtctgcatctgtaggagacagagtcaccatcacttgccaggccagtcagagca
ttagtagttggttatcctggtatcagcagaaaccagggaagcccctaagctcctgatctacagggtcaccactctggcatctggg
gtcccatcaagggtcagtggaagtggatctgggacagatttactttaccatcagcagcctgcagcctgaagatattgcaacatat
30 tactgtcaatccacttatggtggtgtgtggcagtaactgtatgataatcctttggcggagggaaccaagggtggagatcaaacg
gaccgtggctgcaccatctgtcttcatcttcccgcctctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaat
aacttctatcccagagaggccaaagtacagtggaggtggataacgccctccaatcgggtaactcccaggagagtgacaga

gcaggacagcaaggacagcacctacagcctcagcagcacccctgacgctgagcaaaagcagactacgagaaacacaaagtcta
cgctcggaagtcacccatcagggcctgagctcgccgctcacaagagcttcaacaggggagagtg

Exemplified DNA for Expressing Antibody 25F7 Heavy Chain of SEQ ID NO:9

5 (SEQ ID NO: 39)

cagggtgcagctgggtcagctctggggctgaggtgaagaagcctggggcctcagtgaaagtttctgcaaggcatctggattctcc
ctcagtacctatgcaatgaactgggtgcgacaggccctggacaagggcttgagtgatgggaatcattagtgatgatgggtacca
catactacgcgacctgggcgaaaggcagagtcacatgaccaggagacacgtccacgagcacagtctacatggagctgagcag
cctgagatctgaggacacggccgtgtattactgtgcgagagatgctgggtgctgggtgtcgaagactacttaaccttggggc
10 cagggcacccctggtcaccgtctcctcagcttctaccaagggcccatcggtcttcccgctagcgccctgctccaggagcacctcc
gagagcacagccgacctgggctgcctggtcaaggactacttccccgaaccgggtgacggtgtcgtggaactcaggcgccctga
ccagcggcgtgcacaccttccggctgtcctacagtctcaggacttactccctcagcagcgtggtgacctgcccctccagca
gcttgggcacgaagacctacacctgcaacgtagatcacaagcccagcaacaccaagggtggacaagagagttgagtcctaatat
ggtcccccattgccaccctgccagcacctgagggccggggggaccatcagttctctgttcccccaaaaccaaggacac
15 tctcatgatctccggaccctgaggtcacgtgcgtgggtgggtggacgtgagccagggaagaccccgaggtccagtcaactggta
cgtggatggcgtggaggtgcataatgccaagacaaagccgcgaggagcagttcaacagcacgtacctgtggtcagcgctc
ctcaccgtctgcaccaggactggctgaacggcaaggagtacaagtcaagggtctccaacaaggcctccgctctccatcga
gaaaaccatctccaaagccaaaggcagccccgagagccacaggtgtacacctgcccccatccaggaggagatgaccaa
gaaccaggtcagcctgacctgcctggtcaaggcttctacccagcgacatcgccgtggagtgggaaagcaatgggcagccg
20 gagaacaactacaagaccacgcctcccgctgctggactccgacggtccttctctctacagcaggctaaccgtggacaagagc
aggtggcaggaggggaatgttctcatgctccgtgatgcatgaggctctgcacaaccactacacagaagagcctctccctgt
ctctgggt

Exemplified DNA for Expressing Antibody 25F7 Light Chain of SEQ ID NO:10

25 (SEQ ID NO: 40)

Gacatcgtgatgaccagctctccagactccctggctgtgtctctggcgagagggccaccatcaactgccaggccagtgagaa
tattacaacttttggcctggtaccagcagaaaccaggacagcctcctaagctgctcatttactctgcacccactctggcatctggg
gtccctgaccgattcagtggcagcgggtctgggacagatttactctcaccatcagcagcctgcaggctgaagatgtggcagttt
attactgtcaacaggggtctagtaataagtaattgataatccttccggcgagggaccaagggtggagatcaaacggaccgtggct
30 gcaccatctgtcttcatcttccgccatctgatgagcagttgaaatctggaactgcctctgttgctgtgctgaataacttctatccc
agagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagtgacagagcaggacagc

aaggacagcacctacagcctcagcagcacccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaa
gtcacccatcagggcctgagctcgcccgtcacaaagagctcaacaggggagagtgc

Human HVEM (SEQ ID NO: 41)

5 MEPPGDWGPPPWRSTPKTDVLRLLVLTFLGAPCYAPALPSCKEDEYPVGSECCP
KCSPGYRVKEACGELTGTVCEPCPPGTYYIAHLNGLSKCLQCQMCDPAMGLRASR
NCSRTENAVCGCSPGHFCIVQGDHCAACRAYATSSPGQRVQKGGTESQDTLCQ
NCPPGTFSPNGTLEECQHQTCSWLVTKAGAGTSSSHWVWWFLSGSLVIVIVCST
VGLIICVKRRKPRGDVVKVIVSVQRKRQEAEGEATVIEALQAPPDVTTVAVEETIP
10 SFTGRSPNH

WE CLAIM:

1. An antibody that binds BTLA, comprising HCDR1 having the amino acid sequence of SEQ ID NO: 13, HCDR2 having the amino acid sequence of
5 SEQ ID NO: 16, HCDR3 having the amino acid sequence of SEQ ID NO: 19, LCDR1 having the amino acid sequence of SEQ ID NO: 22, LCDR2 having the amino acid sequence of SEQ ID NO: 25, and LCDR3 having the amino acid sequence of SEQ ID NO: 28.
2. The antibody of Claim 1, comprising a heavy chain variable region
10 (HCVR) having the amino acid sequence of SEQ ID NO: 3, and a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO: 4.
3. The antibody of Claim 1 or 2, comprising a heavy chain (HC) having the amino acid sequence of SEQ ID NO: 1, and a light chain (LC) having the
15 amino acid sequence of SEQ ID NO: 2.
4. The antibody of any one of Claims 1 to 3, comprising two HC and two LC, wherein each HC has the amino acid sequence of SEQ ID NO: 1, and each light chain (LC) has the amino acid sequence of SEQ ID NO: 2.
5. A pharmaceutical composition comprising the antibody of any one of
20 Claims 1 to 4, and one or more pharmaceutically acceptable carriers, diluents, or excipients.
6. A method of treating a patient having a rheumatic, neural, and/or dermatological disease, comprising administering an effective amount of an antibody of any one of Claims 1-4.
- 25 7. The method of Claim 6, wherein the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis.
8. The method of Claim 6, wherein the dermatology disease is at least one of atopic dermatitis and psoriasis.
9. The method of Claim 6, wherein the neural disease is multiple sclerosis.
- 30 10. The antibody of any one of Claims 1 to 4 for use in therapy.
11. The antibody of any one of Claims 1 to 4 for use in the treatment of one or more of rheumatic, neural, and dermatology disease.

12. The antibody of Claim 11, wherein the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis.
13. The antibody of Claim 11, wherein the dermatology disease is at least one of atopic dermatitis and psoriasis.
- 5 14. The antibody of Claim 11, wherein the neural disease is multiple sclerosis.
15. Use of the antibody of any one of Claims 1 to 4 in the manufacture of a medicament for the treatment of one or more of rheumatic, neural, and dermatology disease.
- 10 16. The use of the antibody of Claim 15, wherein the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis.
17. The use of the antibody of Claim 15, wherein the dermatology disease is at least one of atopic dermatitis and psoriasis.
18. The use of the antibody of Claim 15, wherein the neural disease is multiple sclerosis.
- 15 19. A pharmaceutical composition for use in treating a condition selected from lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis, comprising an effective amount of the antibody of any one of Claims 1 to 4.
- 20 20. A pharmaceutical composition for use in treating a condition selected from atopic dermatitis and psoriasis, comprising an effective amount of the antibody of any one of Claims 1 to 4.
21. A pharmaceutical composition for use in treating multiple sclerosis, comprising an effective amount of the antibody of any one of Claims 1 to 4.
- 25 22. A DNA molecule comprising a polynucleotide that encodes the antibody heavy chain given by the amino acid sequence of SEQ ID NO:1.
23. A DNA molecule comprising a polynucleotide that encodes the antibody light chain given by the amino acid sequence of SEQ ID NO:2.
- 30 24. A DNA molecule comprising a polynucleotide sequence that encodes the antibody heavy chain having the amino acid sequence of SEQ ID NO: 1

- and a polynucleotide sequence that encodes the antibody light chain having the amino acid sequence of SEQ ID NO: 2.
25. The DNA molecule of Claim 24, wherein the polynucleotide sequence that encodes the antibody heavy chain is SEQ ID NO:35, and the
- 5 polynucleotide sequence that encodes the antibody light chain is SEQ ID NO:36.
26. A mammalian cell comprising the DNA molecule of Claim 22 and the DNA molecule of Claim 23, wherein the cell is capable of expressing an antibody comprising two heavy chains and two light chains, wherein the
- 10 amino acid sequence of each heavy chain is given by SEQ ID NO:1 and the amino acid sequence of each light chain is given by SEQ ID NO:2.
27. A mammalian cell comprising the DNA molecule of Claim 24 or 25, wherein the cell is capable of expressing an antibody comprising two heavy chains and two light chains, wherein the amino acid sequence of
- 15 each heavy chain is given by SEQ ID NO:1 and the amino acid sequence of each light chain is given by SEQ ID NO:2.
28. A process for producing an antibody, wherein the amino sequence of each of the two HCs is SEQ ID NO: 1, and the amino acid sequence of each of the two LCs is SEQ ID NO: 2, and wherein the process comprises: a)
- 20 cultivating the mammalian cell of Claim 26 or 27 under conditions such that the antibody is expressed, and b) recovering the expressed antibody.
29. An antibody obtainable by the process of Claim 28.
30. An antibody that binds BTLA, comprising HCDR1 having the amino acid sequence of SEQ ID NO: 14, HCDR2 having the amino acid sequence of
- 25 SEQ ID NO: 17, HCDR3 having the amino acid sequence of SEQ ID NO: 20, LCDR1 having the amino acid sequence of SEQ ID NO: 23, LCDR2 having the amino acid sequence of SEQ ID NO: 26, and LCDR3 having the amino acid sequence of SEQ ID NO: 29.
31. The antibody of Claim 30, comprising a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO: 7, and a light chain variable region (LCVR) having the amino acid sequence of SEQ ID
- 30 NO: 8.

32. The antibody of Claims 30 or 31, comprising a heavy chain (HC) having the amino acid sequence of SEQ ID NO:5, and a light chain (LC) having the amino acid sequence of SEQ ID NO: 6.
- 5 33. The antibody of any one of Claims 30 to 32, comprising two HC and two LC, wherein each HC has the amino acid sequence of SEQ ID NO: 5, and each light chain (LC) has the amino acid sequence of SEQ ID NO: 6.
34. A pharmaceutical composition comprising the antibody of any one of Claims 30 to 33, and one or more pharmaceutically acceptable carriers, diluents, or excipients.
- 10 35. A method of treating a patient having a rheumatic, neural, and/or dermatological disease, comprising administering an effective amount of an antibody of any one of 30 to 33.
36. The method of Claim 35, wherein the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis.
- 15 37. The method of Claim 35, wherein the dermatology disease is at least one of atopic dermatitis and psoriasis.
38. The method of Claim 35, wherein the neural disease is multiple sclerosis.
39. The antibody of any one of Claims 30 to 33 for use in therapy.
40. The antibody of any one of Claims 30 to 33 for use in the treatment of one or more of rheumatic, neural, and dermatology disease.
- 20 41. The antibody of Claim 40, wherein the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis.
42. The antibody of Claim 40, wherein the dermatology disease is at least one of atopic dermatitis and psoriasis.
- 25 43. The antibody of Claim 40, wherein the neural disease is multiple sclerosis.
44. Use of the antibody of any one of Claims 30 to 33 in the manufacture of a medicament for the treatment of one or more of rheumatic, neural, and dermatology disease.
- 30 45. The use of the antibody of Claim 44, wherein the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis.

46. The use of the antibody of Claim 44, wherein the dermatology disease is at least one of atopic dermatitis and psoriasis.
47. The use of the antibody of Claim 44, wherein the neural disease is multiple sclerosis.
- 5 48. A pharmaceutical composition for use in treating a condition selected from lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis, comprising an effective amount of the antibody of any one of Claims 30 to 33.
- 10 49. A pharmaceutical composition for use in treating a condition selected from atopic dermatitis and psoriasis, comprising an effective amount of the antibody of any one of Claims 30 to 33.
50. A pharmaceutical composition for use in treating multiple sclerosis, comprising an effective amount of the antibody of any one of Claims 30 to 33.
- 15 51. A DNA molecule comprising a polynucleotide that encodes the antibody heavy chain given by the amino acid sequence of SEQ ID NO:5.
52. A DNA molecule comprising a polynucleotide that encodes the antibody light chain given by the amino acid sequence of SEQ ID NO:6.
- 20 53. A DNA molecule comprising a polynucleotide sequence that encodes the antibody heavy chain having the amino acid sequence of SEQ ID NO: 5 and a polynucleotide sequence that encodes the antibody light chain having the amino acid sequence of SEQ ID NO: 6.
- 25 54. The DNA molecule of Claim 53, wherein the polynucleotide sequence that encodes the antibody heavy chain is SEQ ID NO:37, and the polynucleotide sequence that encodes the antibody light chain is SEQ ID NO:38.
- 30 55. A mammalian cell comprising the DNA molecule of Claim 51 and the DNA molecule of Claim 52, wherein the cell is capable of expressing an antibody comprising two heavy chains and two light chains, wherein the amino acid sequence of each heavy chain is given by SEQ ID NO:5 and the amino acid sequence of each light chain is given by SEQ ID NO:6.

56. A mammalian cell comprising the DNA molecule of Claim 53 or 54, wherein the cell is capable of expressing an antibody comprising two heavy chains and two light chains, wherein the amino acid sequence of each heavy chain is given by SEQ ID NO:5 and the amino acid sequence of each light chain is given by SEQ ID NO:6.
57. A process for producing an antibody, wherein the amino sequence of each of the two HCs is SEQ ID NO: 5, and the amino acid sequence of each of the two LCs is SEQ ID NO: 6, and wherein the process comprises: a) cultivating the mammalian cell of Claim 55 or 56 under conditions such that the antibody is expressed, and b) recovering the expressed antibody.
58. An antibody obtainable by the process of Claim 57.
59. An antibody that binds BTLA, comprising HCDR1 having the amino acid sequence of SEQ ID NO: 15, HCDR2 having the amino acid sequence of SEQ ID NO: 18, HCDR3 having the amino acid sequence of SEQ ID NO: 21, LCDR1 having the amino acid sequence of SEQ ID NO: 24, LCDR2 having the amino acid sequence of SEQ ID NO: 27, and LCDR3 having the amino acid sequence of SEQ ID NO: 30.
60. The antibody of Claim 59, comprising a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO: 11, and a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO: 12.
61. The antibody of Claim 59 or 60, comprising a heavy chain (HC) having the amino acid sequence of SEQ ID NO: 9, and a light chain (LC) having the amino acid sequence of SEQ ID NO: 10.
62. The antibody of any one of Claims 59 to 61, comprising two HC and two LC, wherein each HC has the amino acid sequence of SEQ ID NO: 9, and each light chain (LC) has the amino acid sequence of SEQ ID NO:10.
63. A pharmaceutical composition comprising the antibody of any one of Claims 59 to 62, and one or more pharmaceutically acceptable carriers, diluents, or excipients.

64. A method of treating a patient having a rheumatic, neural, and/or dermatological disease, comprising administering an effective amount of an antibody of any one of Claims 59 to 62.
- 5 65. The method of Claim 64, wherein the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis.
66. The method of Claim 64, wherein the dermatology disease is at least one of atopic dermatitis and psoriasis.
67. The method of Claim 64, wherein the neural disease is multiple sclerosis.
68. The antibody of any one of Claims 59 to 62 for use in therapy.
- 10 69. The antibody of any one of Claims 59 to 62 for use in the treatment of one or more of rheumatic, neural, and dermatology disease.
70. The antibody of Claim 69, wherein the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis.
71. The antibody of Claim 69, wherein the dermatology disease is at least one of atopic dermatitis and psoriasis.
- 15 72. The antibody of Claim 69, wherein the neural disease is multiple sclerosis.
73. Use of the antibody of any one of Claims 59 to 62 in the manufacture of a medicament for the treatment of one or more of rheumatic, neural, and dermatology disease.
- 20 74. The use of the antibody of Claim 73, wherein the rheumatic disease is at least one of lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis.
75. The use of the antibody of Claim 73, wherein the dermatology disease is at least one of atopic dermatitis and psoriasis.
- 25 76. The use of the antibody of Claim 73, wherein the neural disease is multiple sclerosis.
77. A pharmaceutical composition for use in treating a condition selected from lupus nephritis, systemic lupus erythematosus, and rheumatoid arthritis, comprising an effective amount of the antibody of any one of Claims 59 to 30 62.

78. A pharmaceutical composition for use in treating a condition selected from atopic dermatitis and psoriasis, comprising an effective amount of the antibody of any one of Claims 59 to 62.
- 5 79. A pharmaceutical composition for use in treating multiple sclerosis, comprising an effective amount of the antibody of any one of Claims 59 to 62.
80. A DNA molecule comprising a polynucleotide that encodes the antibody heavy chain given by the amino acid sequence of SEQ ID NO:9.
- 10 81. A DNA molecule comprising a polynucleotide that encodes the antibody light chain given by the amino acid sequence of SEQ ID NO:10.
82. A DNA molecule comprising a polynucleotide sequence that encodes the antibody heavy chain having the amino acid sequence of SEQ ID NO: 9 and a polynucleotide sequence that encodes the antibody light chain having the amino acid sequence of SEQ ID NO: 10.
- 15 83. The DNA molecule of Claim 82, wherein the polynucleotide sequence that encodes the antibody heavy chain is SEQ ID NO:39, and the polynucleotide sequence that encodes the antibody light chain is SEQ ID NO:40.
- 20 84. A mammalian cell comprising the DNA molecule of Claim 80 and the DNA molecule of Claim 81, wherein the cell is capable of expressing an antibody comprising two heavy chains and two light chains, wherein the amino acid sequence of each heavy chain is given by SEQ ID NO:9 and the amino acid sequence of each light chain is given by SEQ ID NO:10.
- 25 85. A mammalian cell comprising the DNA molecule of Claim 82 or 83, wherein the cell is capable of expressing an antibody comprising two heavy chains and two light chains, wherein the amino acid sequence of each heavy chain is given by SEQ ID NO:9 and the amino acid sequence of each light chain is given by SEQ ID NO:10.
- 30 86. A process for producing an antibody, wherein the amino sequence of each of the two HCs is SEQ ID NO: 9, and the amino acid sequence of each of the two LCs is SEQ ID NO: 10, and wherein the process comprises: a)

cultivating the mammalian cell of Claim 84 or 85 under conditions such that the antibody is expressed, and b) recovering the expressed antibody.

87. An antibody obtainable by the process of Claim 86.